A DISSERTATION FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BIOLOGICAL CHARACTERS OF KOREAN *NEOSEIULUS CALIFORNICUS* MCGREGOR (ACARI: PHYTOSEIIDAE) IN RELATION TO ENVIRONMENTAL PARAMETERS

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General Introduction

The increase of apple production in Korea has been one of the most noticeable that once expanded up to 53,000 ha of cultivating area in 1992 and now settled down around 30,000 ha (MAF, 2007). NIAST (1988) described 312 arthropod pest species in Korean apple orchards where two spotted spider mite (TSSM), *Tetranychus urticae*, European red mite (ERM), *Panonychus ulmi* are considered as most difficult to manage.

Traditionally, spider mites have been controlled with acaricides resulting chemical resistance in mites and residues in the products. There are numerous reports on the occurrence of acaricide resistant in both the ERM and TSSM, and on the failure in mites control with chemicals (Kimura, 1999; Nauen et al., 2001; Sato et al., 2004; Suh et al., 2006). Intensive pest management with chemicals in apple resulted in the destruction of natural enemy complex in the systems (Parent and Lord 1971) and increase of nutritive elements in the leaves have been led population build-up of TSSM or ERM quicker and negatively affected the productivity and quality of apples and the formation of the flower bud for next year (Lee, 1965; Zwick, 1972; McGroarty and Croft, 1978; Kim et al., 1981). As a logical alternative to acaricides, biological control has been attempted on apple prone to spider mites. Many species of predatory mites have been used as control agents with varying success. *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) was reported to occur on various crops and deciduous tress in Europe, Africa, North and South America and Asia (McMurtry and Croft 1997; Schausberger and Croft 2000; Copping 2001, Tixier et al., 2008). This species also has the potential for effective biological control of tetranychid species as well as other pest mites and insects (Swirskii et al., 1970; McMurtry 1977; Friese and Gilstrap 1982; McMurtry and Croft 1997). *N. californicus* shows tolerance to some pesticides and spontaneously colonizes the crops (Escudero and Ferragut, 1998, 1999)

The various alien, indigenous and commercial strains of *N. californicus* (UK, USA, Italian, Spanish, African, Japanese, Spical strains) are distributed throughout the world. Fecundity (Castagnoli and Simoni, 1991; Gotoh et al., 2004), the intrinsic rate of increase (r_m) (Ma and Laing, 1973; Mesa et al., 1990), and diapausing ability (Castagnoli et al., 1996; Hart et al,. 2002; Jolly, 2000) vary among those strains of *N. californicus*. However, in Korea, one population of *N. californicus* was first recognized during the season of 2004-2005 from the citrus orchards in Jeju Island (Jung et al., 2006), but there are no records of *N. californicus* being endemic in the main land. Many information on the general and laboratory biology of *N. californicus* strains from Europe, the United States, Africa and Japan have been published (Castagnoli et al., 1995; McMurtry and Croft 1997; Walzer and Schausberger 1999a,b; Raworth et al., 1994; Canlas et al., 2006; Kim et al., 2009). However, no data are available on *N. californicus* collected from Jeju citrus orchards. Additionally, in respect of abiotic factors, this study compared the Korean *N. californicus* with the dominant native phytoseiid mite *Neoseiulus womersleyi* Schicha (Acari: Phytoseiidae) (Jung et al., 2003; 2004; Kim et al., 2005), and the most frequent and commercially successful spider mite biocontrol agent in the world *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (McMurtry and Croft 1997).

Our current efforts include the evaluation of these predatory mites is to find promising biological control agent of pest mites in apple pest management system. Considering the potentiality of the predators, the study provides (1) the effect of temperatures on life-history of the predatory mite, *N. californicus* fed on *P. ulmi*, (2) the interactions of phytoseiids under different ambient and food shortage conditions for effective use in the field and greenhouse conditions, (3) information on diapause expression in *N. californicus* as the advantageous physiological mechanism to survive in the harsh environmental conditions, and (4) information on cold tolerance of *N. californicus* respective to *N. womersleyi*.

Literature Review

Arthropod pest infestations are the most concern and recognized problems in Korean apple orchards. NIAST (1988) enlisted 312 pest species from Korean apple orchards. Spider mites (Acari: Tetranychidae) became worldwide economic pests mainly due to the increased use of broad-spectrum pesticides after the Second World War (Huffaker et al., 1970). National Horticultural Research Institute (NHRI, Suwon, Korea) described the historical changes of population abundances of European red mite (ERM), *Panonychus ulmi* (Koch), and two-spotted spider mite (TSSM), *Tetranychus urticae* (Koch) (Acari: Tetranychidae) in Korean apple orchards based on research reports from 1958 to 1998. ERM was an abundant species up to 1970s, and TSSM became a dominant species after 1980. The change occurred around mid 1970s. In the past decade, it is remarkable that there have been increasing problems with spider mites, replacing the dominant position from TSSM to ERM in Korean apple orchards (Kim et al., 2005). However, ERM attracted attention in the U.S. as early as in 1920s and by the 1930s the species was a persistent enough pest in northeastern orchards to require treatment (Garman and Townsand, 1938). The increase in pest status of ERM in the 1930s in Connecticut was thought to be a result of dry summers and cold winters or changing grower practices (Garman and Townsand, 1938). In Canada it became an important pest after 1945 when DDT began to be used extensively (Parent and Lord, 1971). ERM is currently an economic pest on a wide variety of agricultural crops and significant resources are spent on its control.

1. Biology of *Panonychus ulmi* (Koch) and *Tetranychus urticae* (Koch)

Taxonomy of *P. ulmi* and *T. urticae*

Kingdom: Animalia
  Phylum: Arthropoda
   Sub- phylum: Chelicerata
    Class: Arachnida
      Sub-class: Acari

Super-order: Acariformes
  Order: Prostigmata
    Family: Tetranychidae
      Genus and Species: *Panonychus ulmi* (Koch, 1836)
        Tetranychus urticae (Koch, 1836)

The two spotted spider mite *Tetranychus urticae* Koch (Acarina: Tetranychidae) is a serious pest on many crops and ornamental plants. This mite feeds on more than 150 species of host plants, including most deciduous fruit trees and vegetables (Jepson et al., 1975; Krips et al., 1998; Sabelis, 1981; Van de Vrie et al., 1972). The ERM is a major pest in almost all fruit growing regions of the world (Hardman *et al*., 1985).The European red mite has a wide host range includes deciduous bushes and trees belonging to the family Rosaceae, but it is in association with fruit trees such as apple, pear, plum, peach, prune and cherry that it reaches economic importance.

Species in the Tetranychidae undergo three immature stages; a six-legged larval stage, and eight-legged protonymph and deutonymph stages. Each of these stages is followed by a quiescent stage known as the protochrysalis, deutochrysalis and the teleiochrysalis respectively (Van de Vrie et al., 1972). Mated females will produce either female or male offspring, while unmated females produce males only (Cagle, 1946). ERM lays two types of eggs. Summer eggs laid on the leaves of the host plant are of the non-diapause type and develop without interruption. The winter eggs are deposited predominantly on the bark of twigs and branches of the host plants (Beament, 1951). Egg type is governed by photoperiod, temperature and nutritional status of the female, as 'summer' females exposed to cool conditions lay diapausing eggs (Van de Vrie et al., 1972).

P. ulmi development, survival, and reproduction are driven mainly by temperature and precipitation with lesser effects from food supply and host nitrogen status (Wermelinger et al., 1992). Rainfall can have a significant negative impact on spider mites, and, also, that may be exacerbated by high temperatures (Van de Vrie et al., 1972; Simpson and Connell, 1973). Dispersal is largely by wind and they can also readily crawl within trees (Garman and Townsand, 1938; Smitley and Kennedy, 1985).

Damage to the host is done by both immature and adult mites. They pierce the leaf epidermal cells and extract cell contents which causes chlorosis or a bronzing effect and in severe cases, necrosis (Pfeiffer and Schultz, 1986). This damage can result in leaf abscission and small fruit with poor color (Garman and Townsand, 1938), although injury caused by ERM is not as severe as that by *Tetranychus* spp. (Youngman et al., 1986; Mobley and Marini, 1990). Several workers have reported effects on growth, yield, fruit colour and fruit bud production of apple trees which they have attributed to infestations of ERM (Chapman *et al.,* 1952; Van de Vrie, 1956; Gould, 1965; Briggs and Avery, 1968; Avery and Briggs, 1968; Coghill, 1969). In commercial orchards, the potential of ERM to cause severe economic damage necessitates chemical control several times a year (Croft, 1975). However, it is considered the most destructive mite in apple orchards where it has been shown to affect fruit size, color, number of fruit the following season, and tree vigor (Croft and McGroarty, 1977).

Many mite species overwinter in diapause (Veerman, 1992) in protected habitats. Shorter day length and cool temperatures are the main inducers of diapause in spider mites (Van de Vrie et al., 1972). The ERM overwinter in diapausing egg stage in the crevices of twigs (Kim et al., 2005; Jepson et al., 1975; Lee et al., 1989; Lee et al., 1994) in apple and pear orchards. In early spring the overwintering eggs hatch and the young larvae move to the foliage and start feeding (Pfeiffer and Schultz, 1986; Jeppson et al., 1975). TSSM overwinter in facultative adult diapause (Veerman, 1992) and use tree trunk barks as a major overwintering habitat (Lee, 1990; Kim and Lee, 2003) and also in ground vegetation or on fallen leaves (Kim et al., 2005).

Spider mites have a tendency to develop their resistance to many classes of pesticides (Brader, 1977; Dennehy et al. 1983; Herron et al., 1994). Bowie et al. (1988), and Collyer and Geldermalsen (1975) observed the development of resistance in the ERM against some pesticides, e.g. organotin miticides, Azocyclotin, dicofol. During the 1960's and 70's *P. ulmi* became resistant against many compounds like omethoate and dimethoate (Sterk, 1994).

Many authors investigated the pesticides resistance in the TSSM and reports on the occurrence of acaricide resistant *T. urticae* as well as on the failure to control *T. urticae* using different pesticides (Edge and James, 1986; Fergusson-Kolmes et al., 1991;

Herron et al., 1993; Cho et al., 1995; Beers et al., 1998; Stumpf and Nauen, 2001; Sato et al., 2004; Suh et al., 2006). TSSM has a relatively high possibility of developing pesticides resistance due to haplo-diploid parthenogenesis, short life cycle and high reproductive potential (Boudreaux, 1963; Huffaker et al., 1969, 1970; Cranham and Helle, 1985).

2. Biology of *Neoseiulus californicus* (McGregor)

Mites belonging to the family Phytoseiidae are generally known as predators of phytophagous mites and are regarded as the most important natural enemies of spider mites (Helle and Sabelis, 1985). The generalist predatory mite of the Phytoseiidae *Neoseiulus californicus* (McGregor) has the potentials to feed on spider mites, tarsonemid mites, other small arthropods and pollen (Castagnoli et al., 1999). For this reason this predatory mite has been studies extensively with respect to its basic biology and potential application as biological control agents (Hoy, 1982; Helle and Sabelis, 1985).

A. Taxonomy of *N. californicus*

Kingdom: Animalia
  Phylum: Arthropoda
    Class: Arachnida
      Sub-class: Acari
        Super- order: Parasitiformes
          Order: Mesostigmata
            Family: Phytoseiidae
              Genus and species: *Neoseiulus* (*Amblyseius*) *californicus*
              (McGregor, 1954)

Synonyms: *Neoseiulus marinus*, *N. chilenesis*, *N. mungeri*
Other names: *Typhlodromus californicus*, *Cydnodromus*
californicus (de Moraes et al., 2004).

N. californicus was first described by McGregor in 1954 from lemon in California as *Typhlodromus californicus* (de Moraes et al., 2004). In 1958, this species was renamed as *Typhlodromus chilenensis* (Dosse, 1958). After 1958, it was moved to the genus *Amblyseius* as *Amblyseius chilenensis* (Ehara, 1964) and later to the genus *Neoseiulus,* and *Neoseiulus chilenensis* is also considered a synonym of *N. californicus* (Moraes et al., 1986; Chant and McMurtry, 2003; Ehara and Amano, 2004). Ehara and Amano (1998) considered this species as *Amblyseius californicus*.

B. Distribution and natural habitat of *N. californicus*

N. californicus is distributed all over the world, both in arid and humid areas of subtropical and temperate zones. The predatory mite naturally occurred in USA, Mexico, Brazil, Guatemala, Peru, Argentina, Chile, Cuba, Colombia, Japan, Taiwan (introduced), Portugal, Italy, France and Spain (de Moraes et al., 2004; McMurtry, 1977; Rencken and Pringle, 1998; Ferreira and Carmona, 1994). Jung et al. (2006) reported a natural population of *N. californicus* distributed in citrus orchards with ERM in Jeju Island for the first time from the outdoor field in Korea.

The natural habitat of *N. californicus* is very diverse. The predatory mite is found on apple, peach, grape, strawberries, avocado, cassava, wheat, pine, mallow, citrus, alfalfa, poplar, lemon fruit, vegetables and ornamental plants. The mites can even be found between ground cover such as dry leaves (Castagnoli and Simoni, 2003).

C. Host range and specificity

N. californicus feeds on Tetranychids (e.g. TSSM, ERM) and on Tarsonemids. Adults eat all stages of spider mites; larvae eat mainly eggs, while nymphs eat eggs, larvae and nymph. *N. californicus* shows a feeding preference for the larval and nymphal stages of the TSSM when the pest is present at low densities (Malais and Ravenberg, 2003). However, *N. californicus* can survive for a few days without eating a prey by feeding solely on a diet of pollen (Malais and Ravensberg, 2003). Castagnoli and Simoni (2003) recorded successful development and reproduction in laboratory trials on the following prey species:

(1) Mites

a) Tetranychidae: *Tetranychus pacificus* (McGregor), *Panonychus ulmi* (Koch), *Tetranychus cinnabarinus* (Boisduval), *Eotetranychus orientalis* (Klein), *Mononychellus tanajoa* (Bondar) eggs, *M. progresesivus* Doreste, *Oligonychus pratensis* (Banks), *O. perseae* Tuttle, Baker *et* Abbatiello, *O. ilicis* (McGregor)

b) Tarsonemidae: *Polyphagotarsonemus latus* (Banks) and *Phytonemus pallidus* (Banks)

c) Eriophydidae: *Aculops lycopersici, Aculus schlenchtendali* (Nalepa) and *Aceria dioscoridis* (Solimati and Abou-Awad).

d) Tenuipalpidae: *Brevipalpus phoenicus* (Geijskes)

e) Pyroglyphididae: *Dermatophagoides farinae* (Hughes)

In laboratory trials nymphs were not able to mature on the Eriophid mite *Phyllocoptruta oleivora* (Swirskii)

(2) Insects

N. californicus accepted *Frankliniella occidentalis* (Pergande) as alternative food, although their oviposition dropped dramatically. Limited maturation was found on eggs of the moth *Prays citri* Milliere, but females did not produce eggs. On a diet of crawlers of the red scale *Aonidiella aurantii* (Mask.) nymphs matured quite well and females produced few eggs (Castagnoli and Simoni, 2003). In laboratory trials, nymphs of *N. californicus* could not mature on eggs of *Spodoptera littoralis, Ectomyelois ceratoniae* and *Ceroplastes floridensis*, nor on *Bemisia tabaci* or *Retithrips syriacus* (Swirskii *et al.*, 1970).

D. Life cycle and description of *N. californicus*

N. californicus is an oblong, tiny, mobile, predatory mite. It has five life stages: the egg, a six-legged larva, eight-legged protonymph and deutonymph stages, and the adult (Sabelis, 1985). Females are slightly larger than males. *N. californicus* eggs are oval shaped, approximately 0.04 mm (0.00016 in) in length, and are pale whitish in color. Larvae are translucent in color. Both nymphal stages, the protonymph and the deutonymph, resemble the adults except that they are smaller and cannot reproduce.

Adult females are approximately 0.1 mm (0.00039 in) in length and oval in shape. Both males and females are translucent and can be pale orange or pink in color.

E. Environmental requirements for development and reproduction of *N. californicus*

(1) Temperature

N. californicus is active over a wide range of temperatures. They can be tolerated much colder temperatures for short periods of time. They reproduce and develop well where the upper and lower temperature limits ranging from 10 to 33°C (50-91°F) (Malais and Ravensberg, 2003). The reproduction rate is optimal at 23°C (Castagnoli and Simoni, 1991).

(2) Relative humidity

Humidity strongly affects egg survival in *N. californicus*, while the larvae are slightly more tolerant than eggs to the same temperature-humidity regimes. They can tolerate a wide range of humidity (40 - 80% relative humidity), but prefer humidity at the upper end of this range. A relative humidity of less than 60% retard the developmental period. The higher the level of relative humidity within the range, the better the predator can maintain themselves in a crop. High temperature (between 29 and 33°C) and high humidity (95 and 100%) minimized mortality and considerably shortened the developmental time of non-feeding stages (Castagnoli and Simoni, 2003; Rott and Ponsonby, 2000).

F. Biological control of spider mites by phytoseiids

Phytoseiids have been used in biological control programs in a wide variety of agricultural systems (McMurtry, 1982). These crops include apple (Croft and MacRae, 1992; Steinburg and Cohen, 1992; Croft and Slone, 1997), grape (Kinn and Doutt, 1972; Duso, 1992; Duso and Pasqualetto, 1993), nursery citrus (Grafton-Cardwell et al., 1997), strawberry (Croft and Coop, 1998), peppermint (Morris et al., 1999) and hops (Strong and Croft, 1995). These experiments used a variety of phytoseiids and had varying degrees of success.

The use of pesticides in orchards is one of the main obstacles to establishing a successful phytoseiid settle down. A strategy that has been used to overcome this problem in other systems is the development and release of resistant strains of phytoseiids to various pesticides (Hoy, 1982, 1985). Pyrethroid-resistant strains of *Typhlodromus pyri* have been successful in controlling ERM in Nova Scotian apple orchards (Hardman et al., 2000; Moreau et al., 2000). Croft and Hoying (1975) released strains of *N. fallacis* that were resistant to organophosphates, carbaryl, or both into Michigan orchards. The predators were easily established, but only the organophosphate resistance persisted in the populations. Some strains of *N. californicus* are highly resistant to pesticides (Croft, 1990; Castagnoli et al., 2005; Escudero and Ferragut, 1998, 1999) allowing the use of *N. californicus* in integrated pest management systems.

G. Potential of *N. californicus* as a biological control agent

The concept of biological control has been defined in many ways. In a broad sense it is the regulation of pest population density by natural enemies at a lower average (Debach, 1974). More specifically, the field of biological control has been defined as the study, importation, augmentation and conservation of beneficial organisms for the regulation of population densities of other organisms (Debach, 1964). Biological control has been practiced in entomology and acarology more than in any other field. When implementing a biological control program, there are certain desirable characteristics to look for in a control agent. Essentially, the natural enemy needs to be adapted biologically, physiologically and ecologically to the host (Doutt and Debach, 1964). What constitutes a successful biological control agent varies depending on the situation, however, common desirable attributes include: high powers of dispersal and searching capacity, prey specificity, the ability to survive at low prey densities, reproductive potential, a power of increase greater than the prey, voracity, synchronous distribution with prey, and the ability to resist pesticides (Messenger et al., 1976; Gerson and Smiley, 1990; McMurtry, 1982). McMurtry and Croft (1997) have suggested that although most studies on the efficacy of phytoseiids as control agents have focused on the rapid rate of increase in predators, the ability of the predators to regulate spider mites at a low density may be just as important. Control agents that regulate mites at these low densities generally have the ability to survive when starved, and can use alternative food, cannibalism, or predation on other phytoseiid species as means of survival during times when the favored prey are scarce (McMurtry and Croft, 1997).

N. californicus possesses many of the characteristics described above. According to a classification of predatory mites based on feeding specialization developed by McMurtry and Croft (1997), *N. californicus* is a type III predator. As a type III generalist, *N. californicus* has a broad diet range that includes not only various arthropods, but also plant sap, honeydew, and pollen (McMurtry and Croft, 1997). *N. californicus* can adapt to fluctuations in prey populations, providing stable pest suppression over time (Croft et al., 1998; Castagnoli et al., 1999; Escudero and Ferragut, 2005; Greco et al., 2005). The ability of *N. californicus* to survive on a broad array of food sources contributes to its stability and may mitigate its effect on community structure and other beneficial arthropods (Jones, 1976; Powers and Mc- Sorley, 2000; Cross et al., 2001; Rhodes et al., 2006). Furthermore, *N. californicus* is successfully used to control spider mites both in the field and in protected crops (Castagnoli and Simoni, 2003). It is able to persist on plants at low or negligible spider mite densities (Schausberger and Walzer, 2001).

H. Overwintering potential

(1) Diapause induction

During winter conditions, phytoseiid mites generally undergo reproductive diapause; females cease laying eggs (Overmeer, 1985). Diapausing females also tend to be less active than non-diapausing mites, feed rarely (Hoy and Flaherty, 1970; Rock et al., 1971; Wysoki, 1974; Van Houten et al., 1988; Morewood and Gilkeson, 1991), and are much more resistant to starvation when in diapause (Croft, 1971; Ivancich, 1990). Females are inseminated before they enter hibernation sites (Putman, 1962); males and juveniles fail to reach the overwintering stage before the onset of the adverse season, and bound to perish (Chant, 1959; Putman, 1959, 1962). Day length, rather than temperature, has been shown to be the factor influencing diapause induction in *N. californicus* (Castagnoli et al., 1996). The phytoseiid mites show a Type I response (Danks, 1987), entering diapause only when exposed to short day lengths. Reported critical photoperiods range from 11.2 hour (Hoy, 1975a) to 15.3 hour (Croft, 1971) and vary among populations as well as among species. Within a species, critical photoperiods are longer for populations from high latitudes or high elevations (Croft, 1971), and this trend is common among insects (Danks, 1987).

Temperature also has a strong influence on diapause induction. The length of the critical photoperiod may be somewhat different at different temperatures (Hoy, 1975a) and diapause is often prevented completely if temperatures remain above 20°C or so (Sapozhnikova, 1964; Rock et al., 1971; Hoy, 1975a; Morewood and Gilkeson, 1991). Temperature alone, in the form of a thermoperiodic cycle, can induce diapause when mites are reared in constant darkness and this thermoperiodic response corresponds very closely to the photoperiodic response (Van Houten et al., 1987, 1988).

Phytoseiid mites generally undergo diapause only after being exposed to diapause-inducing conditions throughout their juvenile development. However, a few species have been reported to "switch" into diapause when exposed to diapause-inducing conditions as adults after being reared under non-diapause inducing conditions (Putman, 1962; Hoy, 1975b; Swift, 1987; Van Houten, 1989).

Finally, the ability to diapause is not universal in phytoseiid mites; rather, some species and some strains or populations within a species have been shown to lack a diapause response or to overwinter without diapausing (Wysoki and Swirskii, 1971a, b; McMurtry et al., 1976; Overmeer, 1985). Hart et al. (2002) mentioned that the *N. californicus* can lay eggs during the UK winter and may therefore be able to overwinter as an egg stage and also reported that the US strain of *N. californicus* survived over three months without entering diapause outdoor in winter under sheltered condition in the UK, where the temperature did not fall below 0°C.

An investigation was carried out by Jolly (2000) into the ability to diapause for three strains of *N. californicus*: from Spain, the USA and one collected from the UK. This study was conducted at 21°C under a short day length (8L: 16D) provided TSSM as food. As a result, the US strain did not show any diapausing symptom whereas Spanish strain exhibited limited diapausing ability (16.1%) and the UK strain was able to diapause about 95.7%. Furthermore, diapause induction in Japanese indigenous and alien (Spical) *N. californicus* strains was determined by Gotoh et al. (2005) under short-day (8L: 16D) and long-day (16L: 8D) conditions at 15°C. All females of both strains under short-day or long-day oviposited within 6 days, indicating that *N. californicus* strains do not have diapause ability at all. Castagnoli et al., (1996) also investigates whether the alternative diets successfully used for mass-rearing of central Italian strain of *A. californicus* affected their responses to diapause inducing stimuli, when the species kept at temperatures and photoperiods close to native geographical areas. At temperatures between 17 and 21°C the length of day affected diapause induction more than temperature. With a ten-hour photoperiod at the tested temperatures, the number of diapausing females of both species exceeded the critical threshold of 50%. With an increase of two hours of light at 17°C the incidence of diapause dropped when fed on TSSM or on pollen.

(2) Cold tolerance

Providing insights into the physiology and ecology of terrestrial arthropods, studies of cold tolerance yielded information of value for such applied areas as pest management and cold hardiness is important adaptation for overwinter survival of terrestrial arthropods that inhibit regions with cold winters. Studies on the relationship between diapause and cold hardiness have demonstrated that either may occur independently of the other but in some cases cold tolerance is a component of the diapause program or is greatly enhanced by diapause (Denlinger, 1991). Very few studies have addressed cold tolerance of phytoseiid mites. Studies by Knisley and Swift (1971) and Wysoki (1974) made cursory comparisons of cold hardiness in diapausing and non-diapausing mites. In both cases diapausing mites survived roughly twice as long as non-diapausing mites at temperature ranging from -1°C to -9°C. Van der Geest et al. (1991) reported cold hardiness in extensively induced *Amblyseius andersoni* (Chant) in terms of LT_{50} at -5°c, the number of days' exposure resulting in 50% mortality. The LT_{50} value for diapausing mites was more than three times that for non-diapausing mites when neither group had been acclimated to low temperatures. In addition, survival times increased markedly when diapausing mites were acclimated prior to exposure. These results indicate that diapausing mites are inherently more cold-hardy and also have a much greater capacity to survive in lower temperature condition. In another study, after acclimation period of 7 days at 0°C, Gotoh (2005) observed that diapause females of *N. womersleyi* survived exposure to -5°C slightly longer (7-10 days) than did non-diapause females of *N. womersleyi* (3-5 days) and females of the Japanese indigenous and alien Spical strains of

N. californicus (3-5 days). In this case, the diapause *N. womersleyi* laid fewer eggs than the indigenous *N. californicus* strain and had little effect on fecundity for those females that survived. Broufas and Koveos (2001a) observed cold hardiness in *Euseius finlandicus* which resulted conspicuous different between diapause and non-diapause females. Most diapause females survived exposure to -4°C for 11 days, while most non-diapause females died within 2 days of that exposure.

I. Dispersal of phytoseiid mites

Dispersal is an adaptive process that permits individual organisms to colonize new habitats and escape unfavorable conditions (Price, 1984). Winged arthropods disperse actively by flight, whereas wingless species have evolved take-off behaviors that enable them to become airborne. Described take-off behaviors (Brandenburg and Kennedy, 1982; Washburn and Washburn, 1983; Margolies and Kennedy, 1985; Weyman, 1993) include one type that involves erect and facing downwind. This behavior occurs among scale insects (Stephens and Aylor, 1978; Washburn and Washburn, 1983), and some phytoseiid mites (Johnson and Croft, 1976; Hoy, 1982; Jung, 2001).

CHAPTER I

Effects of temperature on life-history traits of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) reared on *Panonychus ulmi*

Abstract

Tetranychus urticae and *Panonychus ulmi* are two common mite pests in apple. Many kinds of control methods including use of predatory mites of domestic and imported were tried, yet several attempts attempts on their way for finding satisfactory control particularly in field conditions. This experiment investigated temperature effects on life history parameters of Korean strain *N. californicus* when feeding on *P. ulmi* in laboratory to evaluate the suitability of this predatory mite for control of *P. ulmi*.

Temperature had a significant effect on immature development and adult performance. The developmental time decreased with increasing temperature between 15 and 30°C. Male developmental times were shorter than females at each temperature. The longest adult female longevity and oviposition period were observed at 15°C whereas the shortest at 34°C. At 25°C, females laid maximum number of eggs while minimum was at 34°C. In sex ratio, utmost number of females (0.77) was counted at 25°C and lowest (0.67) at 34°C. Survivorship during immature development varied from 74.29 to 92.86% with the lowest at 34°C.

From life table analysis, the shortest generation time (T = 10.68d) resulted at 34°C. The highest net reproductive rate (R_0 = 49.24; expected progeny per female) was found at 25°C. Intrinsic rate of increase (r_m = 0.29) and finite rate of increase (λ = 1.34) per day was estimated highest at 30°C. Based on these results, it could cautiously be expected to use *N. californicus* as a biocontrol agent of *P. ulmi* in apple orchards management system.

Key words: *Neoseiulus californicus*, *Panonychus ulmi*, temperature, development, fecundity, life table, sex ratio.

Introduction

The phytoseiid mite, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is known to be a promising candidate for biological control of spider mites in various field crops (McMurtry and Croft, 1997), particularly greenhouse crops (Calvitti and Tsolakis, 1992; Smith *et al.*, 1993). *N. californicus* has a broader food range and a higher capability to reside in patches with low pest density than other phytoseiids (Wei-Lan and Laing, 1973; Croft et al., 1997; Pratt et al. 1999). The broader food range may provide advantage to facilitate permanent establishment of *N. californicus*, which can survive and reproduce on alternative food sources including different mites, insects, and even pollen (Swirskii *et al.*, 1970; Castagnoli and Falchini, 1993; Croft *et al.*, 1998), even though it prefers spider mites (McMurtry and Croft, 1997).

Neoseiulus californicus was first reported in 1954 from California where it was collected from lemon trees (de Moraes *et al.*, 2004). The predatory mite is distributed over wide geographical range, both in arid and humid areas of sub-tropical and temperate zones. It was collected in Texas (USA), Mexico, Brazil, Guatemala, Peru, Argentina, Chile, Cuba, Colombia, Japan, Taiwan (introduced), Portugal, Italy, France and Spain (de Moraes *et al.*, 2004; McMurtry, 1977; Rencken and Pringle, 1998; Ferreira and Carmona, 1994). In 2006, *N. californicus* was first reported in Jeju Island of Korea, where it was collected from citrus orchards (Jung et al., 2006) but there are no records of *N. californicus* being endemic in main land of Korea and there is no report on the biological aspects of this predator for controlling the pests.

In commercial orchards, the potential of *P. ulmi* to cause severe economic damage necessitates chemical control several times a year. Acaricide resistance in spider mite populations has been observed in some commercial apple orchards (Lee, 1965; Koh et al., 2009) and the tendency of spider mites to develop resistance to a wide range of pesticides has been well documented (Croft and McGroarty, 1973; Dennehy and Granett, 1982; Dennehy et al., 1983; Helle, 1985; Welty et al., 1987; Herron et al., 1994). In most cases, the need for acaricides results from the destruction of natural enemy complex in the system (Parent and Lord, 1971). Phytoseiid mites have long been used for spider mites control (McMurtry and croft, 1997)

Many species of predatory mites have been used as control agents with varying success. The type III phytoseiid predator (McMurtry and Croft, 1997), *N. californicus* has provided efficient control of *P. ulmi* in apple orchards in South America (Monetti and Fernandez, 1995). This species has the potential to provide effective biological control for both mites (Croft and McGroarty, 1977). There is abundant information on the suitability of spider mite as a food source for *N. californicus*. Yet most studies have been conducted on *Tetranychus* species such as two-spotted spider mite, *T. urticae* (Helle and Sabelis, 1985), the Kanzawa spider mite, *T. kanzawai* Kishida (Hamamura et al., 1976), the Pacific spider mite, *T. pacificus* (Amano and Chant, 1977; Takashi and Chant, 1994), the Tomato spider mite, *T. evansi* (Moraes and McMurtry, 1985) and the Gorse spider mite *T. lintearius* (Dufour) (Pratt et al., 2003). To assess the potentials of a biocontrol agent for *N. californicus*, various abiotic and biotic factors need to be considered (Bale and Walters, 2001; Hart et al., 2002). The most important abiotic factor for invertebrate species would be a temperature, which affects survival, reproduction and development (Bale and Walters, 2001). Although some aspects on *N. californicus* biology has been published abundantly (Canlas et al., 2006; Castagnoli and Simoni, 2003; Escudero and Ferragut, 2005; Gotoh et al., 2004, 2006; Raworth et al., 1994; Rencken and Pringle, 1998; Walzer and Schausberger, 1999a, b), there exists only a few studies have presented information about temperature-dependent development (Canlas et al., 2006; Castagnoli and Simoni, 1991; Gotoh et al., 2004), and further life history information feeding on *P. ulmi* is lacking.

To explore the potential of using *N. californicus* for biological control of European red mite in apple, information on temperature-dependent development when feeding on *P. ulmi* is prerequisite. This study investigated the effects of temperature on the major biological parameters of the predator under laboratory conditions.

Materials and Methods

Neoseuilus californicus rearing

N. californicus was collected in 2006 from Jeju Island on kidney bean plants that were infested with *T. urticae*. The colony has been maintained in the Insect Ecology Laboratory of Andong National University feeding on *T. urticae* under 22-27°C, 65-75% R.H. and 16L: 8D photoperiod. The rearing apparatus (Figure 1) was composed of one small plastic cup inserted to bigger cup with gaps half-filled with water. Inside of the inner cup, *N. californicus* was maintained given periodic supplies of *T. urticae* infested kidney bean leaflets. On the upper inner side of the inner cup, sticky material (Tanglefoot® Company, Michigan 49504 U.S.A.) was banded to prevent mite escape. On top of the bigger cup, a lid with muslin clothes was placed to keep the moisture level as well as prevent contamination.

Immature development and adult performance

To determine the developmental time of *N. californicus*, 50 gravid females for each treatment were taken randomly from rearing unit and transferred to *P. ulmi* infested apple leaf which was placed upside down on wet cotton set on polyurethane foam in a plastic container (9.5 x 4cm). Females were allowed to lay eggs for 12 hours. Individual eggs were transferred by fine hair brush to apple leaf disc (dia. 2.9cm) contained with mixed stages of *P. ulmi* on a wet cotton pad in a small Petri dish (5.5 × 1.5cm) retaining 70-80% R.H.. The Petri dishes were then kept inside the incubators set at a range of temperatures (15, 20, 25, 30 and 34°C) with a photoperiod of 16L: 8D hour. Thirty five replications were maintained for each treatment. As the leaf discs became aged, the mites were transferred to freshly cut discs until the female and male died.

Development from egg to adult was observed at 12 hour intervals to determine stage-specifid developmental time and mortality under the dissection microscope (Olympus Optical Co., Ltd, Tokyo, Japan). Since the inactive period of the immature were short, the active and inactive period of larval and nymphal stages were combined.

Newly-emerged female adults were mated individually by one active male from the same condition. If needed, an additional male was added to ensure successful mating. Oviposition was recorded at 12 hour intervals until they die. Upon counting and collection of newly-laid eggs on each leaf, the eggs of each female were daily transferred to fresh leaf discs where they were reared up to adulthood to determine the sex ratio of the offspring. Mixed stages of *P. ulmi* were provided as food.

Life table analysis

Observations on life history, including adult fecundity and longevity, were used to construct a time-specific life table for *N. californicus* when maintained under laboratory conditions. Actual death occurred in the egg and immature stages were taken into account when the female survival rate at each temperature was determined. Life table parameters for females held at different temperatures were estimated using with Birch (1948) equation:

$\sum e^{-r}{}_{m}x l_x m_x = 1$

Where, x = age in days, l_x = the age-specific survival rate, m_x = the age-specific fecundity, the intrinsic rate of natural increase ($r_m = \ln (\sum l_x m_x)/T$), the finite rate of increase ($\lambda = e^r$), the net reproductive rate ($R_0 = \sum l_x m_x$), the mean generation time ($T = \sum X l_x m_x / R_0$), and the gross reproductive rate ($GRR = \sum m_x$). Doubling time [$DT = (\ln 2)/r$] was calculated as described by Mackauer (1983).

Statistical analysis

Treatment effect on the traits (i.e. developmental time, survival, pre-, oviposition period, longevity and fecundity) were determined by using analysis of variance (ANOVA), and means were separated by using Tukey's honest significant difference test (Tukey's HSD test) (SAS Institute, 2005). Simple linear regression was performed and was used to provide estimates of the threshold temperature (t_0, °C) and the thermal constant required to adult (degree-days or DD) requirement for this species. The jackknife technique was used to estimate mean demographic parameters of $l_x m_x$ of the life table and their SE. This method was first applied to life table analysis as proposed by Meyer et al. (1986). The jackknife procedure estimates standard errors for life table parameter estimates by sequentially removing one female and her offspring from the original dataset and recalculating each life table parameter from the truncated dataset. The method can estimate R_0, T, r_m, λ, DT and GRR and means from five constant temperatures were compared by ANOVA (Tukey HSD test) using SAS.

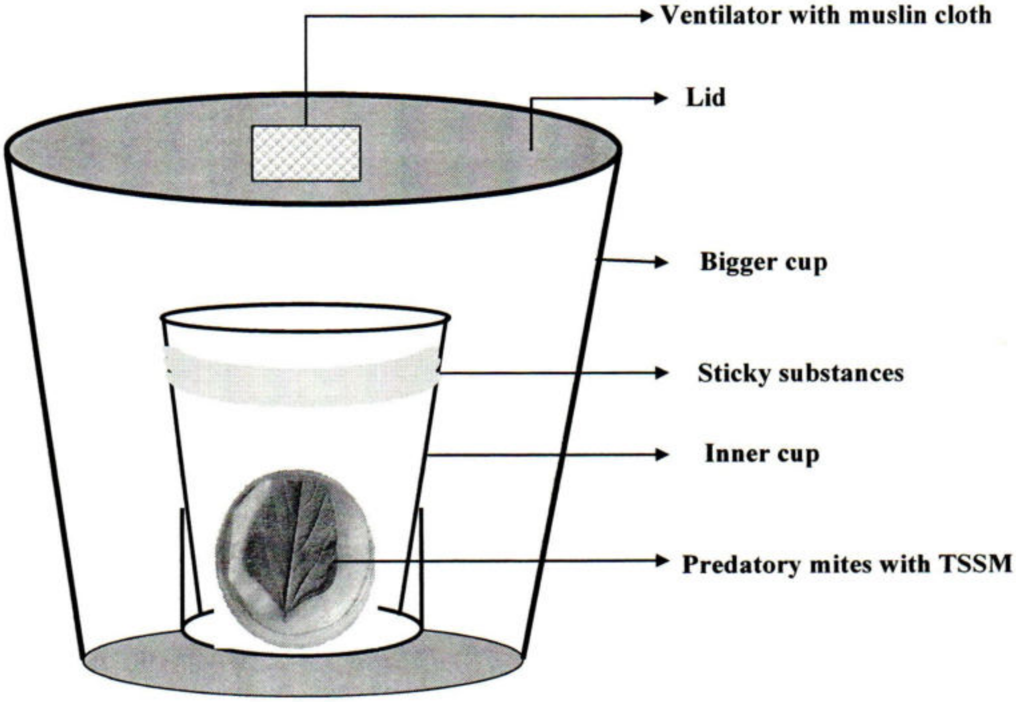

Figure 1. Rearing apparatus of the predatory mite

Results

Immature development

The egg development periods of *N. californicus* were significantly different between temperatures tested. The duration of the egg stage ranged from 5.22 to 0.77 days in females and 5.25 to 0.85 days in males for all temperatures. At 15°C, female egg development time was longest (5.22 days) where only 2.12-0.77 days were taken at 20 to 34°C (Table 1). Significant difference was found at 15 and 20°C (Female: $df = 4, 83$; $F = 594.23$; $P < 0.001$ and male: $df = 4, 38$; $F = 528.05$; $P < 0.001$) but no difference was observed among the temperature ranges from 25 to 34°C.

Newly hatched larvae of *N. californicus* were white in color with three pairs of legs. The five thermal treatment showed the variations in larval periods which presented in Table 1. The developmental times in both the sexes showed significant differences while compared temperatures 15 and 25 with the range from 25 to 34°C (Female: $df = 4,83$; $F = 149.36$; $P <0.001$ and male: $df = 4,38$; $F = 78.64$; $P < 0.001$).

The oval-shape, white color and 8 legged protonymphs were developed immediately after larval molting. Upon feeding, they turned in to reddish color. The development time of protonymph gradually decreased with increasing temperature except at 34°C showing significant differences (Female: $df = 4,83$; $F = 457.31$; $P < 0.001$ and male: $df = 4,38$; $F = 247.14$; $P < 0.001$).

Female and male deutonymphs developed within the range of 1-3.86 and 0.71-3.13 days at all temperatures prior to molting to the adult stage. No significant difference was observed among the temperature ranges from 25 to 34°C but at 15 and 20°C, deutonymphal period significantly differed in both the sexes (Female: $df = 4, 83$; $F = 74.50$; $P < 0.001$ and male: $df = 4,38$; $F = 54.64$; $P < 0.001$)

Development from the egg stage to adulthood of *N. californicus* occurred at five temperatures is shown in Table 1. Temperature influenced the immature development where the development time for completion of the life cycle decreased significantly with increasing temperature. The total development time decreased until the temperature reached 30°C but increased above 30°C. Direct estimates of development time derived from temperature development studies were significantly different (Female: *df* = 4, 83; *F* = 998.96; *P* < 0.001 and male: *df* = 4,38; *F* = 917.13; *P* < 0.001) for all temperatures except 25 to 34°C. The simple linear regression analysis applied to the temperature and developmental rate shows in Table 4. The egg stages of both female and male showed the highest development threshold (11.51 and 10.86°C, respectively), whereas it was the lowest in the female larva and male deutonymph (5.73 and 5.89°C, respectively). The estimated total degree-days required for egg to adult female and male development were 39.61 and 38.57DD, respectively.

Adult performance

Adult longevity: Female and male longevity usually decreased with increasing temperature. Analysis of variance showed that mean adult longevity, the length of time from adult emergence to natural death, was significantly different between temperatures in both the sexes (Female: *df* = 4,83; *F* = 124.02; *P* < 0.001 and male: *df* = 4,38; *F* = 68.62; *P* < 0.001) and was greatest with a value of 70.42 days in female and 43.75 days in male at 15°C, declining to a low of 13.06 days in female and 11.60 days in male at 34°C (Table 1).

Reproduction: Temperature affected the per capita total egg production (*df* = 4,83; *F* = 144.40; *P* < 0.001) of *N. californicus* (Table 2). The total number of eggs was highest (63.94 ± 2.00) at 25°C and there was a significant reduction in egg production per adult at extreme temperatures. The lowest egg production was 10.18 ± 1.64 eggs per adult at 34°C. The mean daily egg production, calculated by dividing the total egg production by oviposition period, was influenced by temperature (*df* = 4,83; *F* = 251.16; *P* < 0.001). Daily oviposition was highest (3.31 ± 0.07 eggs) at 30°C. The total egg production was lowest (10.18) at 34°C, while the daily egg production was lowest (1.01) at 15°C.

Upon adult emergence, the females were arranged to get mated with released males. Temperature had a significant effect on the duration of the preoviposition (*df* = 4, 83; *F* = 64.75; *P* < 0.001) and oviposition period (*df* = 4, 83; *F* = 142.54; *P* < 0.001) (Table 2). The longest preoviposition and oviposition period (4.86 ± 0.32 and 35.83 ± 1.43 days) were observed at 15°C whereas they were shortest (1.53 ± 0.07 and 6.62 ±

0.95 days) at 30 and 34°C, respectively. The duration of the post-oviposition period was significantly longer at 15, 20 and 25°C than at 30 or 34°C (Table 2; *df* = 4, 83; $F = 44.64$; $P < 0.001$). The age-specific fecundity rate (m_x) peaked at earlier ages and the fecundity period became narrower as the temperature increased. The daily reproductive rate at 30 and 34°C declined earlier than at 15, 20 and 25°C (Figure 2).

Survivorship and sex ratio

The survival rate of the *N. californicus* from the immature stages to adult emergence under the tested temperatures ranged from 74.29 to 92.86%, where it was lowest at 34°C. There were no statistically significant differences in immature survival among the temperatures (Table 3; *df* = 4,145; $F = 1.70$; $P > 0.15$). The sex ratio of the phytoseiid species was female biased (provide the range of values) and varied significantly between temperatures (Table 3; *df* = 4,45; $F = 16.32$; $P < 0.0001$).

Life table parameters

Variations of life table parameters were estimated by a jackknife analysis at different temperatures (Table 4). These parameters were significantly affected by temperature. The net reproductive rate (R_0) (*df* = 4,82; $F = 3611.08$; $P < 0.001$), intrinsic rate of natural increase (r_m) (*df* = 4,82; $F = 17660.5$; $P < 0.001$) and finite rate of increase (λ) (*df* = 4,82; $F = 20186.3$; $P < 0.001$) were all significantly different between temperatures. Mean generation time, *T*, was significantly different between temperature and was lowest at 34°C (*df* = 4,82; $F = 5030.67$; $P < 0.001$). Population doubling times, *DT*, showed a statistically significant difference between temperatures (*df* = 4,82; $F = 2819.71$; $P < 0.001$) and were lowest when the predators reared at 30°C. The rearing temperature of 25°C had the highest gross reproductive rate (*GRR*) showing significant differences among the temperatures (*df* = 4,82; $F = 33857.7$; $P < 0.001$).

Table 1. Developmental time and adult longevity (day) of *N. californicus* under five constant temperatures with a 16L: 8D photoperiod

Temp. (°C)	Sex[a]	N[b]	Egg	Larva	Protonymph	Deutonymph	Egg to Adult	Adult longevity
15	F	18	5.22 ± 0.14a	2.00 ± 0.04a	4.53 ± 0.11a	3.86 ± 0.16a	15.61 ± 0.22a	70.42 ± 3.06a
	M	8	5.25 ± 0.09A	2.19 ± 0.09A	4.38 ± 0.16A	3.13 ± 0.18A	14.94 ± 0.20A	43.75 ± 2.60A
20	F	16	2.12 ± 0.06b	0.88 ± 0.09b	1.72 ± 0.06b	1.66 ± 0.08b	6.38 ± 0.07b	55.28 ± 1.58b
	M	10	2.10 ± 0.07B	1.05 ± 0.05B	1.70 ± 0.08B	1.35 ± 0.08B	6.20 ± 0.11B	39.30 ± 1.38A
25	F	18	1.06 ± 0.04c	0.64 ± 0.05c	1.28 ± 0.06c	0.86 ± 0.07c	3.83 ± 0.07c	43.58 ± 1.07b
	M	8	1.13 ± 0.00C	0.75 ± 0.09C	1.00 ± 0.00C	0.94 ± 0.00C	3.81 ± 0.09C	29.06 ± 1.48B
30	F	19	0.84 ± 0.05c	0.55 ± 0.04c	0.97 ± 0.05d	0.76 ± 0.06c	3.13 ± 0.05c	26.34 ± 0.55c
	M	7	0.86 ± 0.09C	0.50 ± 0.00C	0.86 ± 0.09C	0.86 ± 0.09C	3.07 ± 0.13C	17.29 ± 0.99C
34	F	17	0.76 ± 0.05c	0.53 ± 0.03c	1.03 ± 0.04d	0.85 ± 0.05c	3.18 ± 0.08c	12.79 ± 1.05d
	M	10	0.85 ± 0.08C	0.55 ± 0.05C	0.90 ± 0.07C	0.80 ± 0.08C	3.10 ± 0.12C	11.60 ± 1.57C

[a] F means females and M means males.

[b] Number of individuals tested.

All values are means ± SE. Means for each stage of the same sex in the same column followed by different letters are significantly different at $P < 0.05$ (Tukey HSD test).

Table 2. Reproductive phases (day) and fecundity of *N. californicus* under different temperatures with a 16L: 8D photoperiod

Temperatures (°C)	Preoviposition period	Oviposition period	Postoviposition period	Daily fecundity /female	Lifetime fecundity /female
15	4.86 ± 0.32a	35.83 ± 1.43a	29.72 ± 2.57a	1.01 ± 0.02d	36.22 ± 1.57c
20	2.47 ± 0.03b	21.60 ± 0.58b	31.25 ± 1.79a	2.40 ± 0.05b	51.88 ± 1.62b
25	1.69 ± 0.07c	19.94 ± 0.59b	21.94 ± 1.01a	3.21 ± 0.04a	63.94 ± 2.00a
30	1.53 ± 0.07c	14.76 ± 0.29c	10.05 ± 0.49b	3.31 ± 0.07a	48.74 ± 1.19b
34	1.88 ± 0.06bc	6.62 ± 0.95d	4.29 ± 0.66b	1.47 ± 0.09c	10.18 ± 1.64d

Means in the same column followed by the different letters are significantly different at $P < 0.05$ (Tukey HSD test)

Table 3. Survival of immature stages and sex ratio of *N. californicus* under different temperatures with a 16L: 8D photoperiod

Temperatures	Stage specific survival (% ± SE)				Survival to	
(°C)	Egg	Larva	Protonymph	Deutonymph	adulthood (%)	Sex-ratio
15	92.86 ± 4.64	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	92.86 ± 4.6n.s	75.81 ± 1.14ab
20	96.97 ± 2.94	96.67 ± 3.09	96.43 ± 3.19	100.00 ± 0.00	89.65 ± 5.24n.s.	74.13 ± 0.73ab
25	96.43 ± 3.19	100.00 ± 0.00	100.00 ± 0.00	96.30 ± 3.25	92.86 ± 4.43n.s.	77.32 ± 0.56a
30	100.00 ± 0.00	86.67 ± 5.84	100.00 ± 0.00	100.00 ± 0.00	86.67 ± 5.84n.s.	75.97 ± 1.01b
34	97.14 ± 2.86	88.24 ± 5.53	93.33 ± 4.29	92.86 ± 4.43	74.29 ± 7.50n.s.	67.92 ± 0.89c

Means in the same column followed by the different letters are significantly different at $P < 0.05$ (Tukey HSD test).

ns- Not significantly different at $P < 0.05$ (Tukey HSD test)

Table 4. Life table parameters (± SE) for *N. californicus* under five constant temperatures with a photoperiod of 16L: 8D

Temp. (°C)	R_0	r_m	T	λ	DT	GRR
15	26.15 ± 0.59d	0.09 ± 0.0007e	36.58 ± 0.302a	1.09 ± 0.0008d	7.86 ± 0.073a	26.84 ± 0.08d
20	38.38 ± 0.07b	0.19 ± 0.0004c	19.45 ± 0.016b	1.21 ± 0.0001c	3.70 ± 0.002b	38.39 ± 0.10b
25	49.24 ± 0.09a	0.25 ± 0.0000b	15.31 ± 0.020c	1.29 ± 0.0003b	2.72 ± 0.003c	49.24 ± 0.09a
30	31.88 ± 0.89c	0.29 ± 0.0024a	11.81 ± 0.002d	1.34 ± 0.0032a	2.37 ± 0.019d	35.09 ± 0.01c
34	6.80 ± 0.07e	0.18 ± 0.0008d	10.68 ± 0.051e	1.20 ± 0.0009c	3.86 ± 0.017b	8.88 ± 0.10e

Means in a column followed by different letters are significantly different at $P < 0.05$ (Tukey HSD test).

R_0, net reproductive rate; r_m, intrinsic rate of natural increase per day; T, mean generation time in days; λ, finite rate of increase; DT, population doubling time in days; GRR, gross reproductive rate.

Table 5. Linear regression model, developmental threshold and thermal constant for stage-specific development of *N. californicus* reared on *P. ulmi*

Sexes (stages)	Regression equations	r^2 values	Lower threshold (t_0, °C)	Thermal constant (K, degree-days, DD)
Female				
Egg	$Y = -0.8598+0.0693x$	0.98 ($P<0.01$)	12.40	5.99
Larva	$Y = -0.7035+0.0870x$	0.96 ($P<0.01$)	8.09	7.02
Protonymph	$Y = -0.5256+0.0524x$	0.98 ($P<0.01$)	10.03	14.56
Deutonymph	$Y = -0.4952+0.5560x$	0.78 ($P<0.03$)	8.91	10.88
Egg-adult	$Y = -0.1911+0.0174x$	0.99 ($P<0.01$)	10.98	55.15
Male				
Egg	$Y = -0.8230+0.0668x$	0.99 ($P<0.01$)	12.32	6.25
Larva	$Y = -1.0686+0.1002x$	0.99 ($P<0.01$)	10.66	4.87
Protonymph	$Y = -0.7059+0.0645x$	0.97 ($P<0.01$)	10.94	8.04
Deutonymph	$Y = -0.4661+0.0573x$	0.94 ($P<0.01$)	8.13	10.76
Egg-adult	$Y = -0.1906+0.0175x$	0.99 ($P<0.01$)	10.89	49.05

Regression equations were calculated using simple linear regression analysis applied to the developmental data obtained from 15 to 30°C.

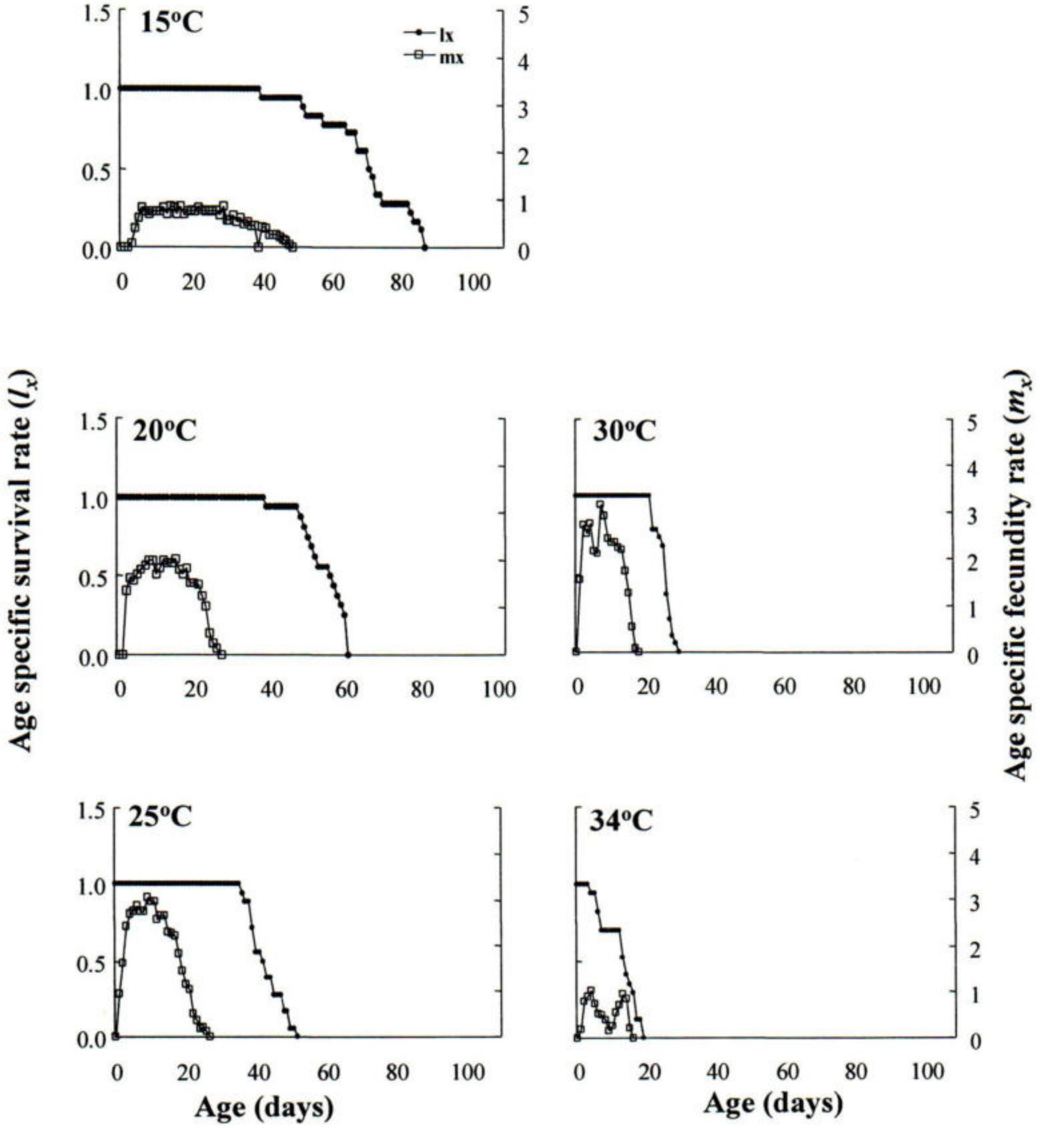

Figure 2. Age-specific survivorship (l_x) and age specific fecundity (m_x) of *N. californicus* under five constant temperatures. *lx* = proportion of females alive at age *x*. *mx* = (proportion of females) × (age-specific oviposition).

Discussion

Results indicate that the Korean *N. californicus* is able to complete their development at the range of temperatures from 15 to 34°C when fed on mixed stages of European red mite. . The population used in this study developed from egg to adult at a faster rate (3.83 days at 25°C) compared with that studied by Gotoh et al. (2006) using *P. ulmi* as prey (4.33-4.39 days at 25±1°C). Castagnoli and Simoni (1991), and Hart et al. (2002) also recorded the egg to adult development period of 5.8 and 8.1days, respectively at 25°C when providing *T. urticae* s prey. But at 25±1°C, Gotoh et al., 2006 observed the development period 3.79 to 3.88 days in the commercial Spical strain while using the prey *T. urticae* which is very close to our result. Kim et al. (2009) recorded the development period of *N. californicus* 8.73 days at 20°C provided *T. urticae* while the current observation was 6.38 days. The faster rate of development observed in this study may be attributable to differences in rearing conditions and the prey (*P. ulmi*). These findings suggest that *N. californicus* has a potential to develop over a wide range of temperatures and on the pest mite *P. ulmi*.

The lifetime fecundity per female (63.94 eggs) was higher at 25°C than the Spical strain (43.80-47.71 eggs, Gotoh et al., 2006) while *P. ulmi* provided as food. At 25°C, the fecundity seemed also higher as compared to other strains, for example, the Spical strain (46.2-46.67 eggs, Gotoh et al., 2006), Riverside strain (43.3 eggs, Ma and Laing 1973; 54.5 eggs, Croft et al. 1998) and the indigenous Japanese strain (41.6 eggs, Gotoh et al., 2004) when fed on *T. urticae*.

The oviposition rate of *N. californicus* on *P. ulmi* observed by Gotoh et al. (2006) was 3.03-3.17 eggs per day at 25±1°C while the current study showed little bit higher (3.21 eggs/day). At 25°C and a diet of *T. urticae*, the egg production rate of the Riverside strain (3.1 eggs per day, Ma and Laing, 1973) and the Japanese strain (3.31-3.42 eggs per day, Gotoh et al., 2006) was almost similar to the Korean population.

The lower threshold temperature (t) from egg to adult of the studied population (10.98°C) was close to the Italian strain (9.0°C, Castagnoli and Simoni 1991), the African strain (8.3°C, Rencken and Pringle 1998) and the USA strain (9.9°C, Hart et al., 2002). However, the thermal constant (K) of the Korean population was 55.15DD which is close to other strains for example the Japanese strain (59.2 and 71.43DD, Gotoh et al. 2004 and Canlas et al., 2006), Italian strain (90.0DD, Castagnoli and Simoni 1991), the African strain (100DD, Rencken and Pringle 1998) and the American strain (123.5DD, Hart et al., 2002).

Life table parameters are good indices of population growth under a given set of conditions. The phytoseiid was able to survive and reproduce on all temperatures tested. The r_m-value of the Korean population was 0.29 at 25°C provided mixed stages of prey, which is lower than Spical strain (0.31, Gotoh et al., 2006) when feeding on only eggs of *P. ulmi*. The reason for the lower r_m-value in the present study might be the prey stages because the r_m-values tend to be higher when fed on eggs than when fed on all prey stages (Ma Laing 1973; Mesa et al., 1990; Castagnoli and Simoni 1991; Castagnoli et al., 1999a; Gotoh et al., 2004). Moreover, working with the same strain and temperature, Canlas et al. (2006) observed a lower r_m- value (0.206) using all stages of *T. urticae* as prey than the value (0.27) found by Gotoh et al. (2004) when fed solely with *T. urticae* eggs in the same Japanese strain. On the contrary, the r_m- value found higher in the Korean population than the Columbian strain (0.19, Mesa et al., 1990, used preys were all stages of *T. urticae* and *Mononychellus progresivus*) the African strain (0.227, Rencken and Pringle 1998, provided prey was all stages of *T. urticae*), the Italian strain on tomato plant (0.118, prey was *T. urticae* but stages not mentioned, Castagnoli and Simoni 1991), and the value was similar with the Italian strain (0.259, Castagnoli and Simoni 1991, prey was *T. urticae* but stages not specified). The r_m-value at 20°C was close in between the present study (0.19) and the Japanese strain (0.173, Gotoh et al., 2004) while only eggs of *T. urticae* were provided.

At 25°C, when fed strictly egg stage of *P. ulmi*, the mean generation time (*T*) of the Spical strain (11.01-11.30days) showed shorter period than the Korean population (15.31days). But Gotoh et al. (2004) provided only egg stage of *T. urticae* as food and found the almost same mean generation time (15.3) in the Japanese strain as the present study. While used all stages of *T. urticae* as prey, the Columbian strain (16.7days, Mesa et al.,

1990) and the Japanese strain (17.5 days, Canlas et al., 2006) showed longer period than the Korean population. However, the number of female progeny produced per female (R_0) was greater in the Korean population (49.24) than those produced per female in the Spical strain (28.81 to 32.20, Gotoh et al., 2006) on a diet of *P. ulmi* eggs alone. Castagnoli et al. (1991) also reported the R_0 value 36.6 when the Italian strain reared on mixed stages of *T urticae*. The variation of the R_0 is may be due to the strain characteristics, because working with the Japanese and African strains, provided mixed stages of *T. urticae*, Canlas et al (2006), and Rencken and Pringle (1998) found the small number of female progeny (22.9 and 11.2, respectively). Xia et al. (1998) reported the R_0 value (42.9) close to the present study while working with *Amblyseius orientalis* supplied *P. ulmi*, honey and water. Takafuji and Chant (1976) and Blommers (1976) also found the R_0 values 50.9 and 50.2 while they were working with *Amblyseius degenerans* (Prey was immatures of *T. pacificus*) and *A. bibens* (All stages of *Tetranychus* as food). Life table statistics, such as those presented here, provide a valuable tool in the evaluation of a potential biological control agent in an area considering the seasonal variations and this approach has demonstrated utility (Bernal and González, 1997)

So, considering the potential characters of a predator, this study explored some prospective characteristics of the Korean population for the successful biological control of pest mites. As compared to other strains or species of *Neoseiulus* in the world, particularly the higher fecundity and R_0, short generation time and moderate r_m of the Korean *N. californicus* seems to be effective for the biological control of the European red mite, *Panonychus ulmi* successfully.

Table 6. Synopsis of developmental periods from egg to adult (female), lower threshold temperatures (t_0) and thermal constants (K, degree-days) under around 15, 20, 25 and 30 and 35°C for some predatory mites

Species[a]	Period of development (days)					t_0	K (DD)	Prey[b]	References
	15±2°C	20±2°C	25±1°C	30±2°C	35±1°C				
N. californicus	11.50[c]	7.30[c]	5.80[c]	4.4[0c]	-	9.00	90.00	*T. urticae* (R), MS	Castagnoli and Simoni (1991)
	-	8.10	-	-	-	9.90	123.50	*T. urticae* (G), E	Hart et al. (2002)
	14.10	7.20	4.30	3.00	3.80	10.90	59.20	*T. urticae* (R), E on Lima bean	Gotoh et al. (2004)
	-	9.20	5.70	4.80	-	8.30[c]	100.10[c]	*T. urticae*, MS	Rencken and Pringle (1998)
	11.40	-	5.00[c]	3.9[0 c]	-	-	-	*T. urticae* (G), E	Ma and Laing (1973)
	21.71	6.74	5.06	3.87	3.78	10.60	71.40	*T. urticae* (G), MS on Kidney bean	Canlas et al. (2006)
	11.48	8.73	6.00	3.82	3.23	10.02	-	*T. urticae*, MS on kidney bean	Kim et al., (2009)
	-	-	3.88	-	-	-	-	*T. urticae* (R), E on Common bean	Gotoh et al. (2006)
	-	-	3.85	-	-	-	-	*T. kanzawai*, E on Tea	Gotoh et al. (2006)
	-	-	4.02	-	-	-	-	*A. viennensis*, E on Cherry	Gotoh et al. (2006)
	-	-	4.07	-	-	-	-	*P. citri*, E on Sour orange	Gotoh et al. (2006)
	-	-	4.33	-	-	-	-	*P. ulmi*, E on Apple	Gotoh et al. (2006)
	15.61	6.38	3.83	3.13	3.18	10.98	55.15	*P. ulmi*, MS on Apple	Present study
N. fallacis	19.70	8.20	5.60	3.90	-	11.20	74.20	*T. urticae* (G)	Genini et al. (1991)
N. cucumeris	20.00	-	-	7.70	-	-	-	*A. corpuzae*	Zhang et al. (2003)
N. womersleyi	13.20	6.30	4.50	3.10	-	11.60	-	*T. urticae*, E	Lee and Ahn (2000)
N. womersleyi	19.60	11.40	6.20	3.90	-	11.50	80.50	*T. kanzawai*	Nakagawa (1984)
N. womersleyi	-	10.40	6.70	3.90	-	13.40	91.80	*T. urticae* (R)	Kadano et al. (1975)
T. Pyri	-	13.10	8.30	6.60	-	9.80[c]	130.80[c]	*T. urticae* (G)	Genini et al. (1991)
P. persimilis	-	7.90	5.00	3.70	-	11.90	71.50	*T. urticae* (R)	Kadano et al. (1975)
P. persimilis	18.90	7.20	4.90	3.50	-	11.60	65.80	*T. kanzawai*	Hamamura et al. (1976)
G. longipilus	-	9.00	6.70	4.90	-	9.90[c]	98.50[c]	*T. urticae* (G)	Genini et al. (1991)

[a] *N., Neoseiulus; T., Typldromus; P., Phytoseiulus; G., Galendromus*

[b] *T., Tetranychus; A., Amphitetranychus; P., Panonychus*; G, green form; R, red form; E- Egg, MS- mixed stages.

[c] Calculated from the original data

Table 7. Synopsis of net reproductive rate (R_0), intrinsic rate of natural increase (r_m) and mean generation time (T) for some phytoseiid species

Species[a]	Temperatures tested (°C)	R_0	r_m	T	Prey[b]	References
N. californicus	25	29.10	0.29	11.70	*T. urticae* (G) (egg stage)	Ma and Laing (1973)
	25	25.30	0.19	16.70	M. *progresivus* (all stages)	Mesa et al. (1990)
	25	29.90	0.19	17.40	*T. urticae* (G) (all stages)	Mesa et al. (1990)
	25	36.60	0.26	13.90	*T. urticae* (G) (not specified)	Castagnoli and Simoni (1991)
	25	11.20	0.23	11.60	*T. urticae* (G) (all stages)	Rencken and Pringle (1998)
	25	-	0.12	-	*T. urticae* (G) (not specified) on tomato	Castagnoli et al. (1999)
	25	-	0.27	-	*T. urticae* (G) (not specified) on strawberry	Castagnoli et al. (1999)
	25	28.60	0.27	15.30	*T. urticae* (R) (egg stage) on lima bean	Gotoh et al. (2004)
	25	22.90	0.21	17.50	*T. urticae* (G) (all stages) on kidney bean	Canlas et al. (2006)
	25±1	32.95	0.31	11.23	*T. urticae* (R) (egg stage) on common bean	Gotoh et al. (2006)
	25±1	33.94	0.31	11.50	*T. kanzawai* (egg stage) on tea	Gotoh et al. (2006)
	25±1	34.04	0.30	11.94	*A. viennensis* (egg stage) on cherry	Gotoh et al. (2006)
	25±1	31.02	0.29	11.68	*P. citri* (egg stage) on sour orange	Gotoh et al. (2006)
	25±1	28.81	0.31	11.02	*P. ulmi* (egg stage) on apple	Gotoh et al. (2006)
	25	49.24	0.25	15.31	*P. ulmi*, (all stages) on apple	Present study
A. bibens	25	50.20	0.33	12.10	*T. neocaledonicus* (all stages)	Blommers (1976)
A. degenerans	25	50.90	0.25	15.90	*T. pacificus* (immatures)	Takafuji and Chant (1976)
A. orientalis	25	42.90	0.33	14.70	*P. citri* (not specified)	Xia et al. (1998)
N. womersleyi	25	12.50	0.17	15.20	*T. urticae* (egg stage)	Lee and Ahn (2000)
P. persimilis	25	63.20	0.32	13.10	*T. pacificus* (immatures)	Takafuji and Chant (1976)
P. persimilis	26	61.90	0.43	13.10	*T. pacificus* (all stages)	Takahashi and Chant (1994)
P. macropilis	25	45.30	0.20	19.50	*T. urticae* (G) (all stages)	Mesa et al. (1990)
T. occidentalis	24-28	31.10	0.24	14.10	*T. pacificus* (eggs)	Bruce-Oliver and Hoy (1990)

[a] *N., Neoseiulus; A., Amblyseius; P., Phytoseiulus; T., Typhlodromus.*

[b] *T., Tetranychus; M., Mononychellus; A., Amphitetranychus; P., Panonychus.* Mite stages provided: G, green form; R, red form.

CHAPTER II

Tolerance of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) to environmental factors in comparison with other predatory mites

Abstract

Temperature and relative humidity are the most important environmental variables for phytoseiid mites given the choice of optimum food. Environmental tolerance of three important predators of spider mites, *Neoseiulus californicus* McGregor, *Neoseiulus womersleyi* Schicha and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) was compared by treating combination of temperatures (15, 20, 25, 30, and 35°C) and relative humidity (33%, 51%, 75% and 95%) for egg hatching, immature survival, and adult female performance. Egg hatching was more influenced by the relative humidity than temperature. Egg hatching rate increased as the relative humidity increased for three species. Temperature effect on egg hatching were only significant to *N. californicus* and *P. persimilis,* but not for *N. womersleyi*. The lethal humidities were in the range of 56-77, 82.0, 66-94% RH, respectively for *N. californicus, P. persimilis,* and *N. womersleyi*. Larval development into protonymph did not require any food intake for the three tested species. For larval survival, *N. womersleyi* showed most susceptible to lower relative humidity followed by *P. persimilis*. The female survival also was limited by the high temperature and low humidity; tolerance 100% mortality at 5th day for *N. californicus* at 35°C under under 51% RH, for *P. persimilis* at 35°C regardless the relative humidity and at 30°C at 33% RH, and for *N. womersleyi,* mortality increased as the temperature increased. For the three tested species, fecundity was influenced both by temperature and humidity. The maximum daily fecundities were shown at 30°C, but only above 75% RH regimes. Under the 75% RH, fecundities were much lower. Implementation of the results was discussed relative to biological control of spider mites in open field and greenhouse crops.

Key words: Egg hatch, immature survival, adult survival, cannibalism, temperature, relative humidity, *Neoseiulus californicus*, *Neoseiulus womersleyi*, *Phytoseiulus persimilis*

Introduction

Abiotic environmental factors largely affect the distribution and abundance of living biota (Krebs, 1985). Arthropods are particularly vulnerable to the unfavorable environmental condition for their performance partly due to the ectothermal nature, lower water-holding capacity, and lower mobility of some immobile stage during their development. For acarine predators in the family of Phytoseiidae, humidity is considered as the second important factor to temperature in determining the development and predation performance (Tanigoshi, 1982; Sabelis, 1985). There is variation among phytoseiid mites in their response to relative humidity and temperature, both in terms of egg hatch success and subsequent survival (Van Dinh et al. 1988; Baier 1991; Bakker et al. 1993; Croft et al. 1993; Van Houten et al. 1995; Shipp and Van Houten 1997; Schausberger 1998). In some circumstances, these responses may limit the utility of phytoseiid mites in biological control (Baier 1991; Gaede 1992; Shipp and Van Houten 1997). Egg and larval stages are more susceptible to high temperature and low humidity (Sabelis, 1985; Zhang and Kong, 1985; Croft et al., 1993; Schausberger, 1998), probably because larger and mobile other life stages could move to more favorable sites and compensate the heat or water deficits by prey consumption or free water uptake. Many reported that the temperature and humidity tolerance of phytoseiid mites would be related to the ambient conditions of their geographic origin and/or on the host plant they inhabit (McMurtry et al., 1976; McMurtry and Croft, 1997). The degree of humidity sensitivity of phytoseiids underlies considerable inter- and intraspecific variation reflecting the adaptation to the climatic characteristics of a given habitat (McMurtry et al., 1976; Dinh et al., 1988; Perring and Lackey, 1989; Bakker et al., 1993; Schausberger, 1998). However, under different ambient conditions the efficacy of phytoseiid species is often insufficient.

The killing and consumption of conspecific individuals, cannibalism, is a common and widespread phenomenon in the animal kingdom. Cannibalism is a common ecological interaction in the animal during scarcity of food. Phytoseiids are well studied group with respect to cannibalism (Schausberger, 2003) and from this habit they may get benefit for their survival. Variations found in phytoseiid mites in response to temperature, humidity and food shortage, in terms of egg hatch, subsequent survival and cannibalism

This study investigated the combined effects of temperatures and relative humidities on immature and adult stages of three important phytoseiids in Korea; *Neoseiulus californicus* McGregor, *Neoseiulus womersleyi* Schicha and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). *N. womersleyi* is native and dominant phytoseiid mites mostly associated with Tetranychid spider mites in fruit orchards and tea plantation (Lee, 1990; Jung et al., 2003). *N. womersleyi* have been mostly implemented in open field crops (Lee, 1990; Kim et al., 1996; Jung et al., 2003). *N. californicus* is one of the most important spider mite predators as well as to some other small insect pests such as thrips and whitefly (McMurtry and Croft, 1997). This species was firstly found from Korea in 2004-2005 seasons in association with *Panonychus citri* in citrus orchards in Jeju, Korea (Jung et al., 2006). This species is widely used as spider mite biocontrol program in greenhouse and has some potential in open field crops (Kishimoto, 2002; Kishimoto et al., 2007). *P. persimilis* is the most commercially successful spider mite biocontrol agent in the world and their use has been restricted to greenhouse to control tetranychid pests (McMurtry and Croft, 1997; Van Lenteren, 2003). The promising characters of *P. persimilis* for successful control of spider mite are a great searching capacity, a high predation rate, a high reproduction rate and a short developmental cycle (McMurtry and Croft, 1997). These characters enable *P. persimilis* to hastily suppress *T. urticae* populations on greenhouse (Simmonds, 1972; Burgess, 1984; Blümel, 1990; Zhang and Sanderson, 1995).

Phytoseiid mites are commercially available and frequently used for biological control of spider mites in green house, field, and orchard crops (Helle and Sabelis, 1985; McMurtry and Croft, 1997), and the ambient conditions of these usable spaces are variable depending upon the seasons or the operation systems. The overall objective of this study was to compare the environmental tolerance of three phytoseiid mites under different ambient and food shortage conditions.

Materials and Methods

Source of mites

Stock cultures of *N. californicus, N. womersleyi* and *P. persimilis* were maintained on detached kidney bean leaves and fed on mixed stages of *Tetranychus urticae* in Insect ecology lab, Andong National University Rearing units were kept in the environmental room at 25°C, 65% RH and 16L: 8D photoperiods. *N. womersleyi* was collected from apple orchard in Andong, Gyeongbuk, Korea in 2006 and maintained in the laboratory for two years. *N. californicus* was collected from citrus orchards in the Jeju in 2006 and maintained in the laboratory. *P. persimilis* was purchased from the commercial supplier (Sesil Company, Korea) in 2007 and then maintained in the laboratory.

Egg hatching and immature development

Combinations of temperatures (15, 20, 25, 30, 35°C) and relative humidities (33, 51, 75, 95%) on egg hatching, immature survival and adult performance (reproduction and survival) of three important phytoseiid mites, *N. californicus, N. womersleyi* and *P. persimilis* were tested. Humidity chambers were constructed from plastic containers (Dia. 15cm, H. 15cm). Saturated solutions of either $Mgcl_2$, $6H_2O$, $Ca(NO_3)_2$, $4H_2O$, NaCl and Na_2HPO_4, $12H_2O$ were used within individual containers to maintain constant humidities of 33±2, 51±2, 75±2, 95±1%, respectively (O'Brien., 1947; Croft et al., 1993). Even-aged eggs (<12 hours) obtained from gravid females fed on *T. urticae* were individually transferred into plexiglas cells (Jung and Croft, 2000). Cells consisted of two holes (1.5×0.3cm DH) in a rectangular piece of plexiglas (8×2.5×0.3 LWH) and closed at the bottom by a fine nylon mesh and covered at the top by slide glass. In each cell, single egg was transferred. To each treatment, 40 eggs were tested. Additionally, transferring five eggs in each cell were repeated to see if there is any difference of immature mortality by cannibalism (Schausberger, 2003) during immature development. In each treatment, 60 eggs were tested for this study. Hatched larvae and other immature did not have access to free water or prey except to conspecifics in five-egg experiments, thus cannibalism could be assessed. Egg hatch and immature development were checked daily until all mites were dead. Cannibalism was determined from five egg experiment by measuring the developmental duration up to final stages as compared to single egg experiment. The degree of cannibalism was calculated by counting the deutonymphs or prolonged survival stages.

Adult female survival and oviposition

For adult performance test, randomly chosen 5-10 days old mated females from rearing units at 25°C, 6o-65% RH and 16L: 8D were individually transferred into each hole of plexiglas provided sufficient prey *T. urticae* stages. When the amount of prey decreased, the predatory mites were transferred to new plexiglas with enough prey. Ten fertilized females were examined in each case. Fecundity and mortality were monitored on a daily basis for consecutive five days.

Statistical analyses

The effects of relative humidity and temperatures on egg hatch and adult performance of three phytoseiids *N. californicus, N. womersleyi* and *P. persimilis* were analyzed by GLM regression analysis. LH_{50} values were estimated from the regression equation. Differences in immatures survival from five and single egg experiment were compared among the three species using χ^2 test. Mean values were compared by LSD using the SAS program, version 9.1 (SAS Institute, 2005).

Results

Egg hatching

For all populations, egg mortality increased as humidity decreased ($P < 0.01$). In all combinations, high mortality occurred at lower than 50% RH, except for *N. californicus* in 15°C (Figures 3, 4 and 5). From the statistics (Table 8), there were significant effects of temperature and humidity on egg hatching rate for *N. californicus*. However, for *N. womersleyi*, only humidity was significantly meaningful for egg hatching. For *P. persimilis*, humidity and the interaction of humidity and temperature were found significant. The proximal cause was the shrinking of the egg chorion by excessive water loss (data not shown here). In high temperature of 35°C, no eggs of *P. persimilis* were hatched even in high humidity level contrasting to *N. californicus* or *N. womersleyi*.

Immature survival

Survival of immatures was measured in the single and five egg experiments provided no food under the different relative humidity and temperature conditions (Figure 6 and 7). No larvae survived to protonymphs below 55% RH for three species. Fifty percent of *N. californicus* larva survived to nymphal stage at 75% RH and almost 100% at 95% RH. No *N. womersleyi* larvae survived at 75% RH but ≈ 80% developed into protonymphal stage regardless of temperature at 95% RH. However for *P. persimilis*, at low temperature, most survived to protonymphal stage, but as temperature increased, larval survival at 75% RH decreased to 10%. At 95% RH, most of the larvae survived into protonymphal stage.

Finding the cannibalism in the three phytoseiid species

Cannibalism within the conspecifics in three species was evaluated by comparison test in different temperature and relative humidity conditions (Figures 6 and 7). Single-egg treatment of all the species was not able to develop beyond larva or protonymph stage in all the temperature and relative humidity combinations whereas eggs from five-egg treatment of all the species developed up to the deutonymph stage or prolonged last stage revealing that the survival and development for longer period or up to deutonymph stage was just because of cannibalism. Survival and development up to the last stage was significantly different between five-egg and one-egg experiment ($P < 0.001$). Development of eggs from five-egg treatment was significant in *N. californicus* in temperature and relative humidity combinations of RH 75% and 95% at 15, 20, 25, 30°C and, 95% at 35°C, respectively. Similarly, five-egg treatment was significant compared to single-egg treatment in attainment of higher developmental stage in case of *N. womersleyi* in above mentioned temperature and relative humidity combinations ($P < 0.001$). Similar result was found in case of *P. persimilis* also in all the temperature and relative humidity conditions except at 35°C ($P < 0.001$). At 35°C eggs of *P. persimilis* from both single-egg and five-egg treatments were unable to develop into further stages. However, the cannibalism trend found higher in *N. californicus* and lower in *N. womersleyi* among the tested phytoseiids (Table 12). Significant differences were found among the species while tested them at different temperature and humidity combinations (ANOVA, $df = 14{,}165$; $P < 0.001$).

Adult female survival and fecundity

The combined effects of different temperatures and relative humidities on the adult mortality for five days were significantly influenced by temperature and humidity were significantly influenced by temperature and humidity (Table 10). *N. californicus* showed no mortality under 30°C and 75% RH. However, at 35°C under 51% RH mortality increased 100%. Similar pattern was shown in *P. persimilis* but the threshold was lower than *N. californicus*. *P. persimilis* experience almost 100% mortality at 35°C regardless the relative humidity and at 30°C at 33% RH. *N. womersleyi* showed different pattern of female mortality. The threshold of relative humidity for *N. womersleyi* mortality increased as the temperature increased.

The fecundity of *N. californicus*, *N. womersleyi* and *P. persimilis* under the different ambient conditions represented at the figures 11, 12 and 13, respectively. Fecundity of all the species was significantly affected by temperature and relative humidity (Table 11). The maximum daily fecundities were shown at 30°C, but only above 75% RH regimes. Under the <75% RH, fecundities were much lower.

Table 8. Parameters from GLM regression of egg hatch rate (0-1) by relative humidity and temperature combination

Species	R^2	Intercept	Humidity (%)	Temperature (°C)	H*Temp[1]
N. californicus	0.84	-0.19 (0.38)[2]	0.017 (<0.001)	-0.018 (0.02)	-[3]
N. womersleyi	0.71	-0.606 (<0.001)	0.0135 (<0.001)	-	-
P. persimilis	0.77	-1.232(0.03)	0.030 (<0.001)	0.0296 (0.1)	-0.0007 (0.01)

[1] Interaction of humidity and temperature

[2] Number in the parenthesis represent the P value of the parameter from T distribution.

[3] "-" represents non significant parameter

Table 9. Estimated lethal humidity (LH_{50}) of three phytoseiid eggs based on the regression analysis for each temperatures

Species	Temperatures (°C)				
	15	20	25	30	35
N. californicus	56.5	61.8	67.1	72.4	77.7
N. womersleyi	82.0	82.0	82.0	82.0	82.0
P. persimilis	66.1	71.3	79.4	93.8	-[1]

[1] "-" represents no data available because of no egg hatching at this temperature

Table 10. Parameters from GLM regression of adult mortality rate (0-1) by relative humidity and temperature combination

Species	R[2]	Intercept	Humidity (%)	Temperature (°C)	H*Temp[1]
N. californicus	0.49	-124.78 (<0.001)	1.34 (<0.001)	6.40 (<0.001)	-0.068 (<0.001)
N. womersleyi	0.60	-108.31 (<0.001)	0.98 (0.006)	7.43 (<0.001)	-0.073 (<0.001)
P. persimilis	0.50	-72.00 (<0.001)	-	3.60 (<0.001)	-[3]

[1] Interaction of humidity and temperature

[2] Number in the parenthesis represent the *P* value of the parameter from T distribution.

[3] "-" represents non significant parameter

Table 11. Parameters from GLM regression of oviposition rate by relative humidity and temperature combination

Species	R^2	Intercept	Humidity (%)	Temperature (°C)	H*Temp[1]
N. californicus	0.72	-60.16 (<0.001)	-[3]	5.99 (<0.001)	0.0033 (<0.001)
N. womersleyi	0.73	-47.09 (<0.001)	-	4.27 (<0.001)	0.006 (<0.001)
P. persimilis	0.73	-69.90 (<0.001)	-0.027 (<0.001)	6.91 (<0.001)	0.004 (<0.001)

[1] Interaction of humidity and temperature

[2] Number in the parenthesis represent the *P* value of the parameter from T distribution.

[3] "-" represents non significant parameter

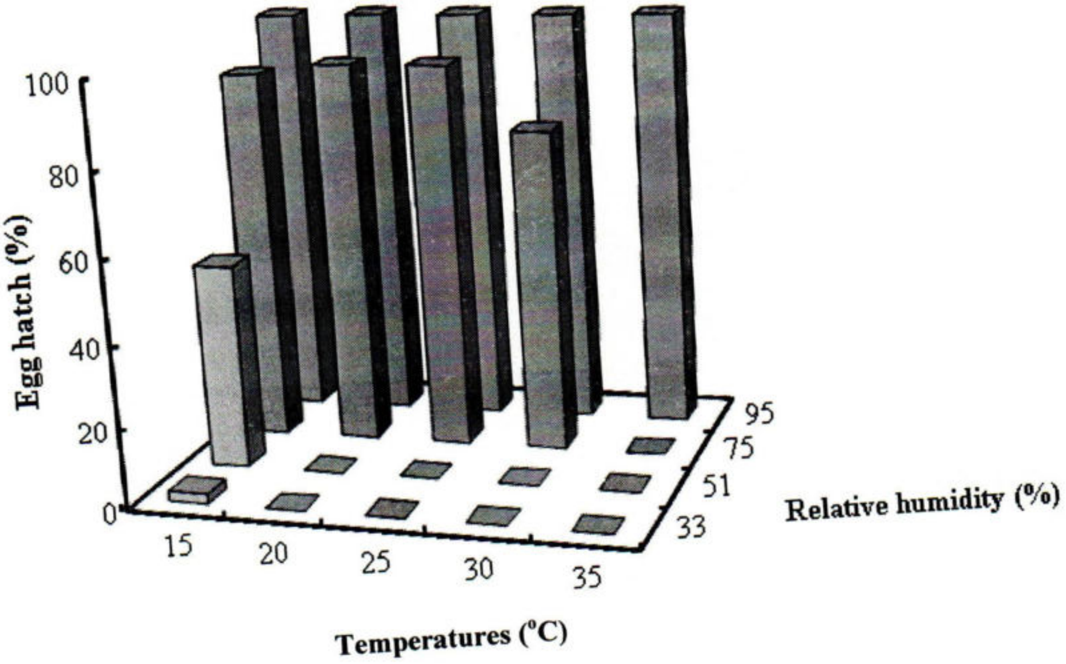

Figure 3. Egg hatch success in *N. californicus* under five different temperatures and four relative humidities.

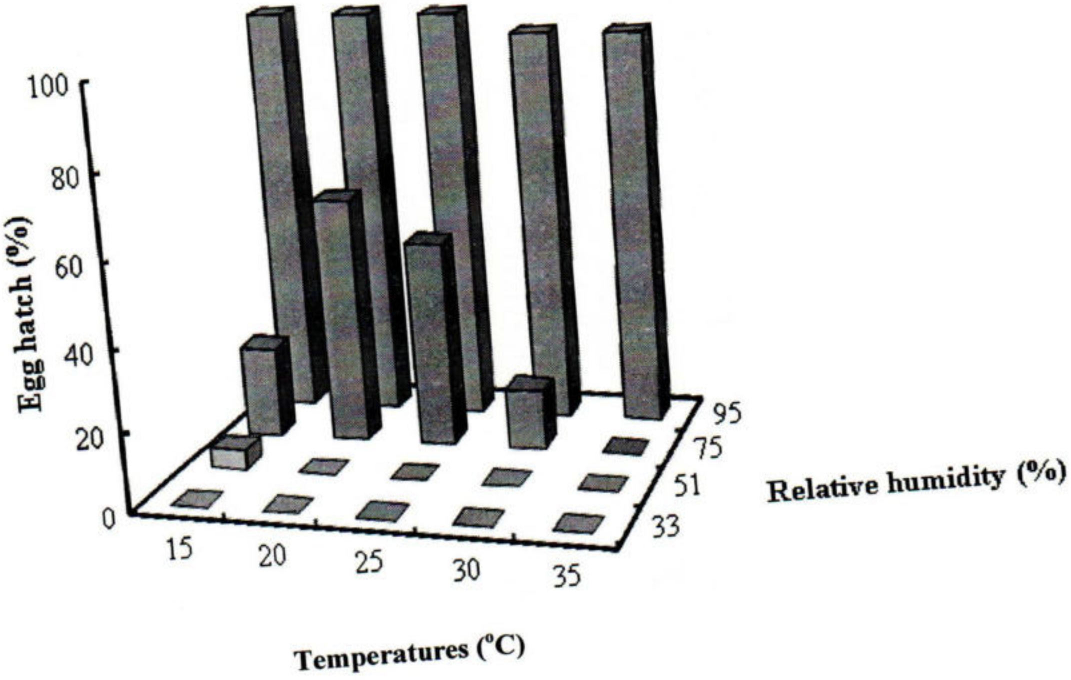

Figure 4. Egg hatch success in *N. womersleyi* under five different temperatures and four relative humidities.

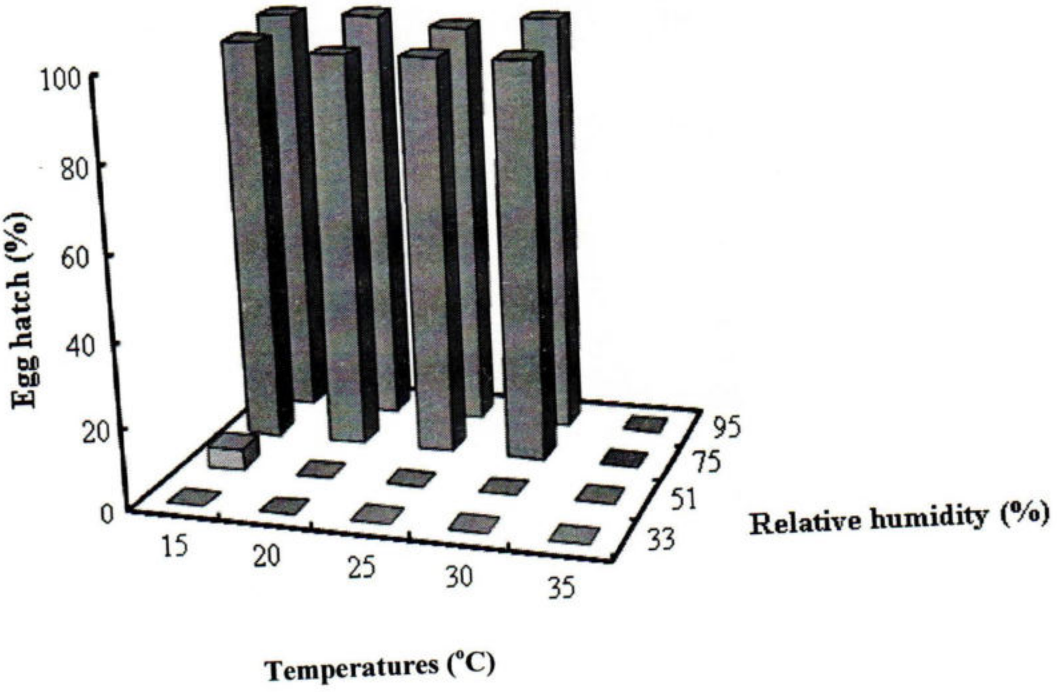

Figure 5. Egg hatch success rates in *P. persimilis* under five different temperatures and four relative humidities.

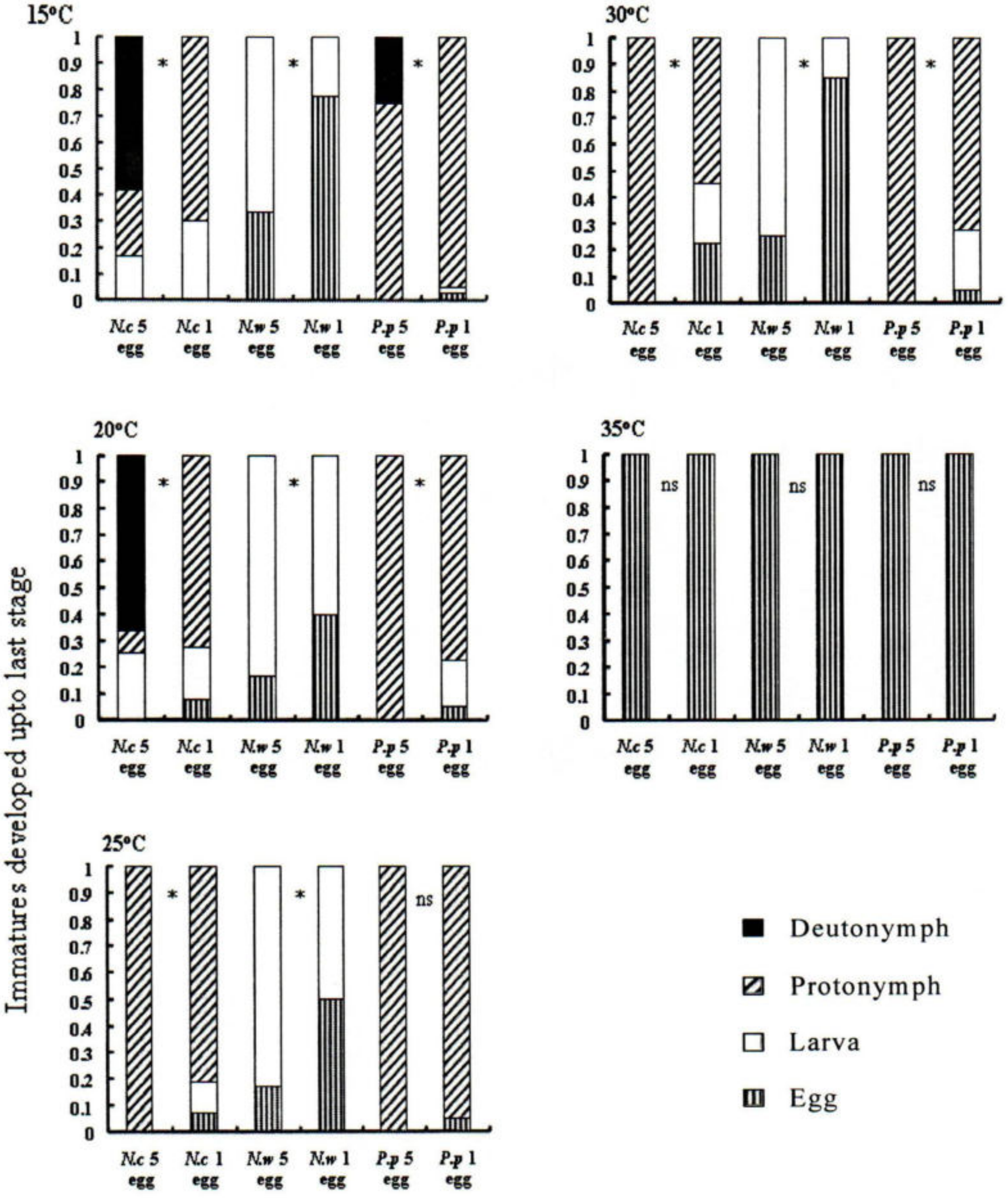

Figure 6. Survivorship (%) of three phytoseiid mites up to last immature stage in five and single egg experiments under five different temperatures and 75% relative humidity. *N.c-N. californicus*, *N.w-N. womersleyi*, *P.p-P. persimilis*. *Means differed significantly at $P < 0.05$ (χ^2 test).

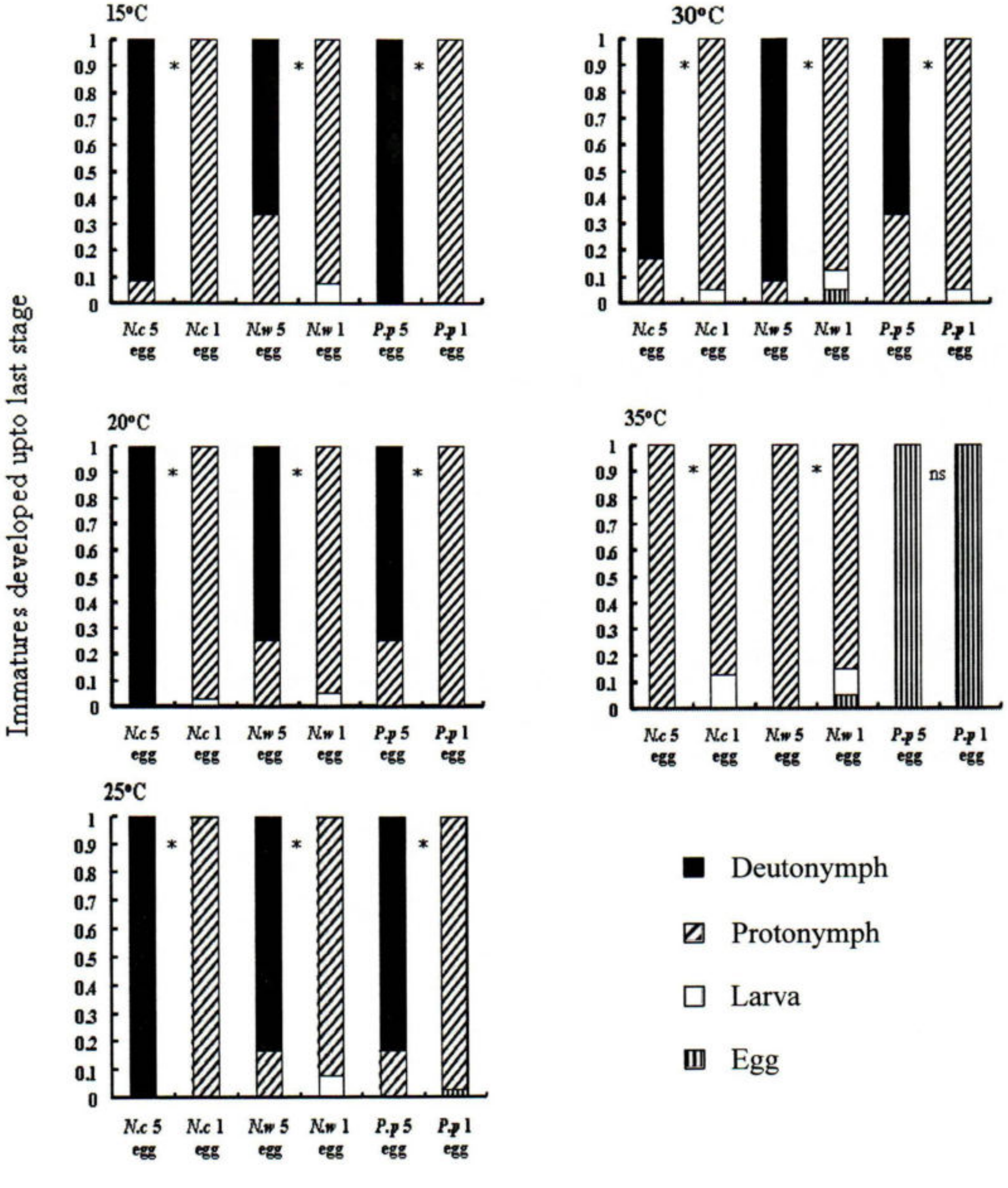

Figure 7. Immature survivorship (%) of three phytoseiid mites up to the last stage in five and single egg experiments under five different temperatures and 95% relative humidity conditions. *N.c-N. californicus*, *N.w-N. womersleyi*, *P.p-P. persimilis*. *Means differed significantly at $P < 0.05$ (χ^2 test).

Table 12. Cannibalism (%) of the three phytoseiid mite species under some different conditions

Species	Temperatures (°C)	Percent cannibalism under different relative humidities	
		75%	95%
	15	13.33 ± 4.49def	46.67 ± 3.59a
	20	16.67 ± 4.82def	43.33 ± 4.14ab
N. californicus	25	0	50.00 ± 3.01a
	30	0	23.33 ± 4.14cde
	35	0	0
	15	5.00±2.61f	23.33 ± 5.69bc
	20	0	13.33 ± 5.12def
P. persimilis	25	0	26.67 ± 6.20cd
	30	0	18.33 ± 4.58de
	35	0	0
	15	0	20.00 ± 0.00de
	20	0	11.67 ± 2.97ef
N. womersleyi	25	0	26.67 ± 4.49cd
	30	0	18.33 ± 1.67de
	35	0	0

Means with different letter represent significant difference (ANOVA, *df* = 14,165; $P < 0.05$)

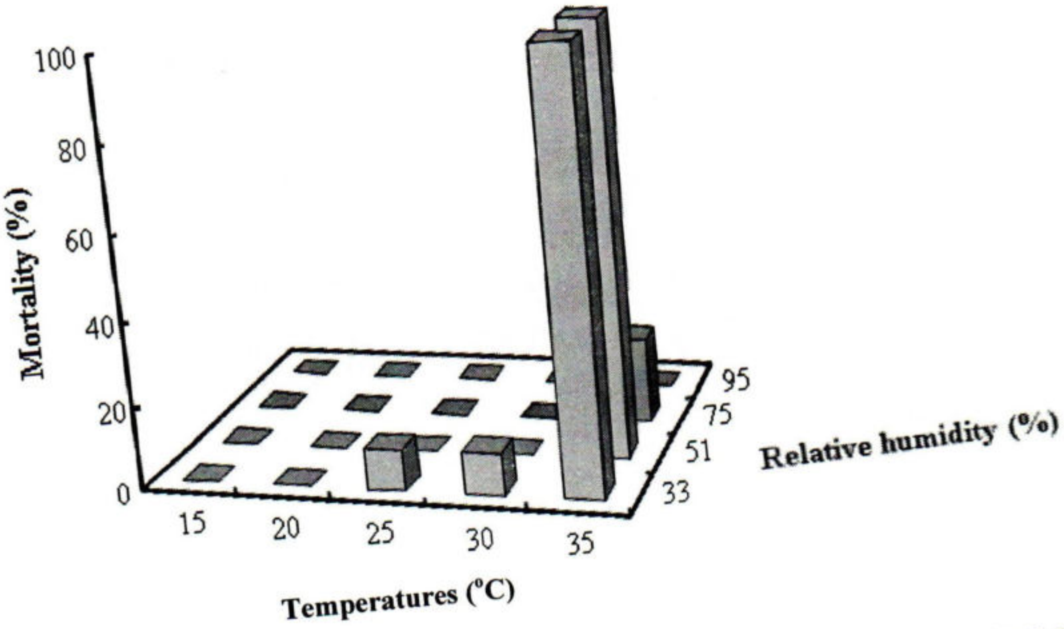

Figure 8. Adult mortality (%) of *N. californicus* under five different temperatures and four humidities.

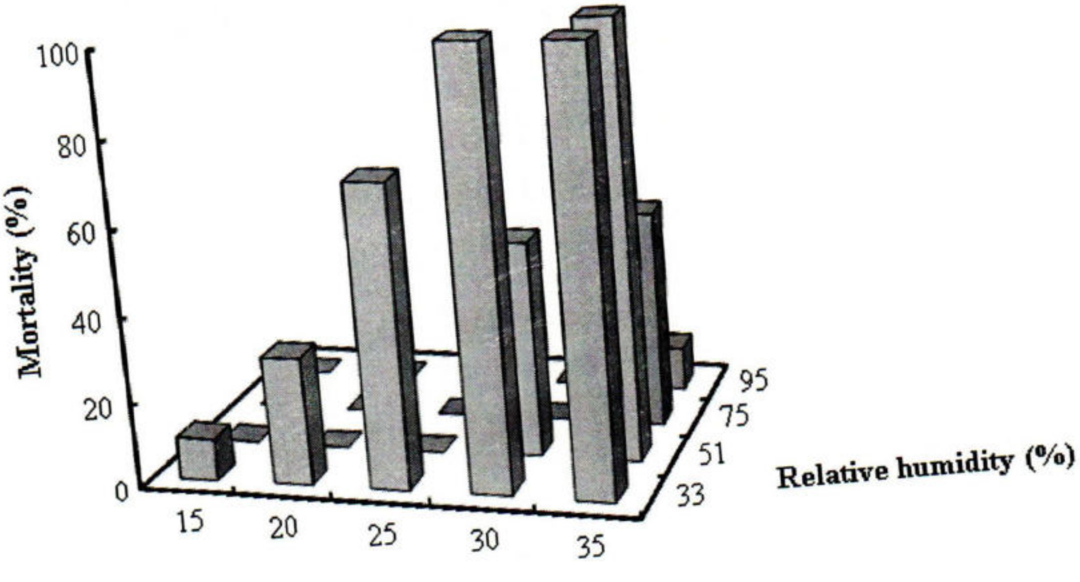

Figure 9. Adult mortality (%) of *N. womersleyi* under five different temperatures and four humidities.

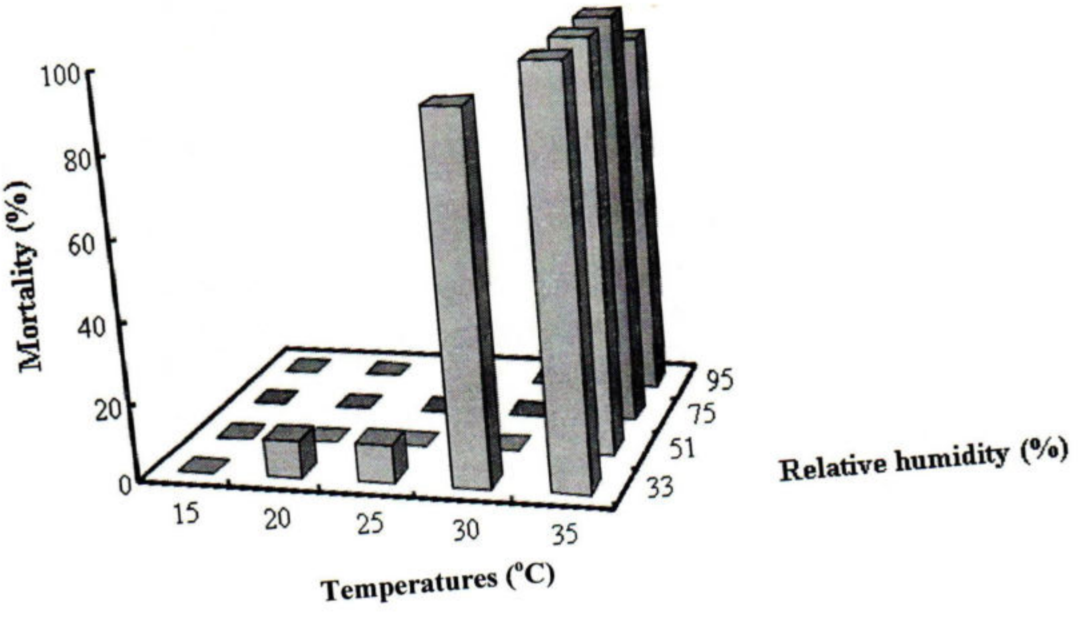

Figure 10. Adult mortality (%) of *P. persimilisi* under five different temperatures and four humidities.

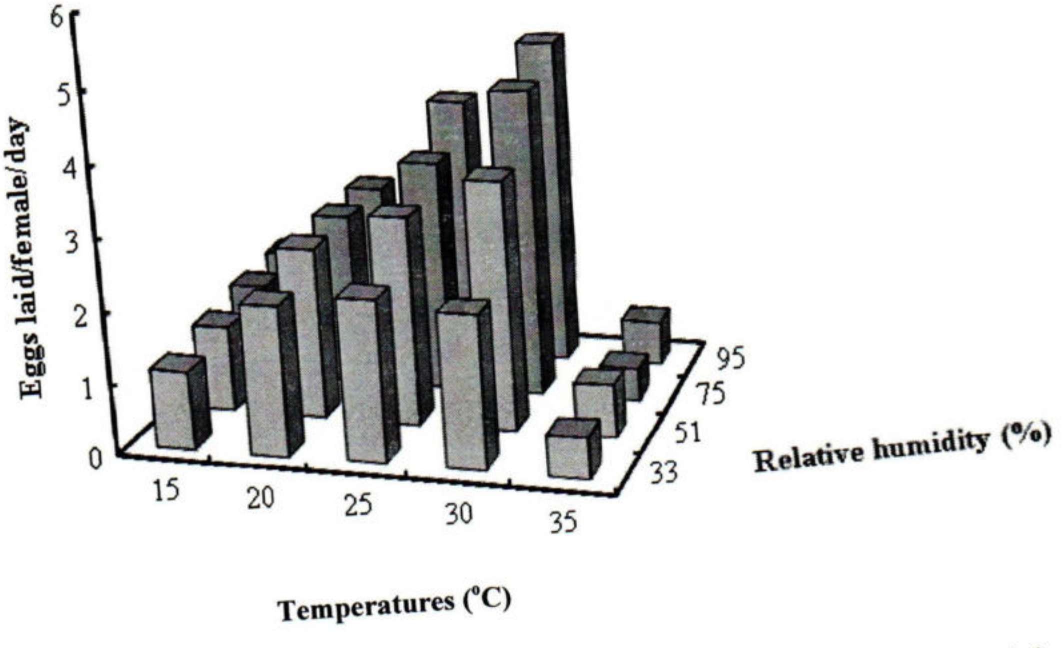

Figure 11. Oviposition by *N. californicus* under five constant temperatures and four humidities.

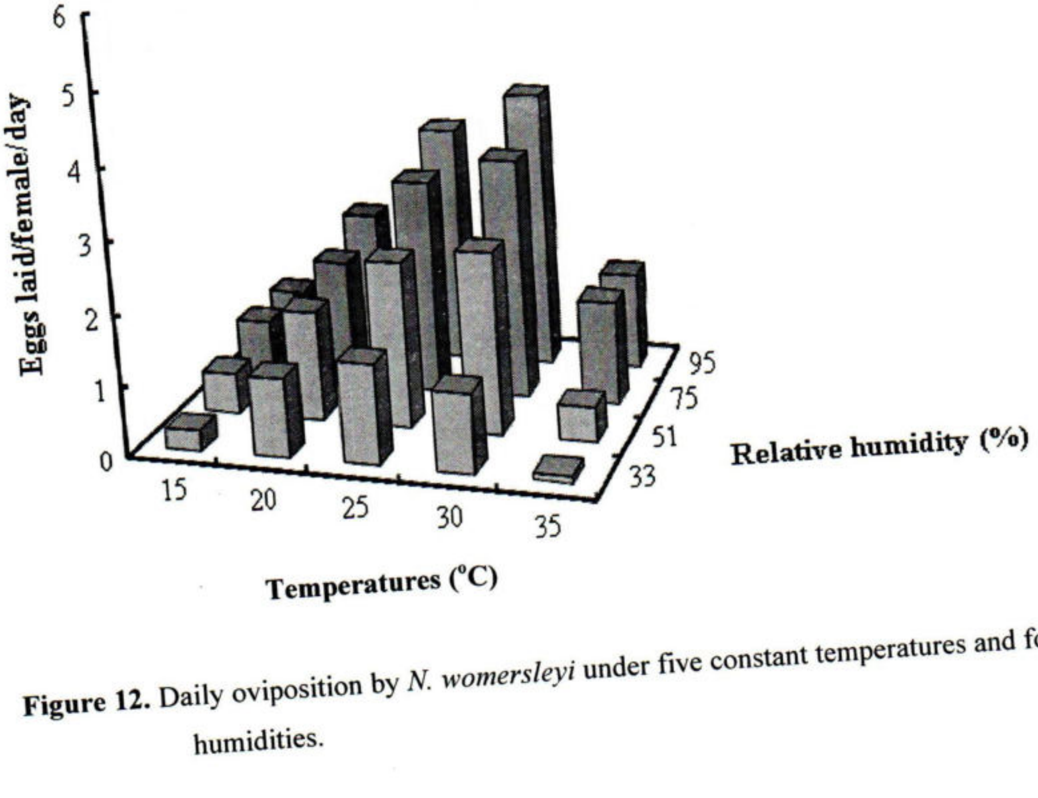

Figure 12. Daily oviposition by *N. womersleyi* under five constant temperatures and four humidities.

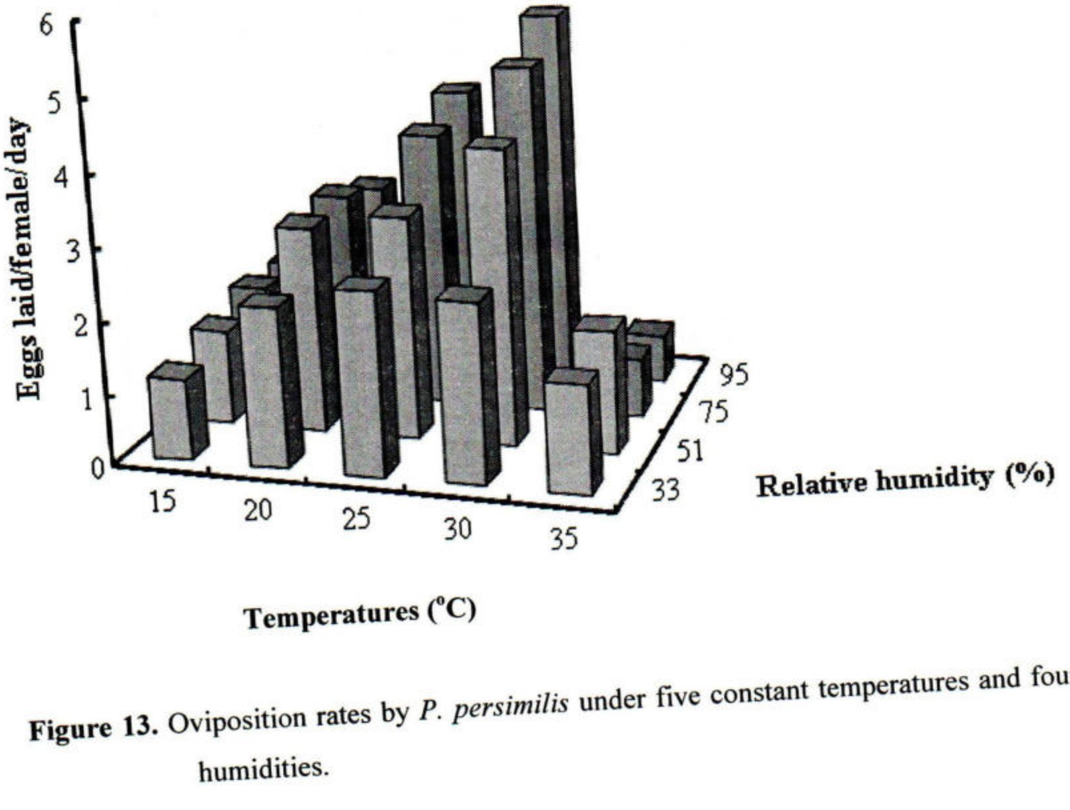

Figure 13. Oviposition rates by *P. persimilis* under five constant temperatures and four humidities.

Discussion

In the experiments, we tested the effect of environmental variables of temperature and humidity on developmental performance of *N. californicus* with comparison to *N. womersleyi* and *P. persimilis*. Egg hatching was influenced by not only temperature but also relative humidity. However, *N.californicus* showed relatively wide range of tolerance to the relative humidty compared to the other species. In the present study all the three tested phytoseiid species showed higher egg mortality as the humidity decreased. Croft et al (1993) also found similar result with *Neoseiulus fallacies*, *Amblyseius andersoni*, *Metaseiulus occidentalis* and *Typhlodromus pyri*. At 95% RH there was almost no mortality of the three species except for *P. persilmilis*. The eggs *of P. persimilis* were less tolerant to higher temperature especially at 35°C. Eggs of *N. californicus* were most tolerant to low humidity and had the greatest range of response as compared to *N. womersleyi* and *P. persimilis.* These traits should enable *N. californicus* to persist in the fields where broader range of environmental conditions prevailing. Another finding we observed in this study that at the lower humidity lower temperatures are effective but as the temperature increased caused the eggs shrinking very quickly.

Data for the larval stage of three phytoseiid mites indicated different types of response between single and five egg experiments. Developed larvae of *N. californicus*, *N. womersleyi* and *P. persimilis* from single egg experiment suffered mortality than five-egg treatment. This study confirmed that larval stage of those tested species does not need to feed to develop into protonymph. However, from protonymph, these predators need feeding to further development. In one-egg experiments, no protonymph has developed into deutonymph. However, in five-egg experiments, significant proportion of protonymphs was successfully developed into deutonymphs. The only source of food intake was the conspecific immatures.

Results suggest single-egg treatment developed beyond larval stage; however five-egg treatment developed beyond larva or protonymph stage in all the temperature and relative humidity combinations. not a scientific argument; rephrase (Schausberger, 2003). In this study eggs from single egg treatment could not develop because of unavailability of food source. However the phytoseiids could develop to certain life stage in five-egg treatment revealing that cannibalism is prevalent. The cannibalism trend was found higher in *N. californicus* and lower in *P. persimilis* among the tested phytoseiids. Based on diet width, phytoseiids like *P. persimilis* and *N. californicus* can be classified as a specialist (broadly, mono- and oligophages) and a generalist (polyphages) (Walzer and Schausberger, 1999). They found that the *N. californicus* can discriminate con- and heterospecific and prefer the latter, but *P. persimilis* do not discriminate and feeds on both con- and heterospecific. However, in this study in the absence of heterospecifics cannibalism in *N. californicus* was found more severe compared to that of *P. persimilis*.

Adult mortality showed similar patterns observed in egg hatching experiment. The result clearly provides some evidence of the higher tolerance of *N. californicus* to low humidity and high temperature (Croft et al., 1993). Also, data on *N. womersleyi* is even more surprise that adult survival as well as egg hatching were significantly influenced by temperature and humidity. Many of the laboratory studies on *N. womersleyi* have only dealt with temperature as an important environmental factor for the development and predation performance (Lee and Ahn, 2000), and for modeling prey-predator dynamics (see Jung et al., 2005). However, many field specialists argued that this species is vulnerable to low humidity. This also has been discussed as a factor responsible to later colonization of the fruit tree canopy and delayed biological control performance (Kim, Y., 2007). The tolerance was decreased at 33 and 51% RH and 35°C temperature. However, survival of *N. californicus* under lower humidity at 30°C was higher compared to survivability of other phytoseiids. Walzer et. al. (2007) found less effect of RH in *N. californicus* adult survival but found that RH affected significantly in case of egg hatch and development. In this study also significant effect of RH was not recorded on adult survivability in the temperature range of 15°C to 25°C. *N. womersleyi* however was less tolerant to low humidity and high temperature condition. RH above 75% and temperature range of 25 -30°C was found most favorable for survivability of the phytoseiid adults.

Fecundity of all the species was significantly affected. Fecundity of all the species was less at 15°C and 35°C at all RH conditions. With the increase in temperature there was increase in number of eggs laid at all RH conditions. Fecundity of *P. persimilis* was significantly higher at most of the temperature and humidity combinations compared to the other two phytoseiid species. The result suggests that optimal temperature (> 25 °C -30 °C) and higher relative humidity promotes biological attributes of the predatory mites. In the field though there is ambient dry condition, other factors help in promoting the survivability of both predatory and phytoseiid mites (Zhang and Kong, 1985). This study has revealed that predatory mite *N. californicus* better resist widerange of RH and temperature conditions whereas *N. womersleyi* resist higher temperature. So, these predatory mites could be employed in the field condition as biological control agents depending on their biological attribute and environmental conditions of the crop.

CHAPTER III

Evaluation of diapause incidence of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) isolated from the Jeju Island, Korea.

Abstract

The population of *Neoseiulus californicus* McGregor collected from Jeju Island, Korea is a prospective generalist biological control agent of spider mite and insect pests. In this study, diapause induction was measured as the reproductive arrest in female under different temperature and photoperiod given *Tetranychus urticae* as food. Oviposition and prey consumption were also assessed for the predator's potentiality. Five photoperiods (L:D of 6:18, 8:16, 10:14, 12:12 and 14:10) combined with four temperatures (15, 18, 21 and 24°C) were subjected. All of the females oviposited within 8 days from one and two consecutive generation exposure studies, indicating the Jeju population of *N. californicus* is non-diapausing one. Preoviposition period was longer in lower temperature (5.68 ± 0.23 days at 15°C) and gradually shortened as temperature increased (1.06 ± 0.04 days at 24°C) but it was not differ among photoperiod treatment given temperature. Higher temperatures showed higher reproduction and prey egg consumptionBased on these results, we further discussed the potential implication of this population in biological control of spider mites in greenhouse and field crops relative to overwintering in temperate weather system in Korea.

Key Words: Diapause, *Neoseiulus californicus*, overwintering, fecundity, biological control.

Introduction

Neoseiulus californicus (Acari: Phytoseiidae) is one of the world-widely used biological control agent commercially available for the control of phytophagous mites and small arthropod pests (McMurtry and Croft, 1997). *Neoseiulus californicus* feed on a broad range of foods such as spider mites, tarsonemid mites, other small arthropods and pollen (Castagnoli et al., 1999; McMurtry and Croft, 1997; Vantornhout et al., 2005). *Neoseiulus californicus* is found on various economic crops and deciduous trees in Europe, North and South America, Africa, and Asia (Tixier et al., 2008). In Japan, it is established as dominant predatory mite in pear of central Japan and citrus in the south (Kishimoto, 2002). Jung et al. (2006) first reported the natural occurrence of *N. californicus* in subtropical Jeju Island of Korea, where the predator is closely associated with citrus red mites. The distribution of this predatory mite is restricted to only Jeju Island which is warmer than the rest of the mainland Korea. There are many possibilities utilizing *N. californicus* in the mainland, where is much colder during winter. To establish the predatory mite in the fields of the mainland, the mite would benefit if it possesses diapause characteristics in short day with low temperatures, and synchronizes the seasonal phenology with its prey (Hoy and Flaherty, 1970; McMurtry and Flaherty, 1977; Mori and Saito, 1979).

Diapause is one of the survival mechanisms in a harsh environment for poikilothermal arthropods. The unfavorable environmental conditions such as lower temperature, short photoperiod, limited foods and shelter would be challenging condition for winter survival of the predatory mites (Croft et al., 1998; Kim et al., 2005). For phytoseiid mites, diapause occurs only in adult females and characterized as reproductive arrest after mating once the inducing conditions are met (Overmeer, 1985; Veerman, 1992), perhaps appropriately, a lingering preoviposition period compared to non-diapausing mites. So, the absence of egg formation or prolonged preoviposition period have been used as a criterion for determining diapauses incidence in phytoseiid mites, and the environmental determinants would be low temperature and short day length (e.g. Sapozhnikova, 1964; Hoy and Flaherty, 1970; Wysoki, 1974; Collyer, 1976; Kashio and

Tanaka, 1980; Van Houten, 1990; Veerman, 1992; Van Houten et al., 1988). Kim et al. (unpublished) proposed that both of temperature and photoperiod are important diapause inducing factors to *N. womersleyi*, the dominant indigenous phytoseiid mite of Korea.

For *N. californicus*, diapause has been reported with variations depending on populations or strains ranging from 0% to 100% (Castagnoli et al., 1996; Hart et al,. 2002; Jolly, 2000). For example, populations of UK strain (95.7%), Italian strain (54-100%) and few individuals of the Spanish strain (16.1%) were reported as diapausing, but populations of US strain, Japanese indigenous strain, and the commercial strain in Japan (Spical®, Arysta Life Science Co., Ltd) as non-diapausing (Castagnoli et al., 1996; Jolly, 2000; Gotoh et al., 2005). However, overwintering biology of Jeju population of *N. californicus* is unknown. Since, there are growing interests in utilizing *N. californicus* in deciduous fruit trees as well as greenhouse crops. This study investigated if combinations of temperatures and photoperiods can induce reproductive diapauses of adult female *N. californicus* by measuring preoviposition period. Reproduction and food consumption also assessed during the experimentation.

Materials and Methods

Sources and rearing of *N. californicus* and prey

A population of *Neoseiulus californicus* was collected from the Jeju Island of Korea in 2006 and was reared on kidney bean plants (*Phaseolus vulgaris* L.)with Tetranychus urticae as food reared at 25 ± 2°C, 70 ± 5% R.H. and 16L: 8D photoperiod in the Insect Ecology Laboratory of Andong National University.

Treatment for diapauses induction

Induction of diapauses was investigated by maintaining different life stages of *N. californicus* in various combinations of temperatures and photoperiods with different exposure period. For each experiment, 70 adult females of *N. californicus* were separated from the stock culture and transferred to a fresh kidney bean leave which was placed as upside down to the moist cotton in a plastic container (9.5 × 4 cm). Ample mixed staged *Tetranychus urticae* were provided, and then the transferred adult females of *N. californicus* were allowed to lay eggs at 25°C and 16L: 8D for 24 hours.

First generation (F_1): After removing females, eggs were allowed to develop to adulthoods under the series of 20 combinations of five photoperiods (6, 8, 10, 12 and 14 h of light period per day) coupled with four temperatures (15, 18, 21 and 24°C). As the female adult emerges, it was separated daily. Total thirty-five female *N. californicus* were transferred individually on to a kidney bean leaf disc (dia. 2.9cm) supplied with mixed staged *Tetranychus urticae* in the petri dish (dia. 5.5cm, H.1.5cm). During transferring females, two active males from the same treatment condition were released for each female for mating. These females were checked for oviposition as an induction of reproductive diapause.

Second generation (F_2): We tested this experiment only at 18°C and L6:18D for the second generation study since this is the diapauses-inducing condition to *N. californicus* as well as to many other phytoseiid mites (Fitzgerald and Solomon, 1991; Morewood and Gilkeson, 1991; Veerman, 1992; Morewood, 1993). Individuals from each developmental stage; egg, larva, nymph and adult were sequentially subjected to the condition for consecutive two generations. Later on, passing two consecutive generations, thirty-five adult females were transferred individually in to small Petri dishes with the same procedures as above to check diapause induction.

Measurement of diapause, oviposition and prey consumption

The females were observed daily during the light period under a microscope (40X, Olympus Optical Co., Ltd, Tokyo, Japan) after mating was confirmed for 14 days at 15°C; or for 10 days at 18, 21 and 24°C. Females which fail to oviposit during these periods would be considered as diapause-induced (Veerman, 1992; Kishimoto and Takafuji, 1994). Once the female began to lay egg, then daily oviposition was recorded to determine the average number of eggs for 10 ovipositing days. During this later period, prey consumption was estimated. To determine the prey consumption of *N. californicus*, only egg stage of *T. urticae* was provided at the density of 15, 20, 25 or 30 in relation to temperatures of 15, 18, 21 and 24°C, respectively. The number of prey consumed was recorded for two days.

Statistical analyses

The effects of temperatures and photoperiods on preoviposition period, daily oviposition and food consumption by the first generation of *N. californicus* were analyzed by two-way analysis of variance (ANOVA). Other treatment effect was analyzed by one-way ANOVA. Means among treatments were separated by Tukey's honest significant difference test (Tukey's HSD test). All statistical analyses were performed using the SAS system version 9.1 (SAS Institute, 2005).

Results

Diapause induction

Under the tested conditions, diapause was not induced in the first generation exposures as well as the second generation study of Korean *N. californicus* (Table 13). In the first generation, the longest preoviposition period was observed at 15°C and 6L:18D, and the shortest period was at 24°C under 14L: 10D (Table 15). The temperature and photoperiod had a significant effect on preoviposition period and noted disparities of preoviposition periods were experienced under different temperatures (F = 599.29; df = 3,524; P < 0.001) and photoperiods (F = 3.97; df = 4,524; P = 0.002). The interaction of temperature and photoperiod had not influenced the preoviposition period (Table 14; Two way-ANOVA; F = 0.97; df = 12,524; P = 0.48).

In the second generation study, the preoviposition period was the highest for the nymph to adult and the lowest for the egg-to-adult under the photoperiod of 6L: 18D at 18°C (Figure 14). The preoviposition period of the second generation exposure treatment was shorter compared to the first generation exposure treatment (ANOVA; F = 5.08, df = 4,146; P = 0.007). Trivial influence of photoperiod was observed on preoviposition periods under the same temperature in both the cases. From all experimental conditions, female *N. californicus* oviposited, indirectly representing no diapause induction. The first oviposition occurred within 8 day (Table 15).

Reproduction

The effects of temperatures and photoperiods on the fecundity of the first generation exposure are shown in the table 16. The daily fecundity of *N. californicus* was higher at 24°C under 14L: 10D and lower at 15°C and 8L: 16D. Nevertheless, the temperature and photoperiod showed notable differentiations among the treatments (Table 14; temperature, F = 1419.79, df = 3,524; P = 0.001 and photoperiod, F = 8.32, df = 4,524; P = 0.001). However, the interaction of temperature and photoperiod did not affect the daily fecundity of *N. californicus* (Two way-ANOVA; F = 1.74; df = 12,524; P = 0.06). In the second generation, the highest fecundity was noted (1.37 ± 0.03) at the adult to adult cycle (Figure 15). Remarkable divergences were experienced in the fecundity of the first and the second generation at the egg to adult, and the adult to adult cycle (ANOVA; F = 6.45, df = 4,145, P = 0.001).

Prey egg consumption

Table 17 shows the amount of food consumed by the studied mite under the diverse thermo-photoperiodic regimes. Daily prey egg consumption of *N. californicus* females was highly influenced by temperatures and photo phase within the tested conditions. The maximum eggs (14.20 ± 0.3) exploited by a female was at 24°C under 14L: 10D, but the least amount (4.59 ± 0.28) was at 15°C under 6L: 18D. The consumption rate of prey eggs was significantly influenced by temperatures (F = 536.84, df = 3,202; $P < 0.0001$) and photoperiod (F = 8.60, df = 4,202; $P < 0.001$). The prey consumption also significantly influenced by the interaction of both the factors (Table 14; Two way-ANOVA; F = 0.82, df = 12,202; P = 0.02). The utmost prey egg (8.70 ± 0.38) was consumed by the adult to adult cycle of the second generation (Figure 16). The first and the second generations also feed the similar quantity of food under unlike photoperiods of 18°C with the exception of the egg to adult, and the adult to adult cycle (ANOVA; F = 5.30, df = 4,49, P = 0.014).

The positive correlation between the quantity of egg consumption and the number of egg production suggests that the parameters are not independent. Conversion rate from *T. urticae* egg to *N. californicus* egg was estimated as 0.22 (P = 0.001) while the intercept value is -0.314 (P = 0.011) in the first generation (Figure 17).

Table 13. Exposed stage, conditions and results to induce diapause of Korean *N. californicus*

	Exposed stage*	Exposed conditions**	Diapause induction
First generation	E – A	5 photoperiods × 4 temperatures	no
Second generation	A – E - A	18°C and L6:18D	no
	N - A – E- A	18°C and L6:18D	no
	L - A – E- A	18°C and L6:18D	no
	E - A – E- A	18°C and L6:18D	no

* E, Egg; L, Larva; N, Nymph; A = Adult.

**Five photoperiods (6, 8, 10, 12, and 14 hours of light period) coupled with four temperatures (15, 18, 21, and 24°C)

Table 14. Results of a two-way ANOVA on factors (temperature and photoperiod) affecting on the preoviposition period, daily oviposition and prey consumption of *N. californicus*

Source	SS	*df*	MS	*F*	*P*
Preoviposition period					
Temperature	1412.60	3	470.87	599.29	< 0.001
Photoperiod	12.49	4	3.12	3.97	0.004
Temperature × photoperiod	9.14	12	0.76	0.97	0.478
Error	411.71	524	0.79		
Daily oviposition					
Temperature	309.07	3	103.02	1419.79	< 0.001
Photoperiod	2.41	4	0.60	8.32	< 0.001
Temperature × photoperiod	1.52	12	0.13	1.74	0.054
Error	38.02	524	0.07		
Prey consumption					
Temperature	2442.49	3	814.16	536.84	< 0.001
Photoperiod	52.16	4	13.04	8.60	< 0.001
Temperature × photoperiod	38.10	12	3.17	2.09	0.019
Error	306.35	202	1.52		

Table 15. Preoviposition period (day, mean ± SE) of *N. californicus* (F_1) exposed to the different photo-thermal conditions from egg stage

Photophases (hr.)	Preoviposition period at different temperatures (°C)			
	15	18	21	24
6	5.69 ± 0.23a	3.76 ± 0.16b	2.24 ± 0.09c*	1.38 ± 0.11d*
8	5.44 ± 0.31a	3.63 ± 0.14b	2.08 ± 0.14c*	1.19 ± 0.09d
10	5.03 ± 0.21a	3.64 ± 0.23b	1.82 ± 0.10c	1.22 ± 0.09d
12	5.53 ± 0.28a	3.50 ± 0.25b	1.72 ± 0.09c	1.14 ± 0.07d
14	5.21 ± 0.21a	3.04 ± 0.14b	1.96 ± 0.04c*	1.06 ± 0.04d*

Mean ± SE within the rows followed by the different letters were significantly different at $P < 0.05$.

*Mean ± SE within the columns were significantly different at $P < 0.05$.

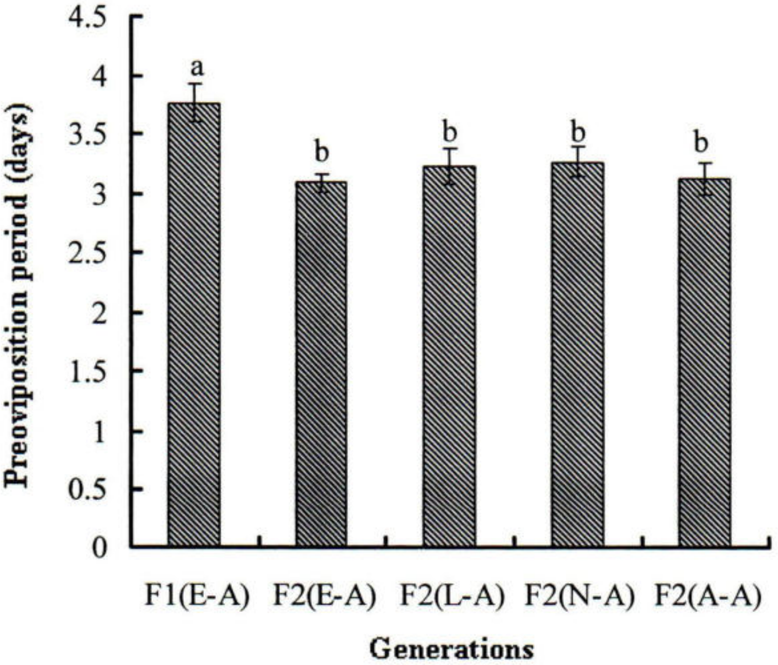

Figure 14. Preoviposition periods (days) of *N. californicus* (F_1 and F_2 generations) under 18°C and 6L: 18D. Vertical lines on bars are ± SE. Bars with different letters are significantly different ($P < 0.05$, Tukey HSD test). Life cycle stages: E, Egg; L, Larva; N, Nymph; A = Adult.

Table 16. Fecundity of *N. californicus* (first generation, F_1) under the combinations of various temperatures and photoperiods

Photophases (hr.)	Fecundity under different temperatures (°C)			
	15	18	21	24
6	0.79 ± 0.04d	1.15 ± 0.04c	2.11 ± 0.07b	2.67 ± 0.05a*
8	0.76 ± 0.05d*	1.26 ± 0.04c	1.93 ± 0.06b*	2.71 ± 0.06a
10	0.92 ± 0.04d	1.24 ± 0.06c	2.21 ± 0.04b	2.91 ± 0.07a
12	0.92 ± 0.04d	1.30 ± 0.04c	2.19 ± 0.06b	2.76 ± 0.06a
14	0.94 ± 0.03d	1.31 ± 0.05c	2.09 ± 0.05b	2.92 ± 0.05a

Values within the rows followed by the same letter were not significantly different at $P < 0.05$.

*Mean ± SE within the columns were significantly different at $P < 0.05$.

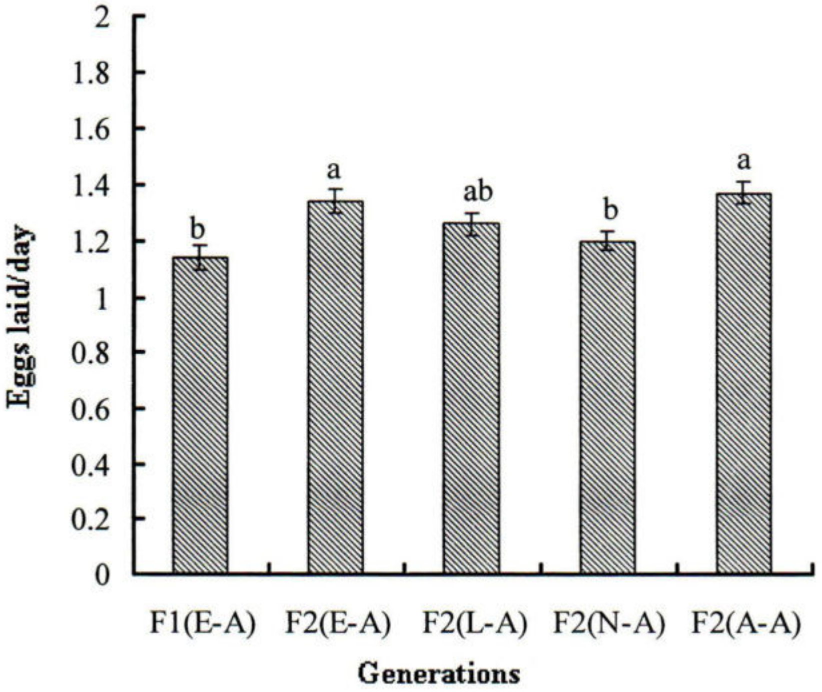

Figure 15. Fecundity in the first (F1) and second (F2) generations of *N. californicus* under 18°C and 6L: 18D. Vertical lines on bars are ± SE. Bars with common letter are not significantly different ($P > 0.05$, Tukey HSD test). Life cycle stages: E, Egg; L, Larva; N, Nymph; A = Adult.

Table 17. Prey eggs consumption by *N. californicus* (F_1) under four temperatures and five photoperiodic combinations

Photophases (hr.)	Temperatures (°C)			
	15	18	21	24
6	4.59 ± 0.28d	6.65 ± 0.26c*	9.80 ± 0.25b*	12.95 ± 0.34a*
8	5.31 ± 0.30d	8.75 ± 0.26c	10.80 ± 0.37b	13.15 ± 0.33a
10	4.69 ± 0.27d	8.50 ± 0.50c	11.90 ± 0.34b	13.40 ± 0.22a
12	5.20 ± 0.38d	8.16 ± 0.73c	11.75 ± 0.47b	13.90 ± 0.17a
14	4.84 ± 0.27d	9.30 ± 0.39c	11.85 ± 0.43b	14.20 ± 0.30a

Mean ± SE with the same letter within the rows were not significantly different at $P < 0.05$.

*Mean ± SE within the columns were significantly different at $P < 0.05$.

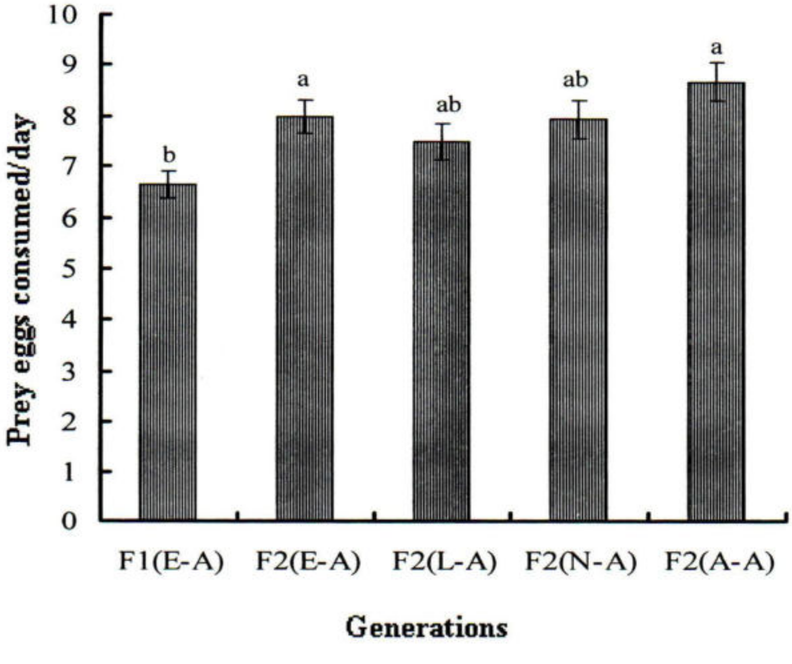

Figure 16. Prey egg consumption (mean ± SE) by the F_1 and F_2 *N. californicus* under 18°C and 6L: 18D. Bars with common letter are not significantly different ($P < 0.05$, Tukey HSD test). Life cycle stages: E, Egg; L, Larva; N, Nymph; A = Adult.

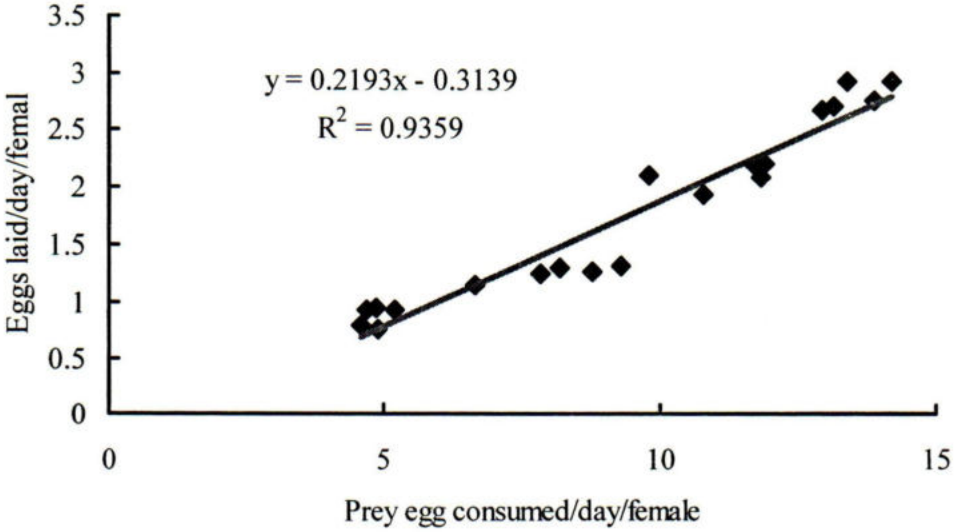

Figure 17. Relationship between prey egg consumption and number of eggs laid by *N. californicus* (n = 20, *R* = 0.97, *P* = 0.0011).

Discussion

Diapause of organisms is characterized as a period during which growth or development is suspended and physiological activity is diminished usually in response to a number of environmental stimuli that precede adverse conditions. Diapause usually occurs during a genetically determined stage of development, and its full expression develops in a species-specific manner in the different geographical contexts of the world. This study showed that diapause was not induced for Korean *N. californicus* by temperature and photoperiodic combinations tested. Such phenomenon was also found from the Japanese indigenous strains of *N. californicus* (Gotoh et al., 2005), and US strains (Hart et al., 2002; Jolly, 2000). On the contrary, 95.7% of the UK, 16.1% Spanish (Jolly, 2000) and 54-100% of the Italian strain of *N. californicus* demonstrated the diapause ability (Castagnoli et al., 1996). In addition, Morewood (1993) argued that the diapause of phytoseiid mites would be facultative, and some species or some populations have no diapause but overwinter successfully (Wysoki and Swirskii, 1971a, b; McMurtry et al., 1976; Overmeer, 1985). Since *N. californicus* overwinter in citrus orchard of the Jeju Island (Kawashima and Jung, unpublished), it was suggested that females of Jeju population might survive winter (average winter temperature of the Jeju Island ranges from 6.6 to 10.1°C, http://www.kma.go.kr/index.html) without diapause. So far it is not clear if the Jeju population is indigenous or invaded from outside Jeju (Jung et al., 2006). The trait of non-diapause would render the possibility that the Korean strain (Jeju population) may be close to US, Japan or commercial strain such as Spical®. Further studies on ecological traits and molecular study which is currently pursued by collaboration with M. Tixier will provide some clue on the geographic origin.

The daily oviposition was 1.93-2.21 and 2.67-2.92 at 21 and 24°C. This range is a bit higher than the reports from Japanese population (Katayama et al., 2006; Toyoshima and Hinomoto, 2004; Gotoh et al., 2004). Escudero and Ferragut (2005) reported the oviposition rate of the Spanish population to be 2.70 at 25 ± 1°C which is very close to our findings. The second generation showed little higher fecundity as compared to first generation study. The longer exposure to the stable photo-thermal condition as compared to first generation exposure is probably responsible for this variation.

The degree of adaptation of *N. californicus* to the prey species and its efficiency in controlling mite populations vary with the strain of the species and environmental conditions (Helle and Sabelis, 1985). Understanding of the phytoseiid feeding specialization especially regarding the various photo-thermal conditions is vital for the biological control program. The daily consumption rate of *T. urticae* egg of female *A. californicus* (= *N. californicus*) was 13.4 (Gotoh et al. 2004), 16.2 (Ma and Laing, 1973), 17 (Castagnoli and Simoni, 1999) at 25°C. Present study measured the prey consumption rate which ranges from 12.95 to 14.2 at 24°C. Katayama et al. (2006) also observed the amount (11.7) of *T. urticae* egg consumption by adult females of *N. californicus* at 25 ± 1.0 °C. Further, Toyoshima and Hinomoto (2004) indicated that the daily prey consumption with regard to *T. urticae* eggs was 11.4-12.2, which is similar to the present study.

Present results indicated that the Korean *N. californicus* is non-diapausing, and the prey consumption and fecundity were similar to Japanese strain. As *N. californicus* has successfully overwintered in central Japan as well as in southern part, successful overwintering in Jeju citrus orchards (Kawashima and Jung, unpublished) is not surprising. However, overwintering in mainland in Korea is still in question. Further study on cold tolerance, overwintering behavior and special habitat requirement would be needed for implementation of *N. californicus* in Korean mainland crop system.

Table 18. Diapausing ability of *N. californicus* in different strains or populations of the world

Strains/Populations	Latitude	Development conditions	Food	Diapause ability	References
Italian strain	42° 50'N	17°C, 10L:14D	*T. urticae*	71%	Castagnoli et al., 1996
		12L:12D	*T. urticae*	0%	Castagnoli et al., 1996
		10L:14D	Quercus pollen	100%	Castagnoli et al., 1996
		12L:12D	Quercus pollen	60%	Castagnoli et al., 1996
		19°C, 10L:14D	*T. urticae*	84%	Castagnoli et al., 1996
		10L:14D	Quercus pollen	64%	Castagnoli et al., 1996
		12L:12D	Quercus pollen	27%	Castagnoli et al., 1996
		21°C, 10L:14D	*T. urticae*	82%	Castagnoli et al., 1996
		10L:14D	Quercus pollen	54%	Castagnoli et al., 1996
UK strain	51° 32'N	21°C, 08L:16D	*T. urticae*	96%	Jolly, 2000
Spanish strain	40° 00'N	21°C, 08L:16D	*T. urticae*	16%	Jolly, 2000
USA strain	38° 00' N	21°C, 08L:16D	*T. urticae*	0%	Jolly, 2000
Japanese indigenous strain	35° 28' N	15±1°C, 16L:08D	*T. urticae*	0%	Gotoh et al., 2005
Japanese indigenous strain		15±1°C, 08L:16D	*T. urticae*	0%	Gotoh et al., 2005
Commercial strain (Syngenta Bioline, USA)		19°C, 06L:18D	*T. urticae*	0%	Hart et al. 2002
Commercial Spical® strain (Arysta Life Science Co. Ltd.)		15±1°C, 16L:08D 15±1°C, 08L:16D	*T. urticae*	0%	Gotoh et al., 2005
Korean	33° 30' N	15°C (6L, 8L, 10L, 12L) 18°C (6L, 8L, 10L, 12L) 21°C (6L, 8L, 10L, 12L) 24°C (6L, 8L, 10L, 12L)	*T. urticae*	0%	Present study

CHAPTER IV

Cold tolerance of the predatory mite, *Neoseiulus californicus* McGregor compared to *Neoseiulus womersleyi* Schicha (Acari: Phytoseiidae)

Abstract

Phytoseiid mites are economically important as biological control agent of the spider mites. The overwintering strategies of the predatory mites, e.g. diapauses and cold hardness, are vital for the successful biological control of spider mites in field crops such as apple. The population of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is naturally abundant in the warmer Jeju Island but no record in the relatively cooler main domain of Korea. But *Neoseiulus womersleyi* Schicha (Acari: Phytoseiidae) is naturally available in the mainland. So the question is why *N. californicus* is not and *N. womersleyi* is available in the mainland of Korea. Here, we tested the cold tolerance of the two species which might be responsible for the variations in natural occurrence. In the study, the survival and reproduction of *N. californicus* and *N. womersleyi* to sub zero temperatures varies depending on temperature and exposure duration. *N. californicus* survived up to 7th days whereas *N. womersleyi* survived up to 10th days exposed to -6°C. At -9°C, the survival of *N. californicus*, *N. womersleyi*, and diapauses-induced *N. womersleyi* was observed up to 6th, 7th and 10th days, respectively. *N. californicus*, non diapause and diapause *N. womersleyi* survived only the first exposure day while exposed to -12°C. Exposure of adult females to -6, -9 and -12°C for various periods significantly affect the fecundity of the two species. So far, *N. californicus* can survive at all tested subzero temperatures and especially at -12°C they survived more than non-diapausing *N. womersleyi*. These results imply that there is a possibility to imply this native biocontrol agent in the cooler environment of the Korean mainland.

Key words: *Neoseiulus californicus*, *Neoseiulus womersleyi*, cold tolerance, survival, fecundity

Introduction

The Republic of Korea has distinct four seasons with a wet monsoon/summer in the middle of the year, and a cold winter from November to March. The winter temperature (normally -10 to 5°C) hovers around freezing and sometimes it has snow in mainland. The northern and the eastern parts of Korea are subject to heavy snow. This weather condition is critical to survival of poikilothermal arthropods such as phytoseiid mites. On the contrary, the Island of Jeju off the southern coast is the warmest (winter temperature ranges from 6.6 to 10.1°C) place with high humidity (64.8 to 64.6% RH) in the country.

Many overwintering arthropods in temperate zones survive through temperature fluctuations by developing cold hardiness and/or entering diapause (Tauber et al., 1986; Storey and Storey, 1991; Leather et al., 1993). Cold tolerance is characterized as adaptation that enhances survival at low temperatures, and it is thought to be achieved by an array of physiological mechanisms including accumulation of cryoprotectants and production of antifreeze proteins (Zachariassen, 1985). Some studies report that cold tolerance is closely associated with diapause (Wyatt, 1967; Asahina, 1969; de Wilde, 1970; Mansingh, 1974; Tauber et al., 1986; Saunders and Hayward, 1998), whereas others arguing as independent of diapause (Salt, 1961; Baust and Miller, 1970; Nordin et al., 1984; Tanaka, 1997).

Freeze tolerance and freeze intolerance or avoidance are the common strategies for survival at low winter temperatures in terrestrial arthropods (Lee, 1991; Salt, 1961). Most insects and mites are freeze intolerant (Broufas and Koveos, 2001a) and ice formation within their tissues is fatal (Morewood, 1992; Sømme, 1982). However, several freeze intolerant species are killed or fatally injured after a brief and rapid exposure to non-freezing low temperature (Broufas and Koveos, 2001a; Denlinger, 1991; Knight et al., 1986; Lee, 1991; Lee and Denlinger, 1985; Morewood, 1992). Physiological adaptation of cold hardiness is extensively studied in several arthropods (Leather et al., 1993; Lee, 1991) and activated mainly by the onset of low temperature (Baust and Lee, 1982; Lee, 1991) and short photoperiod (Horwath and Duman, 1982).

Mites belonging to the family Phytoseiidae are generally known as important natural enemies of spider mites and insect pests (Helle and Sabelis, 1985). As potential biological control agents, they have been extensively studied during the past few decades with respect to their basic biology and promising application (reviewed in Hoy, 1982; Helle and Sabelis, 1985).

Overwintering mite diversity and their habitats were studied from apple and pear orchards in Korea. Phytoseiid species found in both the orchards were *Neoseiulus (Amblyseius) womersleyi*, *A. makuwa*, *A. orientalis*, *A. rademacheri*, *A. obtuserellus*, and *A. eharai*. Among them, *N. womersleyi* is a dominant position species in both apple and pear orchards (Jung et al., 2003, 2004; Kim et al., 2005). However, *N. californicus* McGregor (Acari: Phytoseiidae) is restricted to the citrus orchards of the Jeju Island (Jung et al., 2006). The discrepancy of the distribution may partly be due to the factors involved in overwintering such as diapause and cold hardiness.

The Jeju population of *N. californicus* does not have diapauses (El Taj and Jung, unpublished) even though the diapause of *N. californicus* varies depending on strains (Castagnoli et al., 1996; Hart et al., 2002; Jolly, 2000). On the other hand, *N. womersleyi* from the Korean main terrain enter diapause in the condition of low temperatures and short photo period (Kim et al., Unpublished). In this study, cold tolerance of *N. californicus* and *N. womersleyi* compared by measuring survival and reproduction after experiencing low temperatures with different exposure times.

Materials and Methods

Source of phytoseiids

In the study, we tested two phytoseiid mites, *N. californicus* and *N. womersleyi*. *N. californicus* was collected from citrus orchards in the Jeju Island, *N. womersleyi* from apple orchard in Andong, Gyeongbuk, Korea in 2006. Both species was maintained according to the method presented in the first chapter.

Cold tolerance

Mated adult females of non-diapausing *N. californicus* (NNC), and diapause-induced and diapause-not-induced *N. womersleyi* (DNW and NNW) were obtained by rearing the mites from eggs to adults and letting mate with males from the same condition at 15°C, 8L: 16D photoperiod for NNC and DNW or 16L: 8D for NNW (Kim et al., unpublished). After allowing the mating for several days (5 to 10), females were placed individually in microtubes (1Ø by 4mm H) with a piece of bean leaf (3 × 0.7 mm) containing mixed stages of *Tetranychus urticae*. The lid of the microtube had a narrow puncture for ventilation and it was covered by fine muslin cloth to prevent the escape of mite. Fifteen microtubes were kept in a plastic container with moist foam and tissue paper at the bottom. Ten plastic containers were prepared for each testing population and kept at 0°C for 7 days under continuous darkness. And then these were transferred to -6, -9 and -12°C incubator (KCL-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) with exposure period of 0, 1, 2, 3, 4, 5, 6, 7, 10, and 12 day. The 0 day treatments of each group were considered as control. After the corresponding exposure, the plastic container was kept at 20°C under a 16L: 8D photoperiod for 24 hours, and checked the survival of the females that could walk. Live females were released individually on a leaf discs and provided with *T. urticae* as food. Fecundity of females was checked at 20°C under 16L: 8D for consecutive 10 days.

Statistical analyses

Data were analyzed by ANOVA using GLM procedure. Mean values were compared by using Tukey's honest significant difference test (Tukey's HSD test). All statistical analyses were performed using the SAS system version 9.1 (SAS Institute, 2005).

Results

Female survival

In control, there was no mortality in *N. californicus* and *N. womersleyi* regardless of diapauses induction. The survival of the phytoseiids at -6°C is shown in figure 18. More than 90% DNW survived up to 12 days of cold exposure. For initial five days of exposure, mortalities were low in NNW and NNC. However, LT_{50} of NNW was longer (ap. 9 days) than NNC (7 days) and the same to LT_{90}. *N. californicus* survived up to 7th days whereas non diapause *N. womersleyi* survived up to 10th days. The survival ability of *N. californicus* was 100% up to 2nd days which is similar to control but significant differences were found for the remaining treated days ($df = 9,140$; $F = 28.08$; $P < 0.001$). On the other hand, NNW showed significant differences as compared to control in all the treated days ($df = 9,140$; $F = 13.91$; $P < 0.001$). Furthermore, 100% DNW female survived upto 7 days of cold exposure and there after decreased in 93.33% which showed no significant differences with the control ($df = 9,140$; $F = 0.89$; $P < 0.537$).

At -9°C, survival was always higher for DNW than NNW or NNC. LT_{50} of DNW was 9 day while for NNW and NNC short were 6 and 5 days, respectively (Figure 19). No mortality was found in *N. californicus* up to second days and then the mortality gradually increased during the following days which were significantly differed from control ($df = 9,140$; $F = 37.07$; $P < 0.001$). Non-diapaused *N. womersleyi* 100% survived 100% on the first exposure day, but after that mortality sharply increased compared to control ($df = 9,140$; $F = 15.61$; $P < 0.001$). In the same temperature of exposure, no individuals of diapaused *N. womersleyi* were died up to third days. The survival of the diapaused *N. womersleyi* started to decline from fourth days and significant difference was found in comparison with the control ($df = 9,140$; $F = 24.07$; $P < 0.001$).

Figure 20 shows the survival ability of the phytoseiids while exposed to -12°C for various durations. At -12°C, all populations were dead after second day of exposure. In the first exposure day, survival was higher for DNW and NNC than NNW. The survival of diapaused *N. womersleyi* was insignificant ($df = 1,28$; $F = 3.50$; $P < 0.072$) but significant differences were found in *N. californicus* and non-diapaused *N. womersleyi* in comparison with control (*N. californicus- df* = 1,28; *F* = 5.09; *P* < 0.032; Non diapause *N. womersleyi* – *df* = 1,28; *F* = 38.50; *P* < 0.001).

Reproduction after exposure to low temperatures

The fecundity occurred in females that survived exposure to -6°C for different periods is shown in figure 21. A significant reduction in the number of eggs laid by NNC was found after second day of exposure compared to the control group (*df* = 9,121; *F* = 81.52; *P* < 0.001). In NNW, the fecundity was significantly different between the control group and the different intervals cold shocked females after first day of exposure (*df* = 8,102; *F* = 31.24; *P* < 0.001). On the other hand, there was no significant difference found in between the control and different cold exposure days in DNW (*df* = 9,140; *F* = 1.55; *P* < 0.136).

As shown in figure 22, conditioning of *N. californicus*, non-diapaused and diapaused *N. womersleyi* females exposed to -9°C resulted in a variable fecundity within the species. *N. californicus* had insignificant effect on fecundity up to the second exposure day but significant results were found with other exposure days while compared to control group (*df* = 9,117; *F* = 57.00; *P* < 0.001). Mean number of eggs laid by non diapause *N. womersleyi* sharply declined at the different exposed periods and showed significant difference with control (*df* = 9,106; *F* = 34.18; *P* < 0.001). In diapaused *N. womersleyi*, all the survived individuals laid eggs during the different periods of exposure. The number of eggs gradually decreased at the time increased. Significant differences were found among the oviposited days with the control group (*df* = 9,118; *F* = 28.19; *P* < 0.001).

At -12°C, the survived non diapause *N. womersleyi* did not lay any eggs whereas *N. californicus* and diapause *N. womersleyi* laid eggs (Figure 23). A significant reduction in fecundity occurred in both the *N. californicus* (*df* = 1,22; *F* = 26.39; *P* < 0.001) and diapause *N. womersleyi* (*df* = 1,24; *F* = 45.27; *P* < 0.001) contrasting with the control.

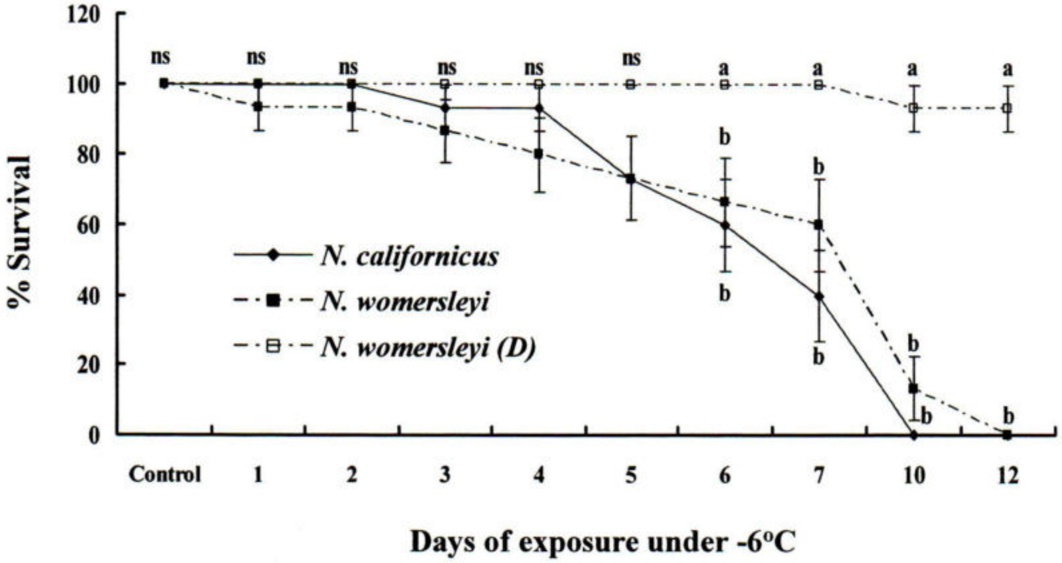

Figure 18. Survival of females of *N. californicus*, diapaused and non-diapaused *N. womersleyi* after exposure to -6°C. Before exposure to -6°C, females were preexposed for 7 days at 0°C under continuous darkness considered as control. Values followed by the same letter are not significantly different at 5% level (Tukey HSD test). ns, not significantly different at 5% level. Numbers of individuals tested were 15 females for each treatment.

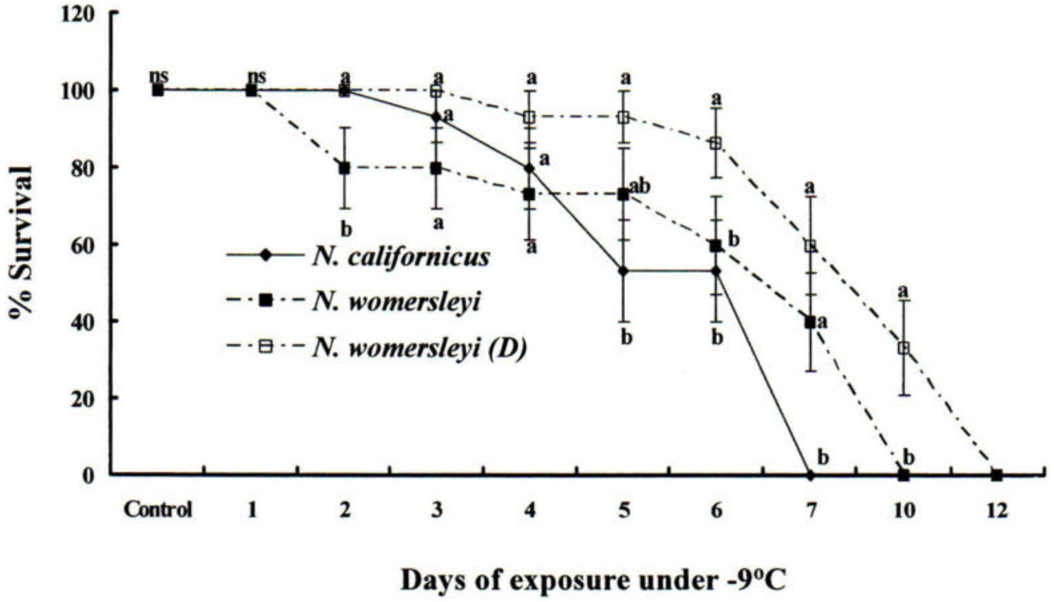

Figure 19. Survival of females of *N. californicus*, diapaused and non-diapaused *N. womersleyi* after exposure to -9°C. Before exposure to -9°C, females were acclimated for 7 days at 0°C under continuous darkness considered as control. Values followed by the same letter are not significantly different at 5% level (Tukey HSD test). ns, not significantly different at 5% level. Numbers of individuals tested were 15 females for each treatment.

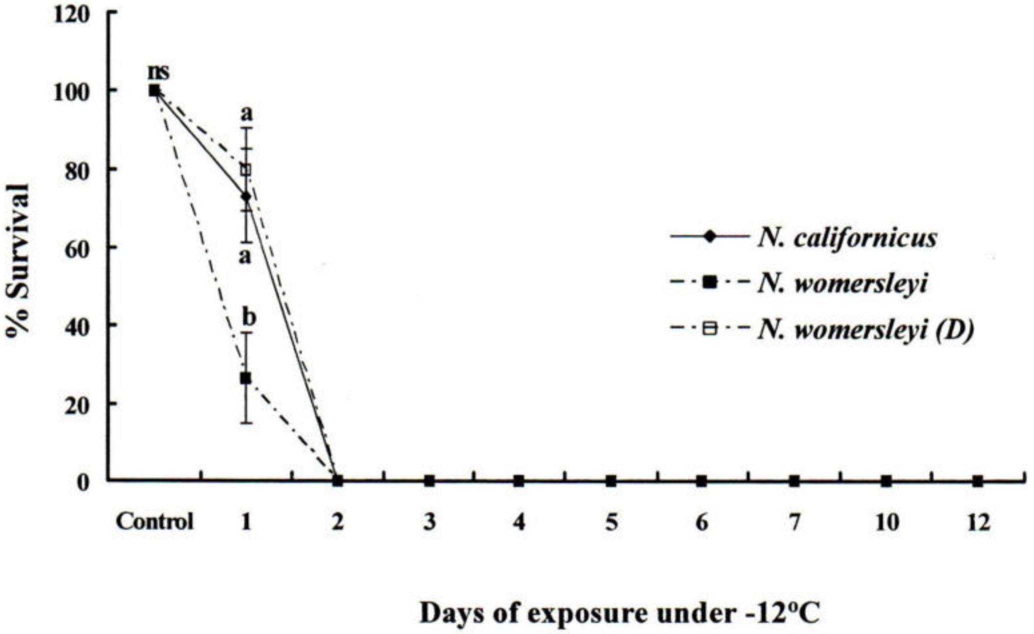

Figure 20. Survival of females of *N. californicus*, diapaused and non-diapaused *N. womersleyi* after exposure to -12°C. Prior exposure to -12°C, females were acclimated for 7 days at 0°C under continuous darkness considered as control. Values followed by the same letter are not significantly different at 5% level (Tukey HSD test). ns, not significantly different at 5% level. Numbers of individuals tested were 15 females for each species.

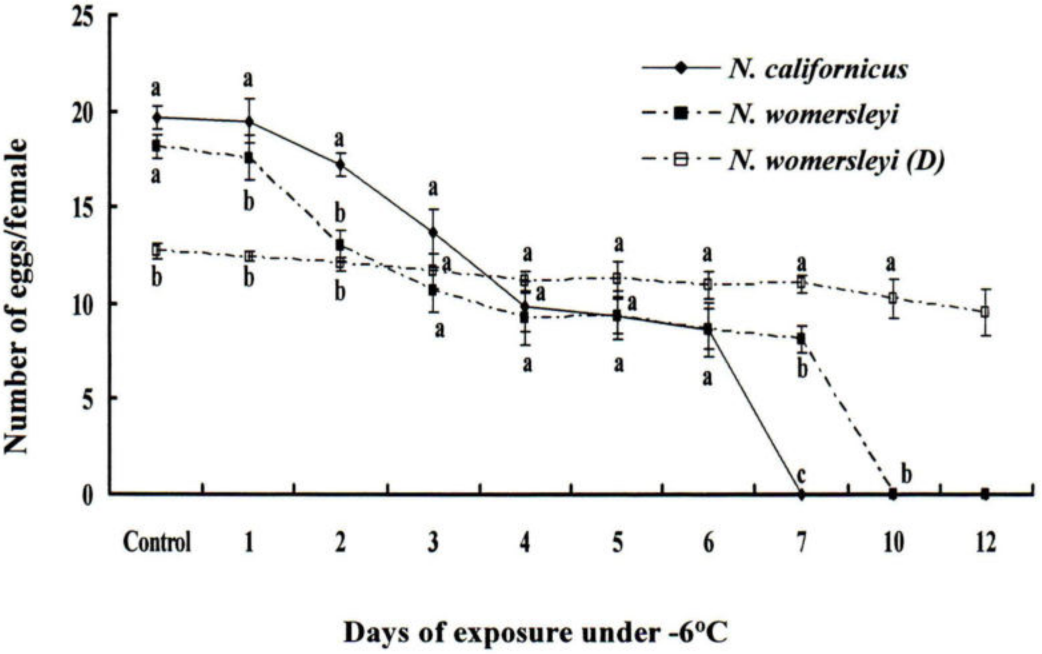

Figure 21. Number of eggs laid by *N. californicus*, and non-diapaused and diapaused *N. womersleyi* at 20°C and L:D of 16:8 for 10 days after exposure to -6°C. Prior exposure to -6°C, females were transferred to 0°C directly from the rearing conditions of 15°C and L:D of 8:16 or 16:8 (Control). Vertical lines on bars are ± SE. Values followed by the same letter are not significantly different at 5% level (Tukey HSD test).

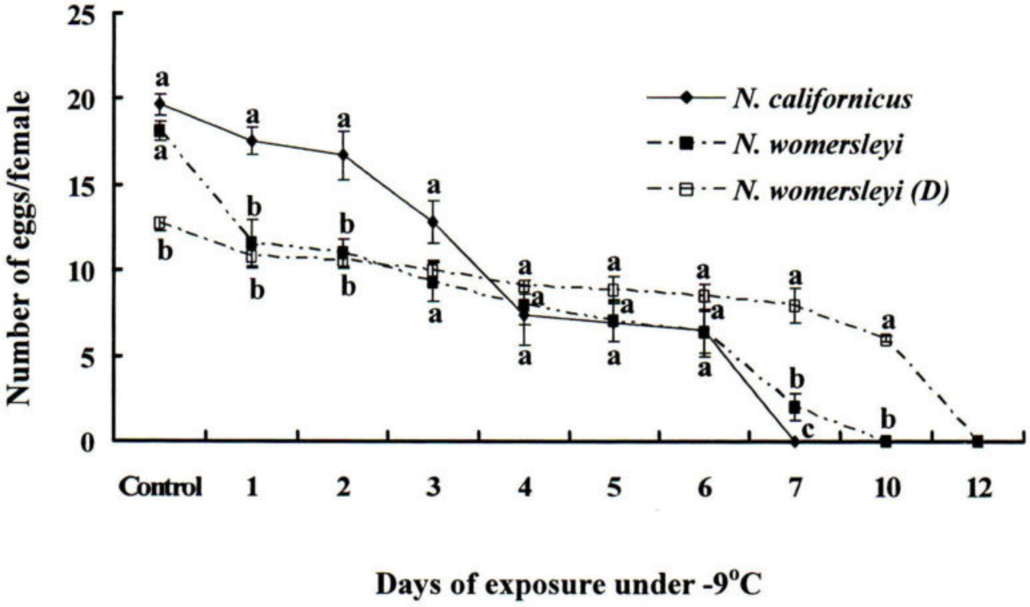

Figure 22. Number of eggs laid by *N. californicus*, and non-diapaused and diapaused *N. womersleyi* at 20°C and L:D of 16:8 for 10 days after exposure to -9°C temperature. Prior exposure to -9°C, females were transferred to 0°C directly from the rearing conditions of 15°C and L:D of 8:16 or 16:8 (Control). Vertical lines on bars are ± SE. Values followed by the same letter are not significantly different at 5% level (Tukey HSD test).

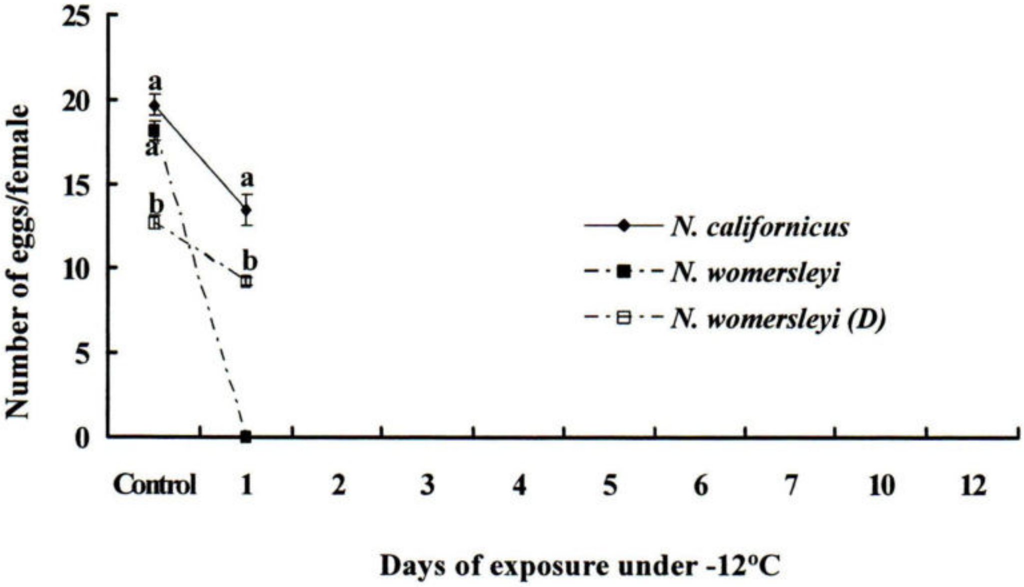

Figure 23. Number of eggs laid by *N. californicus* and, non-diapaused and diapaused *N. womersleyi* at 20°C and L:D of 16:8 for 10 days after exposure to -12°C. Prior exposure to -12°C, females were transferred to 0°C directly from the rearing conditions of 15°C and L:D of 8:16 or 16:8 (Control). Vertical lines on bars are ± SE. Values followed by the same letter are not significantly different at 5% level (Tukey HSD test).

Discussion

This study compared the cold tolerance of non-diapuasing population of *Neoseiulus californicus* and diapauses-induced or not-induced *N. womersleyi.* Results showed that the survival of *N. californicus* was lower than diapause-induced *N. womersleyi*, but similar or higher than diapause-not-induced *N. womersleyi* in sub-zero temperature. Especially in extremely low temperature of -12°C, survival of *N. californicus* was as high as of diapause-induced *N. womersleyi*, and superior than diapause-not-induced *N. womersleyi*. Gotoh et al. (2005) observed the similar survival attitude in between the *N. californicus* and non diapause *N. womersleyi* (up to 5 days) when exposed to -5°C. Additionally, the fecundity of *N. californicus* was higher than non diapause *N. womersleyi* up to 6th days and then the non diapause *N. womersleyi* laid more eggs than *N. californicus*. It means that the *N. womersleyi* is more cold tolerant than *N. californicus* at -6°C temperature.

Diapausing *N. womersleyi* adult females also exhibit higher survival than non diapausing ones, when exposed to -6, -9 and -12°C temperatures. At -6 and -9°C, DNW also survived for longer period than NNC and NNW. Gotoh et al. (2005) also reported the more and longer survival ability of DNW than *N. californicus* and non diapausing *N. womersleyi* at -5°C. Broufas and Koveos (2001a) also reported the cold hardiness of *Euseius finlandicus* was conspicuously different between diapause and non diapause females: most diapaused females survived exposure at -4°C for 11 days, while most non-diapaused ones died after 1-2 days of exposure at the temperature. In addition to that when predatory mites exposed to -9°C, the fecundity of DNW was higher than NNW after second days of exposure and in respect of *N. californicus* they laid more eggs from the fourth day. On the contrary, non-diapaused *N. womersleyi* did not lay any eggs at -12°C, whereas diapaused *N. womersleyi* laid eggs but the number was fewer than *N. californicus*. However, this study revealed that diapause-destined *N. womersleyi* adults were also more cold tolerant than non diapause-destined adults. In conclusion, the cold hardiness in *N. womersleyi* is associated with reproductive diapause for enhancing the survival in winter. Thus, increased cold tolerance can be linked to the diapausing state.

However, to understand the exact relationship between diapause and cold tolerance, when diapause is induced, maintained and terminated needs to be clarified. During the winter, mean monthly temperatures in the mainland Korea ranged from -10 to 5°C and *N. womersleyi* could overwinter successfully in the field condition by taking shelter on ground vegetation as a diapausing state (Kim et al., Unpublished). On the contrary, the southern island of Korea (Jeju) is warmer where temperature ranges from 6.6 to 10.1°C at which the studied strain of *N. californicus* has been recorded. It was found that *N. californicus* overwinter mostly under ground vegetation in Jeju citrus orchard but not in diapause state (Kawashima and Jung, unpublished). Hart et al. (2002) mentioned that the US strain of *N. californicus* survived over three months without entering diapause outdoors in winter under sheltered condition in UK, where the temperature did not fall below 0°C. However, it is interesting that the present study resulted the non diapausing *N. californicus* can survive at all treated sub zero temperatures though the duration is shorter than diapausing and non diapausing *N. womersleyi* and especially at -12°C they survived more than non-diapause *N. womersleyi*. This may imply that in extreme low temperature, *N. womersleyi* may get benefit by evolving diapause while *N. californicus* may have some tolerance. So, considering the cold tolerance of the Korean *N. californicus* strain against the wide range of sub zero temperatures, there is a possibility to survival of this native bio-control agent under sheltered condition of the mainland. Further study is needed to verify the possibility to establish the predator in the natural habitat of the mainland of Korea.

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GENERAL DISCUSSION

The possibility of the species, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) naturally occurred in the southern island of Korea, Jeju, this study investigated its potential as a biocontrol agent of the European red mite (*P. ulmi*) in mainland apple orchards including successful overwintering possibility. There has been a considerable amount published on *N. californicus* with *T. urticae* as the prey species. However, there is little literature concerning predation of *P. ulmi*, although field experiments in Spain and Chile have suggested it does consume this species (Costa-Comelles et al., 1994; Gonzalez, 1971).

If *N. californicus* population were to increase in Korean apple orchards then their ability to control pest mites would be important. Given that the population of *N. californicus* isolated from Korea develops successfully on *P. ulmi* and the rate of development is faster at all temperatures as compared to Japanese indigenous strain developed on mixed stages of *T. urticae* studied by Canlas et al. (2006). It could be suggested that *N. californicus* would be as good a candidate for biocontrol of these two pest species. Further work will be required to determine the efficiency of this predator in the orchards to control of phytophagous mites. In the second chapter, we examined environmental factors such as humidity and temperature on developmental and reproduction performances of *N. californicus* with comparison to *N. womersleyi* and *P. persimilis*. The result showed that *N. californicus* was more tolerant to temperature and humidity than *N. womersleyi* and *P. persimilis*. *N. womersleyi* was more susceptible to humidity and *P. persimilis* was susceptible to high temperature. Even though the reproductive capacity of *P. persimilis* was higher than *N. californicus* but in view of environmental tolerance *N. californicus* could be better to adapt themselves in a wide range of abiotic conditions. This characteristic of *N. californicus* is helpful to establish this predator in the orchard system.

The potential for *N. californicus* to overwinter in the Korea has implications for policy on future introduction as a biocontrol agent. Probably more critical winter conditions of Korean main land than the Jeju Island is responsible for the restricted distribution of this predator only in the Jeju Island. Kawashima and Jung (unpublished) reported that *N. californicus* overwinter mostly under ground vegetation in Jeju citrus orchard as non diapause state. In fact, the establishment of this naturally occurred predator in the main land is of prime importance. The ability to diapause is probably a requirement for successful overwintering in the Korean main domain. In the present study (3rd chapter), *N. californicus* from the Jeju citrus orchards did not enter diapause when exposed to lower temperature and short day length.

However, Hart et al. (2002) mentioned that the US strain of *N. californicus* survived over three months without entering diapause outdoors in winter under sheltered condition in UK, where the temperature did not fall below 0°C. So, the cold tolerance experiment (4th chapter) was deemed as the non-diapausing *N. californicus* can be overwinter successfully or not. The result showed that the non diapausing *N. californicus* can survive at all tested sub zero temperatures though the duration is shorter than diapausing and non diapausing *N. womersleyi* and especially at -12°C they survived more than non-diapause *N. womersleyi*. This may imply that in extreme low temperature, *N. womersleyi* may get benefit by evolving diapause while *N. californicus* may have some tolerance. So, considering the cold tolerance of the Korean *N. californicus* strain against the wide range of sub zero temperatures, there is a possibility to survival of this native bio-control agent under sheltered condition of the mainland. Further study is needed to verify the possibility to establish the predator in the natural habitat of the mainland of Korea. On the basis of this initial investigation, it thus appears likely that the *N. californicus* found to be surviving in overall Korea originated from the Jeju Island.

BIOLOGICAL CHARACTERS OF KOREAN *NEOSEIULUS CALIFORNICUS* MCGREGOR (ACARI: PHYTOSEIIDAE) IN RELATION TO ENVIRONMENTAL PARAMETERS

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Abstract

Spider mite is one of the most important pests of apple in Korea. Two spotted spider mite and European red mite are the most common in apple and they are in competition. Recently European red mite is becoming dominant over two spotted spider mite in Korean apple orchards. As the acaricide application has been the most option employed to control spider mites and as wide range of acaricide resistances have been the concerns, alternative control options are in demand. Biological control of spider mites by predatory mites has been tried on a wide range of crops including apples. This study examined the feasibility of using a Korean isolate *Neoseiulus californicus (*Acari: Phytoseiidae) as a potential biological control agent in apple orchards in the mainland Korea. The distribution of this naturally occurring predatory mite is restricted only to the southern, warmer island, Jeju but not in the relatively cooler main domain of Korea. Scanty of information is available on the biological characters and overwintering phenology of this native predator. Temperature effect on life history parameters of *N. californicus* using *Panonychus ulmi* was experimented to evaluate the suitability of this predatory mite for controlling apple mites. The results specified that the developmental time is decreased with increasing temperature between 15 and 30°C. At 25°C, females laid maximum number of eggs. The highest net reproductive rate (R_0 = 49.24) and the intrinsic rate of natural increase (r_m = 0.29) were found at 25 and 30°C, respectively. Environmental tolerance of *Neoseiulus californicus* was compared to the native dominant phytoseiid, *Neoseiulus womersleyi* and the introduced, most widely used phytoseiid, *Phytoseiulus persimilis* (Phytoseiidae) by treating combination of temperatures and relative humidity for egg hatching, immature survival, and adult female performance. Korean *N. californicus* was most tolerant to high temperature and low humidity, *N. womersleyi* was more humid susceptible and *P. persimilis* showed high temperature susceptible with high reproductive ability. While food limited, cannibalism was higher in *N. californicus* and they may get benefit for their survival. Overwintering phenology was studied with a view to establish this predator in the lion cooler part of Korea. As a part of overwintering phenomena, diapause propensity of the native predator was measured and diapause induction was not found in *N. californicus* isolated from Korea. Cold tolerance of the Korean *N. californicus* was also measured as the non-diapausing population can survive up to certain limit of cold temperatures for a certain period. The survivorship of this predator at all subzero temperatures could assume the possibility to establish in the main domain.

Key words: *Neoseiulus californicus*; *Neoseiulus womersleyi*; *Phytoseiulus persimilis*; *Panonychus ulmi*; temperature; relative humidity; development; fecundity; life table; immature and adult mortality; cannibalism; overwintering; diapause; cold tolerance

한국산 *Neoseiulus californicus* McGregor (Acari: Phytoseiidae)의 환경요인에 따른 생물학적 특성

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초록

잎응애류는 한국 사과에서 가장 중요한 해충들 중 하나이며, 최근 한국의 사과과원에서는 점박이응애보다 사과응애가 주요해충으로 바뀌는 양상을 보여준다. 살비제 살포가 가장 일반적인 방제 방법이나, 지나친 의존은 살비제 저항성, 친환경 농업에서의 거부감 등의 문제를 야기하여 온 바, 새로운 방제체계가 요구되고 있다. 하나의 대안으로 포식응애를 이용한 생물적 방제가 사과를 포함한 여러 작물에서 시도 되어왔다. 이 연구는 제주도에 널리 분포하는 *Neoseiulus californicus* McGregor (Acari: Phytoseiidae)의 한국본토 사과과원에서 생물학적 방제원으로서 이용 가능성을 평가하였다. 이 포식응애의 국내 자연 발생분포는 오직 제주도로 제한적이며 비교적 추운 본토에서 발생은 확인되지 않았다. 따라서 본 계통에 대한 다양한 온습도 환경에서의 생물적 특성과 월동 휴면 여부를 연구하였다.

사과의 주요 해충인 사과응애를 먹이로 한, *N. californicus* 의 온도 발육 실험 결과, 15~30℃의 범위 내에서 온도에 따른 발육시간은 온도가 증가할수록 발육시간이 줄어드는 결과를 보여주었다. 그리고 암컷의 산란수는 25℃에서 가장 많았다. 순증식율(R_0)은 25℃에서 49.24 로 가장 높았고 내적자연증가율(r_m)은 30℃에서 0.29 로 가장 높았다.

기상 환경 중 포식성 이리응애에 가장 영향을 많이 주는 온도와 상대습도에 대한 알, 유충, 성충의 적합도로 평가된 환경내성은 *N. californicus* 와

토착우점 이리응애인 *Neoseiulus womersleyi* Schicha 와 상업적으로 가장 널리 사용되는 *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) 와 비교하였다. 한정된 먹이 조건에서 동종포식은 *N. californicus* 더 높았으며 이러한 점은 그들의 생존율을 높이는 이점이 있는 것으로 생각된다. *N. californicus* 는 높은 온도와 낮은 습도에서 가장 높은 내성을 보였으며, *N. womersleyi* 는 습도가 높은 조건에서 *P. persimilis* 는 높은 온도조건에서 높은 산란능력을 보였다. 본 계통을 대상으로 한 월동을 위한 휴면여부를 조사한 결과, 국내에서 발견된 *N. californicus* 는 휴면을 하지 않는 것으로 조사되었다. 비휴면 개체군인 한국산 *N. californicus* 의 내한성에 대한 연구에서 일정 한계의 낮은 온도에서 일정 기간 동안 생존할 수 있는 것으로 조사되었다. 영하의 온도에서 이 포식응애의 생존력은 한국 본토에서 정착할 수 있는 가능성을 보여준다.

키워드: 한국산 *Neoseiulus californicus*; *Neoseiulus, Panonychus ulmi*; 온도; 상대습도; 산란수; 생명표; 약충과 성충의 사망률; 동종포식; 월동; 휴면; 내한성