Biological performance, response and population dynamics of *Tetranychus evansi* (Acari: Tetranychidae), as influenced by different African nightshade (Solanales: Solanaceae) species

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DECLARATION

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This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

This thesis is dedicated with deepest appreciation to my son Jonathan and my husband George for their love, generosity and support that was always my inspiration during the many months of hard work.

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LIST OF ABBREVIATIONS AND ACRONYMS

AVRDC:	The World Vegetable Centre
ANOVA:	Analysis of Variance
CB & ID	Capacity Building and Institutional Development
DAAD	German Academic Exchange Service
DRIP	Dissertation of Research Internship Program
GC-MS:	Gas Chromatography-Mass Spectrometer
GLV's:	Green Leaf Volatiles
HPR:	Host Plant Resistance
icipe	International Centre of Insect Physiology and Ecology
IPM:	Integrated Pest Management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
Proc GLM:	General linear Model procedure
SAS:	Statistical Analysis Software

ABSTRACT

Despite the vast research on trichome-based resistance as well as plant chemical factors in plant-herbivore interactions in Solanaceous plants, little or no information is available on the association between *Tetranychus evansi* Baker and Pritchard and these mechanisms in African nightshades. The current study which was carried out from December, 2006 to March, 2009 focused on five African nightshade species viz. *Solanum sarrachoides* Sendter, *S. villosum* Mill, *S. tarderemotum* Bitter, *S. americanum* Mill and *S. scabrum* Mill that were evaluated for their resistance to *T. evansi*. The objective was to investigate several host plant characteristics of the five *Solanum* spp and their influence on developmental duration, oviposition, survival, intrinsic population growth rate and other life history characteristics of *T. evansi*. To achieve this objective, the study concentrated on four major areas (i) Effect of five *Solanum* spp on biology and life table parameters of *T. evansi* (ii) Evaluating the effects of morphological and chemical factors in five *Solanum* spp to mite fecundity, repellency and olfactory responses (iii) Population dynamics of *T. evansi* on five *Solanum* spp under greenhouse and field conditions and (iv) Effect of *T. evansi* feeding on growth and yield of five *Solanum* spp grown under field conditions. The results indicated that *S. villosum, S. scabrum*, *S. tarderemotum* and *S. americanum* are the most susceptible to *T. evansi* due to the shorter adult developmental period, longer adult longevity, higher reproduction and intrinsic rate of natural increase ranging between 0.180 - 0.196 females/female/day compared with *S. sarrachoides* which cannot support *T. evansi* populations as the r_m was

negative on this host. Differences in developmental time and life table parameters among the other host plants were also not significant. Five different trichome types were identified among the *Solanum* spp with the glandular types predominant in *S. sarrachoides*. There was a significant negative correlation between fecundity (R = -0.649; P = 0.0019) and distance traveled by mites after every 15 min interval with the density of glandular trichomes. Significantly fewer eggs that decreased with the age of the plant were laid on *S. sarrachoides* in comparison to other *Solanum* spp. Distance traveled by mites was also significantly low in this species indicating that higher densities of glandular trichomes decreased distances walked by mites. In olfactometer bioassays, significantly more females responded to volatiles from intact plants of *S. villosum* than those from other *Solanum* spp. Based on mite fecundity and behavioral response studies, intact plants of two species, *S. sarrachoides* and *S. villosum* were selected for volatile chemical analysis. GC-MS analysis and comparison with authentic standards identified volatile compounds as belonging to the classes of terpenoids, esters, aldehydes, ketones and green leaf alcohols. Quantification of these compounds revealed that except for the ketones, other compounds were significantly higher in *S. sarrachoides* than in *S. villosum*. Population densities of *T. evansi* in screenhouse studies revealed that *S. scabrum* was highly infested by *T. evansi* but the percentage leaf area damaged was very low in comparison to other *Solanum* spp. The highest level of resistance was observed in *S. sarrachoides* where *T. evansi* populations significantly remained low. Field studies revealed significant differences in number of motile individuals of *T. evansi* among the acaricide free and acaricide protected plots.

Significant differences were also found on growth and yield among nightshade species in acaricide free and acaricide protected plots over time and space. Significant seasonal variation in yields of respective nightshade species was detected. Yields in both seasons were negatively correlated to leaf area damaged by *T. evansi* in acaricide free plots. Based on population dynamics findings, *S. sarrachoides* did not support any mite populations over time. However, *S. scabrum* supported high mite populations but no significant reduction in growth and yield was detected. In conclusion, *Solanum americanum*, *S. villosum*, *S. tarderemotum* are suitable host plants for *T. evansi* and severe mite outbreaks are likely to occur under favorable conditions in the field. Since *S. sarrachoides* and *S. scabrum* seem to possess resistance and tolerance attributes respectively to *T. evansi*, these attributes can be investigated further and utilized in programs such as breeding and IPM in order to develop resistant genotypes and reduce *T. evansi* populations in African nightshades.

Key words—Tomato spider mite, *Solanum* spp., host plant resistance, biological parameters, demographic parameters, population dynamics, bioassays

CHAPTER ONE

Introduction

1.1: Background

Many spider mites are serious pests of vegetable and ornamental crops grown under protected structures and in the field (Walter and Proctor, 1999; Gerson *et al*., 2003). When environmental conditions are favorable especially hot and dry weather, spider mite populations multiply fast (Huffacker *et al*., 1969). The nymphs and adults colonize plants and feed on the younger leaves rather than on the tougher lower and older leaves (Leite *et al*., 2003) with their piercing-sucking mouthparts. Under severe infestation, leaves become scorched, fall off prematurely and the plants even die (Smith Meyer, 1996; ICIPE, 2004). Many spider mite species have the ability to produce webbing which is highly evident during severe infestations and protects them against natural enemies and environmental stress (Gerson, 1985).

The two-spotted spider mite *Tetranychus urticae* Koch, is one of the most known and important species in the family Tetranychidae (Pritchard and Baker, 1955; Bolland *et al*., 1998) being common in outdoor and glass house crops in tropical and temperate zones (van de Vrie, 1985; Kennedy and Storer, 2000). *T. urticae* attacks a wide range of garden plants including ornamentals, fruit crops, and vegetables and feeds on more than 200 economically important host plants throughout the world (Bolland *et al*., 1998; Migeon and Dorkeld, 2006). Development from egg to adult can take 10 days at room

temperature, with each female laying up to 100 eggs in its lifetime, allowing its population to build rapidly (Mitchell, 1973).

Another spider mite species which has become one of the most destructive on vegetables and other crops worldwide is the tomato spider mite, *Tetranychus evansi* Baker and Pritchard (Escudero and Ferragut, 2005). *T. evansi* is probably of South American origin (Gutierrez and Etienne, 1986) and was first recorded in Africa in Zimbabwe in 1979 (Blair, 1983) from where it spread northwards (Knapp, 2003a). The tomato spider mite attacks Solanaceous crops such as nightshade, tomato, eggplant, pepper, potato and tobacco (Moraes and McMurtry, 1987; Rosa *et al*., 2005; Fiaboe *et al*., 2006). It has also been found feeding on other crops such as beans, citrus, cotton, castor bean, roses and on many weed species e.g. *Amaranthus*, *Chenopodium*, *Convolvulus*, *Conyza*, *Diplotaxis*, *Hordeum*, *Lavatera* and *Sonchus* (Moraes *et al*., 1987). In eastern and southern Africa, *T. evansi* is considered as the most important dry season pest of tomato and causes severe damage and loss in tomato fields (Knapp *et al*., 2003a, b; Saunyama and Knapp, 2003). When left uncontrolled a farmer can lose the crop within a week (Keizer and Zuurbier, 2001).

African nightshades (Solanales: Solanaceae) are among the top priority African indigenous vegetables that have been earmarked for promotion through further research and improvement because of their role in improving the nutritional and economic status of marginalized and nutritionally vulnerable populations in Africa (Guarino, 1997;

Schippers, 2000). Although they have for long been considered as inedible poisonous plants and troublesome agronomic weeds (Schilling and Andersen, 1989; Edmonds and Chweya, 1997), nightshades have for long been used in western, eastern and southern Africa as well as India, Indonesia and China, as a source of leafy herbs and vegetables, fruits and dye, and for various medicinal purposes (Onyango, 1993; Schippers, 2000).

The species of nightshades utilized by some rural communities in Kenya include *Solanum villosum* M. and *S. sarrachoides* Sendtn (Edmonds and Chweya, 1997; Schippers, 2000). However, there are also other species of nightshades consumed in East, West and Central Africa which include *Solanum scabrum* M., *S. americanum* M., and *S. tarderemotum* (Maundu *et al*., 1999; Onyango, 2007). For example, in East Africa, the demand for *S. scabrum* commonly referred to as the broad leafed nightshade has increased tremendously due to its large leaves that result to higher leaf yields compared to other species (Onyango, 2007).

Although African nightshades are alleged to be tolerant to pests and diseases (Maundu *et al*., 1999), like other vegetables they are susceptible to common herbivorous pests such as the bean aphids (*Aphis fabae* Scop.) (Edmonds and Chweya, 1999), variegated elephant grasshoppers (*Zonocerus* sp.) (Schippers, 2000) and beetles (*Lagria* sp., *Podagrica* sp., *Epilachna* sp.) (Epenhuijsen, 1974; Fontem and Schippers, 2004). *Tetranychus evansi* was first reported on nightshade plants in Mauritius (Moutia, 1958) and in 1965 in California (Harper, 1966, Oatman *et al*., 1967). Recently, it has been

found on nightshades in Italy (Castagnoli, 2006); France (Migeon, 2005); Brazil (Rosa *et al*., 2005); Greece (Tsagkarakou, 2007) and in several localities in Kenya and Tanzania. Field observations in Benin also showed that nightshades and amaranths are both susceptible to attacks by tetranychid mites (Adango, 2006). In addition, other important pests of greenhouse and field grown African nightshades include white flies, thrips, aphids, leaf miners and birds.

In order to manage spider mites, small holder African farmers have mainly relied on frequent application of synthetic acaricides (Saunyama and Knapp, 2003). However, the excessive and inappropriate use of acaricides has been related to environmental and health problems, affecting non-target organisms, and promoting the rapid development of spider mite resistance to acaricides (James and Price, 2002; Herron, 2003).

For instance, soon after *T. evansi* was recorded as a new pest of tobacco in Zimbabwe, it developed resistance to thiophosphate acaricides due to frequent application of these acaricides (Blair, 1989). In addition, use of lower dosages than the recommended rates by manufacturers, combined with lack of proper application techniques has facilitated the acquisition of pest status by populations of this species (Sibanda *et al*., 2000; Saunyama and Knapp, 2003). This has left many smallholder farmers with minimal or no alternatives considering that unlike other closely related spider mite species, biological control of *T. evansi* with predatory mites such as *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor is not effective (Moraes and

McMurtry, 1985 a, b; Escudero and Ferragut, 2005). However, recently a Brazilian strain of *Phytoseiulus longipes* Evans was identified as a promising biological control agent for *T. evansi* (Furtado *et al*., 2007) and it is being reared under quarantine conditions at *icipe* and field tests are planned.

There is therefore an increased demand for alternative pest control strategies that are sustainable and environmentally friendly. Several cultural techniques such as crop rotation, proper field sanitation and reducing planting distance can lower spider mite populations (Tindall, 1983; Saunyama and Knapp, 2003). For instance in Zimbabwe, pruning and trellising of tomatoes was reported to increase yields and profit in high potential tomato growing areas associated with use of acaricides (Saunyama and Knapp, 2003). The use of host plant resistance (HPR) constitutes an alternative environmentally friendly method for control of herbivorous arthropod pests. Efforts to develop cultivars with increased levels of pest resistance are an important component for Integrated Pest Management (IPM) programs leading to reduced overdependence on acaricides for management of spider mites. Research involving the development and use of arthropod resistant cultivars has led to significant food production, alleviation of poverty and hunger and improved human nutrition in the major food producing areas of the world in the past (Khush, 1995). Since small scale farmers in many parts of Africa utilize few external inputs in food production, pest resistant cultivars would be economical since the level of resistance is genetically incorporated in the seed and does not need any additional input (Ampofo, 1995).

When *T. evansi* was found in Kenya for the first time in 2001 and identified as a serious pest of tomato (Knapp, 2002), one of the initial steps was to screen several tomato accessions for resistance to the invasive pest. There are three known major categories of resistance (Horber, 1980) originally described by Painter, (1951) as mechanisms of resistance. These categories include; antixenosis (Kogan and Ortman, 1978) which defines a group of plant characters and arthropod responses that lead to a plant being less damaged than another plant lacking these characters and the arthropod responses to them; antibiosis which defines those plant characteristics that adversely affect the physiology of the feeding arthropod such as reduced fecundity, decreased size, reduced longevity and increased mortality; tolerance which defines an ability of a resistant plant to grow and reproduce in spite of supporting a pest population almost equal to that damaging a susceptible host (Schoonhoven *et al*., 1998; Smith, 2005).

Both antixenosis and antibiosis, denote the presence of morphological plant factors such as trichomes, thickened plant epidermal layers, waxy deposits on leaves, stems or fruits and chemical plant factors such as volatiles and essential oils that adversely alter the behavior of an arthropod resulting in the selection of an alternate host plant (Kennedy, 2003; Smith, 2005). For instance, it was earlier reported that plant resistance to spider mites may be related either to physical leaf attributes such as trichomes or other characteristics (Weston *et al*., 1989) or biochemically-based host plant interactions that affect mite feeding and fecundity (Hesk *et al*., 1991; Elliger and Waiss, 1991). On the other hand, tolerance is distinct from antixenosis and antibiosis (Panda and Heinrichs,

1983) in that it is an adaptive mechanism for the survival of the plant and is more or less independent on the effect of the feeding arthropod (Panda and Khush, 1995). However, tolerance is difficult to measure since it requires simultaneous observations of pest populations and yield potential of mature plants (Frazier and Hanson, 1986).

Trichome-based resistance as a host plant resistance mechanism has attracted much interest in plant-herbivore interactions. Although little or no information is available on the association between *T. evansi* and trichomes in nightshades, several studies have been carried out to establish the role of different trichomes as described by Luckwill (1943) in resistance to spider mites in other Solanaceous plants especially the genus *Lycopersicon*. For instance, glandular trichomes of *Lycopersicon* are known to have 'heads' that release on contact with pests sticky and/or toxic exudates that entrap, irritate and potentially kill the pest by inhibiting feeding, oviposition and larval development while non-glandular trichomes have no 'heads' and affect small arthropods such as spider mites by mechanical means i.e. can act as barrier to movement or access to nutritional tissue (Duffey, 1986; Simmons and Gurr, 2005). For example, the wild tomato species *Lycopersicon hirsutum* f. *glabratum* C. H. Muller has been reported to confer resistance in spider mites (Gonçalves *et al*., 1998; Maluf *et al*., 2001; Resende, 2003). In addition, higher densities of glandular trichomes were reported to decrease distances walked by *T. urticae* females onto the leaf surface in several tomato genotypes (Maluf *et al*., 2007).

Plant chemical factors have also received a lot of attention considering that completion of an arthropods life cycle depends on the ability of the female parent to locate and oviposit on or near a plant that can supply the necessary nutrients for larval growth and development (Juvik *et al*., 1988). Adult females of *T. evansi* reach potential host plants either by random walking and at some point make a choice as demonstrated in olfactometer bioassays with various accessions of tomato (Murungi *et al.*, 2009) or by passive dispersal by wind as tested with *T. urticae* females by Brandenburg and Kennedy (1982) and by Kennedy and Smitley (1985).

The quality of the host plant selected can affect several life-history characteristics by impairing growth and reducing fecundity (Price *et al*., 1980) thus altering population growth (Sabelis, 1985; Walde, 1995). Volatile plant chemicals that affect the olfactory chemoreceptors of the arthropod have been implicated as agents mediating the process of host-plant selection (Kennedy, 1977). For example, the 'green-leaf' volatiles (GLV's) from potato plants were reported by Visser and Avé (1978) to elicit a positive anemotactic response in Colorado potato beetles while Avdiushko *et al*., (1997) reported the GLV's found in tobacco to reduce fecundity in spider mites.

Therefore, a full knowledge of the morphological as well as chemical composition of different African nightshade species and their effect on development, life history characteristics and population dynamics of *T. evansi* has the potential to make host plant resistance a more sustainable approach in management of these spider mites.

1.2: Hypotheses

1. Developmental duration and life history characteristics of *T. evansi* are not affected by different species of African nightshade
2. Reproduction, repellence and olfactory responses of *T. evansi* are not influenced by trichome types, density and volatile chemicals of different African nightshade species
3. The population dynamics of *T. evansi* do not vary on different African nightshade species under greenhouse and field conditions
4. The tomato spider mite, *T. evansi,* does not cause yield and growth reduction in African nightshades

1.3: Objectives

1.3.1: General objective

The general objective of this study was to determine host plant resistance mechanisms in various African nightshade species that can be utilized in management of the economically important tomato red spider mite species, *T. evansi*

1.3.2: Specific objectives

Specific objectives of this study were as follows:

1. To evaluate developmental duration and life history characteristics of *T. evansi* on African nightshade
2. To establish the effect of trichomes and volatile chemicals of African nightshades on reproduction, repellence and olfactory responses of *T. evansi*
3. To determine the population dynamics of *T. evansi* in African nightshade under greenhouse and field conditions
4. To establish the effect of *T. evansi* feeding on growth and yield of African nightshades

1.4: Justification

The tomato spider mite is highly polyphagous and is considered one of the most serious pest of vegetables and other crops worldwide (Escudero and Ferragut, 2005) posing a threat to food security in many economies. The central objective of the Kenya national food security policy as stated in the sessional paper number 4 of 1981 was to ensure adequate supply of nutritionally balanced food available in all parts of the country (Anon, 1981). In order to achieve this objective, the policy advocates use of indigenous food sources (MOA, 1989, 1998). The Food and Agriculture Organization of the United Nations monitoring hunger reduction targets of the World Food Summit (WFS) and Millennium Development Goals (MDG) indicated that 32% of Kenyan population had a high level of undernourishment in 2003 – 2005 with a food deficit for undernourished people of 250kcal/person/day (FAO stat, 2008; 2009). This is one of the recent statistics available regarding the depth of hunger and food security status in Kenya where over 50% of people especially in rural communities have been reported to be living below the poverty line (AICAD, 2003).

Although African nightshades are alleged to be resistant to common pests and diseases, they have been found severely infested by *T. evansi* and other tetranychid species in some countries in East (Oluoch, AVRDC pers. comm.) and West Africa (Adango, 2006). In order to manage these spider mites, many small holder African farmers rely mainly on frequent application of synthetic acaricides. The excessive and inappropriate use of acaricides has not only been related to environmental and health problems but has

also affected non-target organisms, and promoted the rapid development of spider mite resistance to acaricides (Herron *et al*., 2004). Therefore, there is growing public concern over the impact of chemicals on the environment and the greater number of cases of pest resurgence, secondary pest outbreaks and increasing frequency of resistance of arthropods to pesticides (Flexner *et al*., 1995). Since host plant resistance constitutes an alternative environmentally friendly method for control of arthropod pests, efforts to develop African nightshade cultivars with increased levels of arthropod resistance is an important component for IPM programs.

Despite the vast research on trichome-based resistance as well as plant chemical factors in plant-herbivore interactions in Solanaceous plants (Simmons and Gurr, 2005), limited information is available on the association between *T. evansi* and these mechanisms in African nightshades. The current study seeks to investigate several host plant characteristics of different African nightshades that are commonly grown and consumed in East Africa and their influence on oviposition, development, survival, intrinsic population growth rate and other life history characteristics of *T. evansi*. The study concentrated on four major areas (i) Evaluating developmental duration and life history characteristics of *T. evansi* on African nightshade (ii) Establishing the effect of trichomes and volatile chemicals of African nightshades on reproduction, repellence and olfactory responses of *T. evansi* (iii) Determining the population dynamics of *T. evansi* in African nightshade under greenhouse and field conditions (iv) Establishing the effect of *T. evansi* feeding on growth and yield of African nightshades grown under field

conditions. Since arthropod control in resistant cultivars is genetically incorporated (Smith, 2005), development of African nightshades resistant to *T. evansi* could have an economic advantage in that farmers will only pay for the cost of seed. Thus information that will lead to further research on management of *T. evansi* in African nightshades through integrated pest management including the development and the utilization of resistant genotypes is vital.

CHAPTER TWO

Review of literature

2.1: Description of *T. evansi* (Acari: Tetranychidae)

The tomato spider mite belongs to the order Acari, suborder Prostigmata, superfamily Tetranychoidae, and family Tetranychidae. *T. evansi*, commonly referred to as the tomato spider mite, is closely related to two spider mite species; *Tetranychus marianae* McGregor and *T. takafujii* Ehara and Ohashi (Moraes *et al*., 1987; Ehara and Ohashi, 2002). *T. evansi* was initially recorded as *T. marianae* in northeastern Brazil on tomato (Silva, 1954), Mauritius on tomato, aubergine, potato and peanut, (Moutia, 1958), and USA on tomato (Wene, 1956; Schuster, 1959). However, it was later redescribed as *T. evansi* (Baker and Pritchard, 1960; Moraes *et al*., 1987) from the material collected in Mauritius.

Reproduction by tetranychid mites involves arrhenotokous parthenogenesis which contributes to the female bias found in spider mite populations. Females develop from diploid (fertilized) eggs while males develop from haploid (unfertilized) ones. Females can therefore control fertilization which can potentially control the primary sex ratio of the offspring they produce (Young *et al*., 1985; Wrensch, 1993). The actual sex ratio of progeny produced by individual females is dependent upon many factors such as the amount of sperms a female receives during mating, host plant quality, population density and temperature (Wrensch, 1985).

Tetranychid mites are 0.16-0.22 mm long and 0.15-0.18 mm wide. Development of *T. evansi*, like other tetranychid mites, follows five stages i.e. egg, larva, protonymph, deutonymph and adult (Plate 2.1). The two nymphal stages i.e. protonymph and deutonymph have four pairs of legs and the feeding ones are greenish-yellow. The protonymph is 0.16-0.27 mm long and 0.13-0.19 mm wide while the deutonymph is 0.28-0.37 mm long and 0.18-0.26 mm wide. The three active feeding immature stages are each followed by intervening periods of quiescence called the protochrysalis, deutochrysalis and teliochrysalis respectively (Helle and Sabelis, 1985). Adult females are oval, orange red with an indistinct dark blotch on each side of the body (Plate 2.1).

Newly emerged females are 0.31-0.39 mm long and 0.19-0.28 mm wide while, ovipositing females are 0.41-0.49 mm long and 0.27-0.39 mm wide (Qureshi *et al*., 1969). Males are slender and straw to orange colored with long legs and smaller than the females (Plate 2.1). They are 0.25-0.40 mm long and 0.15-0.24 mm wide (Qureshi *et al*., 1969). Fine strands of silk are spun by the adults to form an open web above the leaf surface (Meyer, 1996). There is however, a marked difference between males and females in the rate of development. The early maturing males locate and remain near the female teliochrysalis until the females emerge; copulation takes place almost immediately after emergence of the young females (Helle and Sabelis, 1985). This explains why, in a normal bisexual population, the males mate more than once with either emerging or already emerged females (Smith Meyer, 1996).

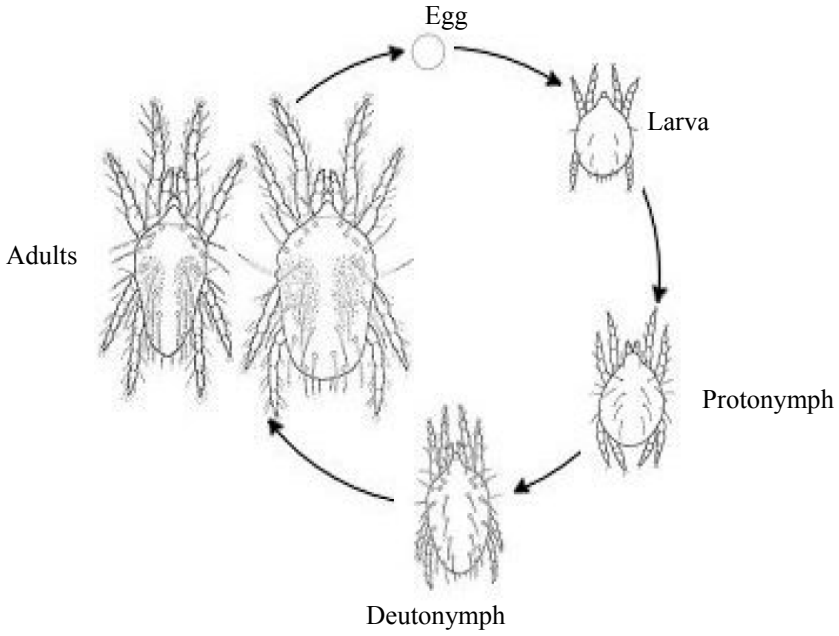

Plate 2.1 Life cycle of *Tetranychus evansi* from egg to adult (the largest is the female) (Source: Migeon, 2005)

Females lay eggs singly on the underside of the leaf in batches of 10-15 eggs per day, after which the rate of oviposition decreases by the fourth day (Qureshi *et al.*, 1969). Eggs may be covered with webs in order to regulate humidity and to protect them from predators (Gerson, 1985). They hatch after 4-7 days at a temperature of 25-30°C. The larval period lasts 3-5 days while the nymphal period lasts 6-10 days. There are no conspicuous changes in morphology after individuals have entered the first nymphal stage. The entire development period is completed within 10-14 days, but this is dependent on temperature and relative humidity (Smith Meyer, 1996).

2.2: Pest status and host plants of *T. evansi*

Tetranychus evansi is presently considered a key pest of tomato in several African countries (Saunyama and Knapp, 2003). In most countries where *T. evansi* occurs, it has shown a pronounced preference for solanaceous plants such as nightshade, tomato, potato, aubergine and tobacco (Moraes and McMurtry, 1985a; Leite *et al*., 2003). This mite species has a high reproductive potential and populations can rise sharply during dry and hot weather (Denmark, 1973; Moraes *et al*., 1986; Moraes and McMurtry, 1987). *T. evansi* can develop within a broad thermal range of 10-36° C (Bonato, 1999).

Generally, spider mites penetrate the leaf surface with their stylets to a depth of 70-120 μm and suck out the cell contents (Tomczyk and Kropczyńska, 1985) while leaving the leaf area intact. Typical symptoms of this feeding are small, light colored punctures that on prolonged feeding develop into irregularly shaped white or grayish-colored spots (Plate 2.2a). This leads to damage of the protective spongy parenchyma, stomata, and the palisade layer of leaves (Huffaker *et al*., 1969; Mothes and Seitz, 1982) which may lead to defoliation, leaf burning, and even plant death under severe infestation. In addition, there are disturbances of plant metabolic processes such as photosynthesis and transpiration, which contribute to a decrease in plant growth, flowering and consequently, yield (Brandenburg and Kennedy, 1987; Mathews and Tunstall, 1994). For instance, in tomato fields in Zimbabwe, yield losses of up to 90% due to *T. evansi* damage have been recorded (Saunyama and Knapp, 2003). When spider mite infestation is severe, fine webbing is clearly visible on plants (Meyer, 1996) (Plate 2.2b).

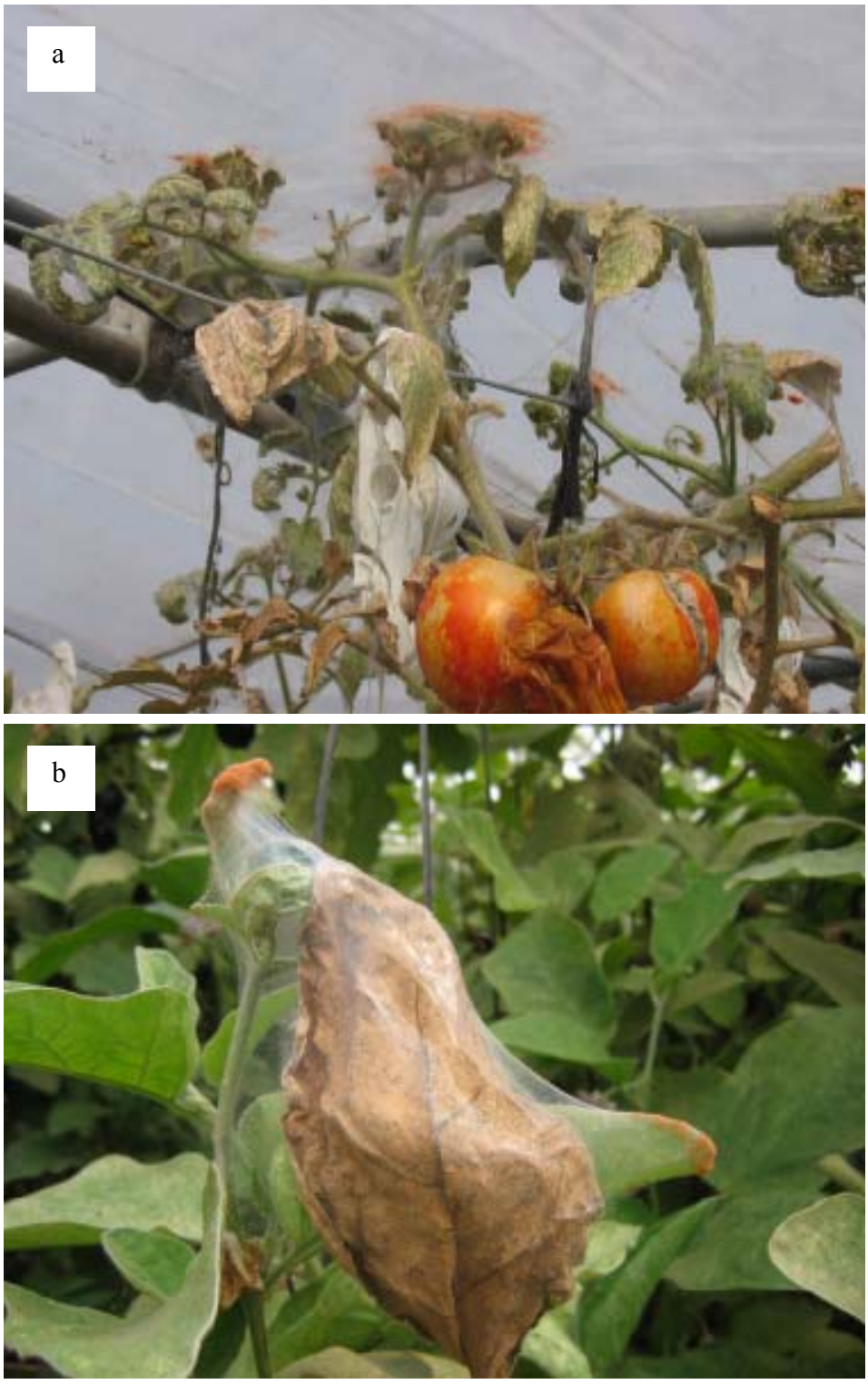

Plate 2.2 Symptoms of *T. evansi* (a) feeding on tomato and its (b) characteristic webbing on egg plant (Photo: Alain Migeon, Montpellier, France, 2005)

2.3: Origin and distribution of *T. evansi*

The tomato spider mite is probably of South American origin and was accidentally introduced to other parts of the world (EPPO, 2004). It was first recorded in Africa on tobacco in Zimbabwe in 1979 (Blair, 1983). It spread to Reunion from Mauritius in the 1970s (Gutierrez and Etienne, 1986). In the 1980s, it spread into southern Africa, and late 1980s and early 1990s it spread into North Africa. *T. evansi* has also been reported in Greece (Tsagarakou, 2007), Italy (Castagnoli *et al*., 2006), Israel (Ben-David *et al*., 2007), Jordan (Palevsky, pers. com. 2007), Portugal (Bolland and Vala, 2000), Spain (Escudero and Ferragut, 2005), France (Migeon, 2005), Congo (Bonato, 1999), Morocco (El Jaouani, 1988), Tunisia (Bolland *et al*., 1998), Kenya (Knapp *et al*., 2003a,b), Virgin Islands and the U.S.A (Moraes *et al*., 1987) (Figure 2.1).

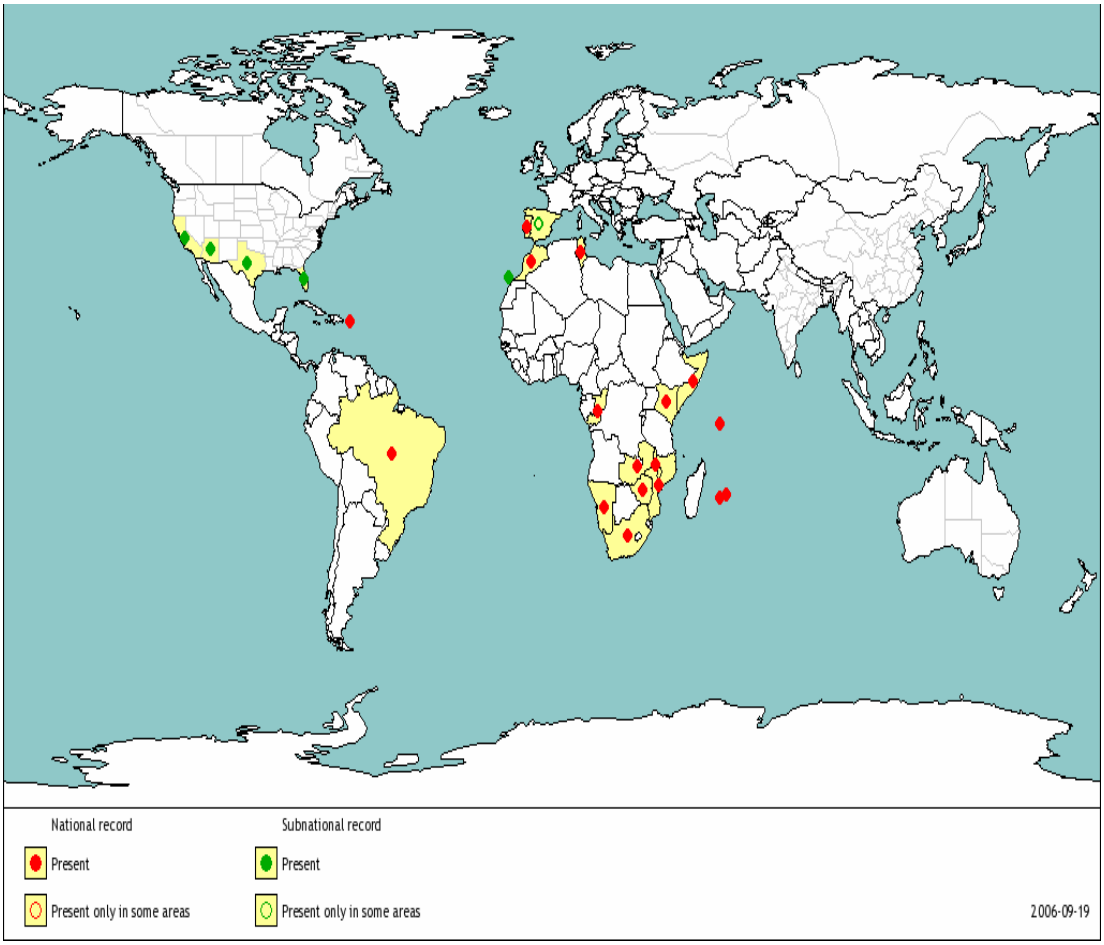

Figure 2.1 World distribution map of *T. evansi* (Source: European Plant Protection Organization, 2006).

2.4: Management of Tetranychid mites

2.4.1: Chemical control

Synthetic acaricide applications are the most common strategies used for control of spider mites (Herron *et al*., 2004). In Kenya, several synthetic acaricides such as dimethoate, cypermethrin, propargite, lamda-cyhalothrin, bifenthrin among others are used for management of *T. evansi* (Machini, 2005; Toroitich, 2006). However, the response of spider mites to these acaricides can range from resistance via metabolic detoxifying mechanisms (Rizzieri *et al*., 1988; Fergusson-Kolmes *et al.*, 1991; Bynum *et al*., 1997) to behavioral responses such as changes in dispersal (Fisher and Wrench, 1986) and oviposition site selection (McPherson *et al.*, 1989).

For instance, *T. urticae* has been reported to develop resistance to acaricides such as bifenthrin (Kolmes *et al*., 1994), dicofol (Rossi and Conti, 1997; Karban and Zalom, 1998), propargite (Goodwin *et al*., 1995) and dimethoate (Jensen and Mingochi, 1988) which are frequently used for its management. In addition, some pesticides also enhance population growth of tetranychids through direct stimulation of fecundity or indirectly by killing predators. Direct stimulation may occur as trophobiosis (Chabousseau, 1966) in which the pesticide improves host plant favorability to tetranychids or by hormoligosis (Luckey, 1968) where pesticides directly stimulate the rate of development and fecundity of tetranychids.

In Africa, pesticide applications to manage *T. evansi* have been found to be often ineffective (Sibanda *et al*., 2000; Saunyama and Knapp, 2003), although not much has been documented on pesticide resistance in *T. evansi* (Knapp *et al*., 2003b). However, Blair (1989) tested 62 acaricide formulations against *T. evansi* on tobacco in the laboratory and found that control with dimethoate and other thiophosphates were ineffective. Other acaricide formulations have also been tested in the laboratory at ICIPE in order to determine their effect against *T. evansi* (Machini, 2005; Toroitich, 2006).

Chemical control of spider mites requires regular distribution of the spray solution on all parts of the plant including the lower surface of the leaves. For example, pruning and staking of tomatoes makes it easier to cover the plants with acaricides and therefore improves spider mite control (Saunyama and Knapp, 2003). Preventive application of dosages lower than the recommended as well as broad spectrum pesticides should be avoided since this may lead to resistance (Smith Meyer, 1996; Varela *et al*., 2003).

2.4.2: Biological control

Natural enemies such as predatory mites, acarophagous insects and entomopathogens have been used in regulating populations of plant feeding mites (Helle and Sabelis, 1985; Smith Meyer, 1996). Predatory mites mainly Phytoseiidae are generally the most effective and widespread predators of plant feeding mites (Moraes *et al*., 2004). This is because a number of them possess attributes such as lower food requirements, short life cycle, ability to survive on alternative food sources and good host-searching ability (McMurtry *et al*., 1970; Overmeer, 1985) that enable them to control spider mite

populations effectively at low densities. For instance, *Phytoseiulus persimilis* Athias-Henriot has been an efficient biological control agent of the two-spotted spider mite *T. urticae* on greenhouse crops in Europe and North America since the late 1960s (Chant, 1961; Lenteren and vanWoets, 1988; Lenteren *et al*., 1992) and on outdoor crops in California and Florida (McMurtry, 1999). However, attempts to control *T. evansi* with *P. persimilis* in Zambia and Zimbabwe failed (Jensen and Mingochi, 1988; Sibanda, 1995).

Earlier reports by Moraes and McMurtry (1986) indicated that the eggs of *T. evansi* contain a feeding depressant such that the predatory mites rarely consume a whole egg and if they do feed, they do it very slowly. More recently, Escudero and Ferragut (2005), investigating the suitability of *T. evansi* as prey for *P. persimilis* and *Neoseiulus californicus* McGregor in Spain compared to three other spider mites, found that none of the phytoseiids were effective predators of *T. evansi*. Their predator performance, fecundity and survivorship were very poor on *T. evansi*. However, search for natural enemies of *T. evansi* in South America revealed that the predatory mite *Phytoseiulus longipes* Evans is a potential candidate for introduction in Africa (Furtado *et al.*, 2006). This predator was exported to Kenya for field releases trials and release (Wekesa *et al*., 2007).

All known species of the genus *Stethorus* are obligate predators of spider mites (Chazeau, 1985; Rott and Ponsonby, 2000; Ullah, 2000) and several species have been reported to be effective biological control agents (Gotoh *et al*., 2004; Mori *et al*., 2005).

They are known to be voracious predators with all motile stages feeding on all prey stages, having high host-finding and high dispersal potential and long-living adults (Roy *et al*., 2003; 2005). For example, the acarophagous lady beetle *Stethorus japonicus* Kamiya is considered to be an important predator of *T. urticae* and the Kanzawai spider mite, *Tetranychus kanzawai* Kishida in apple, citrus, tea, pear, hydrangea and kudzu vine (Gotoh and Gomi, 2000; Kishimoto, 2000). In addition, another acarophagous lady beetle *Stethorus tridens* Gordon was recently found in association with *T. evansi* in the field in Brazil, and the insect was reported to be a promising biological control of this mite species (Fiaboe *et al*., 2007).

Fungal pathogens belonging to the order of Entomophthorales are known to cause epizootics in mite populations (Van der Geest *et al*., 2000). The genus *Neozygites* is the commonest among the fungi that attack mites in the order Entomophthorales. *Neozygites floridana* Weiser and Muma, an obligate pathogen of spider mites, has been observed on several species of spider mites on various agricultural crops. For instance, it was reported on *T. evansi* on tomato in Brazil (Humber *et al*., 1981), on *Tetranychus ludeni* Zacher on bean in India (Rameseshiah, 1971), on *Oligonychus hondoensis* Ehara on cedar in Japan (Nemoto and Aoki, 1975), on *T. urticae* on field corn in North Carolina, USA (Brandenburg and Kennedy, 1982), and on the cassava green mite (CGM), *Mononychellus tanajoa* Bondar, in Venezuela (Agudella-Silva, 1986), Brazil (Delalibera *et al.*, 1992), and Kenya (Bartkowski *et al*., 1988). More recent studies by Wekesa *et al*., (2007) in Brazil revealed that *N. floridana* interacted with the predatory mite *P. longipes*

without causing any epizootics, which implied that both natural enemies of *T. evansi* can be utilized concomitantly in classical biological control programs of this mite species in Africa. Maniania *et al*., (2008) has recently reviewed the role of Entomopathogenic fungi in the control of *T. urticae* and *T. evansi.*

2.4.3: Cultural control

Several cultural control techniques such as crop rotation, proper field sanitation, pruning and trellising, uprooting and burning of old crops have been employed for management of spider mites. These techniques are aimed at disrupting the life cycle and hence reducing mite populations (Tindall, 1983). For example, pruning and trellising of tomatoes associated with acaricides was found to have a positive effect on yields and quality of tomatoes as well as the profitability of tomato production in high potential growing areas in Zimbabwe (Saunyama and Knapp, 2003).

Since hot and dry environmental conditions are favorable to the development of mite populations, reducing the planting distance and applying overhead irrigation reverses these conditions and thus depresses the mite populations. For example, water spray programs in form of mist were reported to inhibit mite infestations (Tulisalo, 1974). In addition, a high relative humidity reduces the female life span and they lay eggs at a slower rate. Attempts have been made to reduce mite build-up by varying the fertilizer regime applied to the crops. For instance, large quantities of nitrogen or a deficiency of potassium increases the amount of soluble nitrogen in the plant so that sharp increases in

the rate of population growth by mites follow such fertilizer regimes (Markkula and Tiittanen, 1969).

2.4.4: Resistant varieties

The most important advance in facilitating IPM has been the breeding of crop cultivars that compromise higher levels of pest resistance (Panda and Khush, 1995; Maluf *et al*., 2007). For instance, a number of wild *Lycopersicon* accessions have been reported to be resistant to a wide array of tomato pests including spider mites (Gentile *et al*., 1969). Rasmy (1985) reported that two species of solanaceous plants, *L. hirsutum* f *glabratum* and *Solanum sarrachoides* Sendter were resistant to *T. urticae*. More recently, Murungi, *et al*., 2009 and Wosula *et al*., 2009 tested several *Lycopersicon* accessions against *T. evansi* in the laboratory and reported that the wild types posses' resistant traits that could be utilized in breeding for resistance against this mite species. The resistance mechanisms are reported to be related to several characteristics such as presence of trichomes that are known to confer resistance either by entrapment (Duffey, 1986) or by secretion of toxic compounds (Patterson *et al*., 1975; Goffreda *et al*., 1989; Chatzivasileiadis and Sabelis, 1997). Thus, introgression of the arthropod resistant traits from the wild into cultivated species can be utilized for spider mite management as an IPM strategy.

2.5: Host Plant Resistance

Plant resistance has been utilized as a means of protecting plants from herbivores for many years (Smith, 1989) and is an appealing means for management of herbivorous pests for several reasons; It is specific, has cumulative effectiveness over several generations of pests, is persistent, environmentally compatible, easy to use and is generally compatible with other pest management tactics (Kogan, 1982). However, the benefits of resistance depend on the modality i.e. antixenosis, antibiosis or tolerance, and the level of resistance against the specific pests and the crop production system under consideration (Kennedy *et al*., 1987). Moreover, many arthropod species including spider mites invade crops in low numbers, but their populations increase gradually over several generations before reaching damaging levels (Panda and Khush, 1995).

A plant with antixenosis and antibiosis-based resistance can reduce the rate of population increase of the herbivore (Zangerl and Bazzaz, 1992; Strauss and Agrawal, 1999; Rautio *et al*., 2002) by shifting populations to other nearby crops, reducing reproduction, survival, and prolonging generation time (Price *et al*., 1980); and such traits may vary both among and within natural populations (Fritz and Simms, 1992). However, plants with tolerance can withstand damage from a pest population which its non-tolerant counterpart cannot (Panda and Khush, 1995).

2.5.1: Effects of antixenosis and antibiosis on arthropod behavior

The physical structures that may influence arthropod behavior include structures such as plant trichomes, surface waxes and tissue hardness (Smith, 2005). Plant trichomes are the first with which arthropods come in contact with after making the decision to alight or walk as in the case with spider mites on the plant surface. However, trichomes vary in structure whereby some may be simple, erect hairs, hook-shaped hairs or complex multi-cellular glandular structures (Smith, 2005). Simple trichomes limit the ability of arthropods to attach themselves to the plant surface, in order to initiate and maintain feeding, while hooked and glandular trichomes either entrap or impale the arthropod body causing it to desiccate and die. For example, leaf hairs of the wild *Lycopersicon* accession *L. hirsutum f. glabratum* was reported to confer antixenosis-related effects to both *T. urticae* (Weston *et al*., 1989) and *T. evansi* (Maluf *et al*., 2001). Patterson *et al* (1974) also showed that resistance in *Nicotiana* species to *T. urticae* was due to a combination of non-preference and antibiosis associated with a viscid secretion from trichomes that was toxic and entrapped the mites. However, some pests may prefer hirsute surfaces for oviposition making pubescence a trait not always useful for pest suppression. For instance, *Phthorimaea operculella* Zeller was reported by Gurr (1995) to prefer to oviposit on densely pubescent *Solanum* species.

Surface waxes also play an important role in the resistance of some crop cultivars to arthropod attack especially when the sense organs of the arthropod perceive negative chemical stimuli from the leaf surface (Smith, 2005). For instance, glossy leafed kale

and brussel sprouts devoid of the wax sustain less feeding by the cabbage aphid, *Brevicoryne brassicae* L. than waxy-leafed cultivars (Thompson, 1963; Way and Murdie, 1965) since the glossy types have a reduced lipid microstructure and quantity as well as altered chemical composition (Eigenbrode *et al*., 1991). Just as plant trichomes may not affect the expression of resistance, in some cases, waxes actually stimulate arthropod feeding and oviposition. For example, Lamb *et al*. (1993) found that no significant effects were derived from glossy leafed plants of rape or kale compared to waxy-leafed mutants of each in resistance to the aphid *Lipaphis eryisimi* Kaltenbach.

The thickness or modifications of the plant physical structure of various plant tissues such as stems, leaves shoots or pods determines the degree of resistance in some crop cultivars (Smith, 2005). For instance, the nymphs and adults of Tetranychid mites prefer to feed on the tender upper and middle leaves of the colonized plant rather than on the tougher lower leaves (Leite *et al*., 2003). In addition, Jiang and Ridsdill-Smith (1996) reported that the mechanical strength of cotyledons of certain subterranean clover cultivars is directly responsible for antixenosis of those cultivars to feeding by the red-legged earth mite, *Halotydeus destructor* Tucker.

Antibiosis may also involve proliferation of cells triggered by herbivore feeding or increased secretion of plant substances known to cause death of eggs or young larvae inside the damaged plant (Panda and Khush, 1995). For example, experimental results by Shapiro and DeVay, (1987) demonstrated that mustard plants produced a necrotized

zone around the base of the eggs of cabbage worm *Artogeia rapae* L., causing them to desiccate.

Allelochemicals may act as repellents (Dethier *et al*., 1960) during the olfactory orientation of an arthropod to a resistant plant or as feeding deterrents (Schoonhoven *et al*., 1982) or feeding inhibitors (Chapman, 1974) when an arthropod tastes a resistant plant. Volatile hydrocarbons emitted by the foliage of resistant plants comprise a great variety of arthropod repellents. For instance, volatiles from strawberry species with high essential oil content repel feeding by *T. urticae* and the strawberry spider mite, *Tetranychus turkestani* Ugarov and Nikolsik (Dabrowski and Rodriguez, 1971) although repellency may also be due to lack of perception of volatile allelochemical attractants. Also Guo *et al*., (1993) and Snyder *et al*., (1993) reported that a unique volatile organic acid, 2,3-dihydrofarnesoic acid, produced by the glandular trichomes of wild tomato, *L. hirsutum F. glabratum* also repels *T. urticae* feeding. Those allelochemicals that most frequently cause deterrence and toxicity include alkaloids, flavonoids, terpene lactones, organic acids, ketones and phenols produced and stored in leaf cell walls, vacuoles or specialized structures such as trichomes and waxes (Norris and Kogan, 1980; Norris, 1986; Schultz, 1988; Panda and Khush, 1995; Smith, 2005). For example, foliar glycoalkaloids in wild *Solanum* species deter feeding of the potato leafhopper, *Empoasca fabae* Harris (Sinden *et al*., 1986). Also, studies on the contact toxicity of the methyl ketone 2-tridecanone found in wild tomato species on *T. urticae* revealed strong acaricidal properties of the methyl ketone (Chatzivasileiadis and Sabelis 1997, 1998).

2.5.2: Effects of tolerance on arthropod resistance

Unlike antixenosis and antibiosis, tolerance involves only plant characteristics and is not part of an arthropod/plant interaction (Horber, 1980; Smith, 2005). Several factors such as; increased net photosynthetic rate, high relative growth rate, increased branching, pre-existing high levels of carbon stored in roots, and the ability to shunt stored carbon from roots to shoots contribute to increased plant tolerance to arthropod infestation (Strauss and Agrawal, 1999). For instance, some cultivars of maize, *Zea mays* L., tolerant to damage by the corn earworm, *Heliothis zea* Boddie and the European corn borer, *Ostrinia nubinalis* Hübner were reported to harbor larger larval populations than susceptible cultivars, due to their increased biomass (Wiseman *et al*., 1972; Hudon *et al*., 1979) and yet provided greater yields than susceptible ones. Tolerance against *T. urticae* also exists in some cultivars of tomato, *L. esculentum* such that tolerant cultivars have high levels of defoliation but yields are similar to those with little defoliation (Gilbert *et al*., 1966).

The partitioning of carbon between chloroplasts and the cytoplasm is highly regulated by endogenous factors such as rate of carbon dioxide assimilation, concentration of substrates etc and exogenous ones such as light and temperature (Trumble *et al*., 1993). Research addressing the mechanisms of plant tolerance has been limited, likely due to the reluctance of plant breeders and producers to use arthropod-resistant crop cultivars that harbor high pest populations (Smith, 2005). However, some studies have shown direct involvement of photosynthesis, hormones and physical structures in the

expression of plant tolerance. For instance, Gawronska and Kielkiewicz (1999) reported that in tomato plants infested by the carmine spider mite *T. cinnabarinus*, mite-tolerant plants had higher leaf abscissic acid (ABA) content compared to mite-susceptible cultivars. Since tolerance is a complex genetic trait, it is necessary to identify the gene sequences of several different components in order to fully understand the contributions of each to the phenotypic effect identified as plant tolerance to arthropods (Smith, 2005).

2.6: African nightshades

Nightshades (Solanales: Solanaceae) comprise of approximately 30 species making up section *Solanum* of the genus *Solanum*; constituting a large number of closely related morphogenetically distinct taxa, and typified by the true black nightshade, *Solanum nigrum* L. (Edmonds, 1977). This species group is one of the largest and most variable groups in the genus *Solanum* L., with species that are distributed from temperate to tropical regions and from sea level to altitudes above 3500 m (Edmonds and Chweya, 1997). However, the taxonomy of nightshades has long been beleaguered by complexity, resulting in extensive synonymy and confusion (Gray, 1968; Schilling and Andersen, 1990). Several reasons such as phenotypic plasticity, genetic variation, polyploidy, natural hybridization and discordant variation have been associated with this complexity (Edmonds, 1977).

Generally, fresh leaves of most African indigenous vegetables contain more than 100% of the recommended daily allowances for vitamins and minerals and 40% proteins for

growing children and lactating mothers (Chweya, 1985). African nightshades are particularly rich in protein, vitamin A, iron and calcium compared to exotic vegetables (Table 2.1) and meet nutritional needs of many rural households. Medicinally, nightshades are used in Kenya for stomach upsets, duodenal ulcers, swollen glands and teething problems (Onyango, 1993; Edmonds and Chweya, 1997). They occur in many parts of Kenya where they are known by a variety of local names such as managu (Kikuyu), ndulu (Kamba), osuga (Luo), lisutsa (Luhya), rinagu (Kisii), mnavu (Giriama and Swahili), ksoyo (Pokot), kisochot (Elgeyo), where they are often cultivated in small kitchen gardens, and occasionally collected from the wild for domestic use and sale in markets (Edmonds and Chweya, 1997).

Both the leaves and berries of nightshades are used as a source of dyes. For instance, Nzioka (1994) reported that nightshade leaves are macerated to extract a dye used to color sisal baskets, while the purple/black berries of both *S. scabrum* and *S. americanum* are used as a source of ink. Moreover, the anthocyanin pigments of *S. scabrum* have been used in the past as a colorant for fruit juices and apple sauce (Francis and Harborne, 1966). These *Solanum* species are also found on sale as vegetables in both rural and urban markets in Cameroon, Ghana, Kenya, Madagascar, Nigeria, Guatemala and New Guinea (Edmonds and Chweya, 1997). These findings reveal the urgency to commercialize African nightshades that will provide a source of income for both rural and urban populations, hence contribute to poverty alleviation.

Table 2.1 Composition per 100 g edible portion of African nightshade compared to cabbage, a widely consumed exotic vegetable

Composition	African nightshade	Cabbage
Moisture Content (g)	87.2	91.4
Iron (mg)	1.0	0.7
Protein (g)	4.3	1.7
Calories (g)	38	26
Carbohydrates (g)	5.7	6.0
Fiber (g)	1.3	1.2
Ascorbic Acid (mg)	20	54
Calcium (mg)	442	47
Phosphorus (mg)	75	40
ß-Carotene (µg)	3660	100
Thiamin (Vitamin B_1) (mg)	-	0.04
Riboflavin (Vitamin B_2) (mg)	0.59	0.1

(Source: Kenya Resource Center for indigenous knowledge, 2003)

The success of nightshades is associated with their wide tolerance of habitat types, their ability to flower at the juvenile stage and their prolific seed production under tropical conditions (Henderson, 1974). However, like other indigenous vegetables, they face some major constraints of production which include poor seed quality, pests and diseases, drought, poor marketing channels, transport to markets and lack of agronomic and utilization packages (Onyango, 2007). In addition, the issues of perishability, lack of awareness of the merits of consuming nightshades as indigenous vegetables (Mnzava,

1997) and stiff competition from exotic vegetables like cabbage, spinach, kale and lettuce (Maundu *et al*., 1999). Also indigenous vegetables are often overlooked by the scientific and development community for research and promotion (Westphal *et al*., 1987).

Nightshades are also well adapted to high moisture conditions, rainfall about 500-1200 mm, temperatures between 20-30° C and in fertile soils that are rich in nitrogen, phosphorus, and high organic matter content (Holm *et al*., 1977; Onyango, 1993). Naturally, the seeds are effectively dispersed by birds all over the world, as well as by various animals and man (Weller and Phipps 1978; 1979). Seeds can also be dispersed by water (Burgert *et al*., 1973) and by contaminating harvested seed crops such as sugar beet (Weller and Phipps, 1978; 1979). Propagation of nightshades is mainly by seed although shoot cuttings may be used as propagules though plants propagated in this way branch, spread and yield less than those propagated by seed (Mwafusi,1992). Plantlets have also been regenerated from *S. nigrum* mesophyll chloroplasts (Wang and Xia, 1983). In Kenya, seeds are marketed by Simlaw Seeds in Nairobi under the name Black Nightshade in 25 g packets and from SACRED Africa, in western Kenya (FORMAT, 2003).

The occurrence of steroidal alkaloid solasonine and of solasonine-like alkaloids in most species belonging to the genus *Solanum* has resulted in a number of phytochemical surveys of various taxa from different geographical regions throughout the world (Edmonds and Chweya, 1997). Furthermore, several chemicals have been reported in the black nightshade and include; solanine, solasonine, solamargine and chaconine (Everist,

1974; Cooper and Johnson, 1984) with solanine occurring in all parts of the plants and the levels increasing as the plant matures (Weller and Phipps, 1978). Nightshades also contain other chemicals such as nicotine, quinine, cocaine and morphine, which are known for their medicinal attributes (Kokwaro, 1993).

Although African nightshades are alleged to be tolerant to pests and diseases, FORMAT (2003) reported mites, aphids and other pests attacking crops of the Solanaceae family on African nightshades, hence low productivity. There are few or no scientific reports that have associated the level of arthropod pest resistance in African nightshade with their relative chemical composition. Other Solanaceous crops, e.g. the wild potato, *Solanum tuberosum,* has been found to be resistant to the wireworm, *A. obscurus*, due to the presence of two foliar glycoalkaloids chalcone and solanine (Jonasson and Olsson, 1994).

CHAPTER THREE

General materials and methods

3.1: Biological material and experimental conditions

The study was carried out between October, 2006 to April, 2009 at the International centre of Insect Physiology and Ecology (*icipe*) and at Jomo Kenyatta University of Agriculture and Technology (JKUAT).

3.1.1: Spider mites

Mites used in this study were obtained from a regularly regenerated colony of *T. evansi* maintained on potted tomato plants variety 'Money Maker' (obtained from the East African Seed Company, Nairobi, Kenya), in a rearing room at a temperature of 25 ± 1°C, 50-70 % relative humidity (RH) and a 12h photoperiod at *icipe*.

3.1.2: Plant establishment

Five African nightshade species namely *Solanum villosum*, *S. sarrachoides, S. scabrum*, *S. tarderemotum* and *S. americanum* that were obtained from the Gene bank of Kenya and the World Vegetable Center (AVRDC, Tanzania) were used (Table 3.1). Seeds were sowed in rows in soil enriched with compost in plastic seed trays. Seedlings were transplanted, one month after sowing, into pots (29-cm diameter) each containing 3 kg of (3:2:1 v/v) red soil: compost: sand and placed on benches in a greenhouse at *icipe*. Plants were watered daily and each pot was nourished with 3 g calcium ammonium

nitrate [CAN (26% N); from Jumbo Agrovet, Nairobi, Kenya] 2 weeks after transplanting.

Table 3.1 African nightshade species, accession code and source

African nightshade species	Accession code	Source
S. villosum	MW 13	AVRDC, Tanzania
S. sarrachoides	GBK 028726	Gene Bank, Kenya
S. scabrum	SS 51	AVRDC, Tanzania
S. tarderemotum	MW 03	AVRDC, Tanzania
S. americanum	SA	AVRDC, Tanzania

3.2: Mite biology tests

Developmental time: Tests on development duration were performed on one-day old mite eggs. Eggs were transferred to respective leaf disks of individual accessions and placed in an incubator maintained at a temperature of 25 ± 1°C, 70-80 % RH and 12: 12 h (L: D) photoperiod. The rearing units (with either eggs or deutonymphs on leaf disks placed in a petri dish) were checked every 24h using a dissecting microscope (Leica MZ8; Leica Microsystems, Wetzlar, Germany) and the development duration and survival were observed for each developmental stage.

Fecundity and longevity: In order to establish fecundity and longevity of *T. evansi* on African nightshades, a single female deutonymph and two males were picked from the colony and transferred to leaf disks of respective species. All rearing units were placed

in an incubator maintained at a temperature of 25 ± 1°C, 70-80 % RH and 12: 12 h (L: D) photoperiod. Fecundity and life length were observed daily under a dissecting microscope until the female died.

3.3: Leaf surface morphology and mite response tests

Trichome type and density studies: Trichome density was evaluated by counting the number of trichomes on the abaxial leaf surface with the aid of a 32x dissecting microscope (Leica MZ8; Leica Microsystems, Wetzlar, Germany) fitted with a square grid lens. To establish trichome types, photos of leaf sections along the midrib were taken with a camera fitted on a 25x light microscope (Leitz Orthoplan; Leitz GmbH, Wetzlar, Germany) and the films developed.

Thumb-tack bioassay (No-choice): The resistance to *T. evansi* was quantified with the thumbtack bioassay (Weston and Snyder, 1990) from leaves of respective species whose trichome density had previously been determined. Ten female mites from the colony were transferred to the head of each thumbtack pin for each leaf of the respective nightshade species. The distances traveled by each mite onto the leaf surface were measured as the shortest distance between the mite and the thumbtack edge, and were recorded at a 15 min interval for 1h.

Olfactometer bioassay: The olfactory response of female *T. evansi* to odor emitted by intact nightshade plants was tested in a closed-system Y-tube olfactometer. The

olfactometer consisted of a glass Y-tube (1 cm inner diameter, 7 cm length of trunk, 8 cm length of the arms). Each olfactometer arm was connected by a Teflon® tube (0.8 cm inner diameter) to glass chambers that were used for holding the source of test odors. A pressure pump (Air Cadet vacuum/pressure station, Cole Palmer Instrument Co., USA) was used to pump air into and out of the system while two flow meters (Cole Palmer Instrument Co., USA) regulated the airflow. Additional Teflon® tubes conveyed air from the inlet pressure pump through an activated charcoal filter to remove impurities, then through one flow meter and into the separate treatment glass jars. The second flow meter was connected between the stem of the olfactometer and the T-junction to the pump, which exhausted air out of the system. Airflow at the entry was set at 175 ml min^{-1} and at the exit 390 ml min^{-1}. Mites were introduced singly at the entrance of the trunk and observed for a maximum of 10 min and response to either arms recorded.

3.4: Collection and analysis of volatiles

Chemical odors emitted by intact nightshade plants established in the greenhouse at *icipe* were collected using a portable volatile collection system. Super Q trap (Analytical Research Systems, Gainesville, FL) was used to adsorb the volatiles. Characterization of the volatile compounds was done by Gas Chromatography-Mass Spectrometer (GC-MS). GC-MS (Agilent Technologies, Wilmington, DE, USA) analysis was carried out on a HP 7890A model series GC coupled to a 5975C mass spectrometer and a Triple Axis Detector. The separation was done on a HP5 MS 5% nonpolar methyl silicon capillary column 30 m (length) x 0.25 mm (internal diameter) x 0.25 µm (film

thickness). The GC was interfaced to a HP monitor (Dell Optiplex x 520) via 3365 MSD Chemstation software (G1701EA E.02.00.493) onto whose screen individual derived chromatographic fractions were acquired and evaluated. The spectrometer was operated in the electron impact (EI) mode at 70 eV and a temperature at the ion source and interface at 230 and 150°C respectively. Helium was used as a carrier gas at a constant flow rate of 1.2 ml min^{-1}.

3.5: Dynamics of mite populations

Screenhouse experiments: Leaves of the respective nightshade species were infested with young female mites sourced from the colony maintained at *icipe*. Leaves were sampled a fortnight after infestation and brought to the laboratory for assessment. Spider mite life stages such as number of motile individuals were counted under a 25x dissecting microscope. Other parameters such as leaf damage, leaf area (cm^2) and mite population densities were evaluated weekly for a period of six consecutive weeks.

Field experiments: The respective nightshade species established in the field were individually infested with spider mites sourced from a colony maintained at *icipe*. One plant was randomly selected per each sampling time and three leaves randomly selected from the upper, middle and lower parts of the plant to assess spider mite densities, leaf area (cm^2) and leaf damage. Selected plants were also excised and brought to the laboratory to measure fresh and dry weight of above ground matter. Data was collected weekly for six consecutive weeks.

CHAPTER FOUR

Effect of African nightshade species on developmental time and life table parameters of *Tetranychus evansi* (Acari: Tetranychidae)

4.1: Introduction

Life history studies of *T. evansi* were first initiated in the laboratory on tomato leaves by Silva (1954) and on nightshade leaves by Qureshi *et al*., (1969) although both authors did not give complete life table studies. However, Bonato (1999) gave complete studies on the effect of temperature on biological and demographic parameters of *T. evansi* on tomato, one of the main host plants of this pest. The potential of increase in a population can be estimated in terms of the intrinsic rate of natural increase (r_m) (Birch, 1948), since it reflects an overall effect on development, reproduction and survival, under given food and climatic conditions (Southwood and Handerson, 2000). Since host plant is one factor with the ability to produce change in biological (e.g. fecundity and development) and demographic parameters (such as intrinsic rate of increase, doubling time, finite rate of increase, mean generation time and net reproductive rate) of spider mites, the effects of different species of nightshade on the main life history parameters were determined. The objective of this study was to determine the biological and demographic parameters of *T. evansi* on five African nightshade species of economic importance.

4.2: Materials and methods

4.2.1: Spider mite culture and plant material

The study was conducted in the laboratory at *icipe*, Duduville campus (S01°13.140'; E036°53.440'), Nairobi, Kenya. A Spider mite colony was maintained in a rearing room under experimental conditions described in section 3.1.1. Plants of African nightshade species used in this study were established in a greenhouse (Plate 4.1) as described in section 3.1.2. The greenhouse temperature was monitored using a HOBO Pro Series Temp, RH monitor (version 32, 1998 ONSET, HOBO data loggers) with a daily average of 23 ± 1° C and 60-70% RH.

4.2.2: Developmental time of mite immatures

To obtain eggs, ten aging leaves of four week-old tomato plants used for rearing the stock colony in section 3.1.1 were excised and placed in equal portions on top of two freshly potted Money Maker plants where *T. evansi* females were allowed to move and oviposit. After 24h, a single egg was carefully picked from the infested plants and transferred to leaf disks (2.5 cm diameter) cut from the respective five African nightshade species. The leaf disks were maintained on petri dishes stacked with cotton wool moistened with tap water. Petri dishes were placed on plastic trays (36 x 23 x 2.3 cm) and incubated (Plate 4.2) at 25 ± 1°C, 70- 80 % RH and a 12h photoperiod. Observations for development from the egg to the adult stage were monitored on a daily basis. The leaf disks were changed every 4d. A total of three replicates each with 48 eggs were studied for each African nightshade species.

Plate 4.1 Seedlings established on plastic seed trays

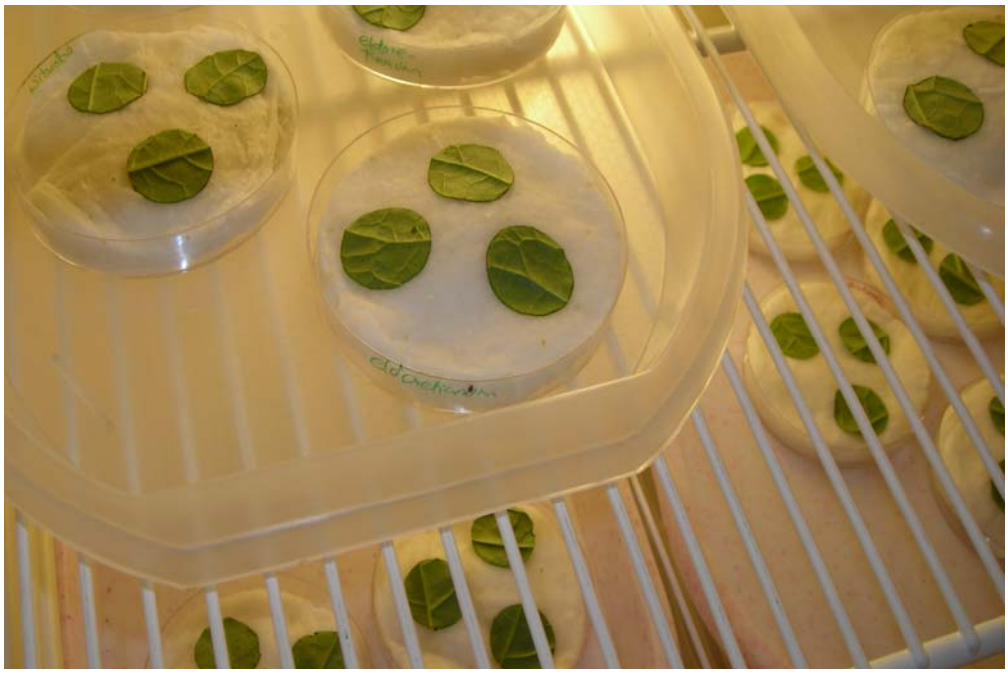

Plate 4.2 Set-up of rearing units on leaf disks placed in the incubator

4.2.3: Mite population development

To determine life table characteristics of *T. evansi* on the five African nightshade species, a female deutonymph, 2nd nymphal stage, was picked from the stock colony and transferred to the abaxial surface of 2.5 cm leaf disks of the respective nightshade species. To ensure mating, two adult males were picked from the same colony and introduced onto the leaf disk since mating occurs immediately the female emerges (Helle and Sabelis, 1985). If females had emerged 24h later, the males were removed. These rearing units were placed in an incubator (Plate 4.2) maintained at 25 ± 1°C, 70 - 80 % RH and 12 : 12 L: D photoperiod. The number of eggs deposited per female was recorded daily until all the females died. A total of three replicates each with 12 deutonymphs were studied for each African nightshade species.

4.2.4: Statistical analysis

Data on developmental time, longevity and fecundity were subjected to the general linear model procedure (Proc GLM) and where significant differences were obtained; means were separated using Student-Newman Keuls (SNK) test (SAS, Institute 2000). For each African nightshade species, a fertility life table was constructed following the method described by Andrewartha and Birch (1954). Age specific survival rates (l_x) and average number of female offspring (m_x) for each age interval (x) were used to construct age-specific fertility life table. From these data, the net reproductive rate (R_0), intrinsic rate of natural increase (r_m), finite rate of increase ($\lambda = e^{rm}$), mean generation time [$T = (\ln R_0)/r_m$] and doubling time [$T= (\ln 2)/r_m$] were estimated using the Jacknife computer

program developed by Maia *et al*. (2000). Differences in life table parameters among species were calculated following the protocol by Dixon (1987) and compared with Newman-Keuls sequential tests (Sokal and Rohlf, 1995) based on Jacknife estimates of variance for each parameter values (Meyer *et al*., 1986).

4.3: Results

4.3.1: Developmental time of mite immatures

Survival of *T. evansi* to adulthood differed among species (Table 4.1). Between 40-45% of the mites on *S. americanum*, *S. villosum*, *S. scabrum* and *S. tarderemotum* reached adulthood, whereas slightly 6% reached that stage on *S. sarrachoides*. *T. evansi* development was likewise affected by the different nightshade species; the egg incubation time was significantly longer on *S. sarrachoides* than the other *Solanum* species tested. On all the nightshade species, egg stage had the longest development time and was generally more than two fold longer than any of the other stages. There were no significant differences among the *Solanum* species in the other developmental stages, except for the protonymph stage where the duration was significantly longer on *S. sarrachoides* and *S. americanum*. Total development time was significantly longer on *S. americanum* and *S. sarrachoides* than the other *Solanum* sp. The sex ratio (% of females) of *T. evansi* was 1:1 when placed on *S. sarrachoides* compared to a higher female bias on other *Solanum* species tested (Table 4.1).

Table 4.1 Duration (± S.E) of various stages, survival (± S.E) and sex ratio (% of female) of *Tetranychus evansi* on five species of African nightshade

Plant species	Egg (d)	Larva (d)	Protonymph (d)	Deutonymph (d)	Egg - Adult[‡] (d)	Survival to adulthood (%)	Sex ratio[§] (% of ♀)
S. sarrachoides	5.2 ± 0.1a (94)[†]	2.6 ± 0.2a (28)	2.3 ± 0.2a (9)	1.9 ± 0.1a (8)	12.1 ± 0.2a	5.6b	50.0 (4)
S. americanum	4.9 ± 0.1b (116)	2.4 ± 0.1a (77)	2.3 ± 0.1a (64)	1.9 ± 0.1a (63)	11.7 ± 0.1a	45.1a	70.0 (43)
S. villosum	4.9 ± 0.1b (108)	2.3 ± 0.1a (86)	2.2 ± 0.1ab (69)	1.8 ± 0.2a (63)	10.9 ± 0.2b	43.8a	60.0 (39)
S. tarderemotum	4.9 ± 0.1b (116)	2.3 ± 0.1a (73)	2.0 ± 0.1bc (60)	1.8 ± 0.1a (59)	10.9 ± 0.1b	40.3a	50.0 (36)
S. scabrum	5.0 ± 0.1b (101)	2.4 ± 0.1a (76)	1.9 ± 0.1c (64)	1.9 ± 0.1a (58)	10.8 ± 0.2b	40.3a	60.0 (35)
F	4.19	1.48	10.84	0.70	7.13	4.51	0.47
P	0.0024	0.2090	<0.0001	0.5901	<0.0001	0.0244	0.7599

For each development stage, durations followed by the same letters within columns are not significantly different (SNK test; $\alpha = 0.05$).
(d) = days it took to complete one stage
[†] Number of individuals completing each respective stage and used in data analysis
[‡] Total development from egg to adult;
[§] (♀/ (♂+♀)

4.3.2: Mite population development

No significant host plant effects were observed on the pre-oviposition period of *T. evansi* (Table 4.2). The oviposition period was however influenced by the different African nightshade species and was significantly shorter for mites allowed to oviposit on *S. sarrachoides.* The post-oviposition period was significantly longer for mites placed on *S. sarrachoides* than on the other species. Again, the longevity of adult female *T. evansi* was significantly shorter on *S. sarrachoides* than on the other nightshade species. In addition, total fecundity and daily oviposition rate were significantly lower on *T. evansi* females allowed to oviposit on *S. sarrachoides* than on other four *Solanum* species (Table 4.2).

Table 4.2 Duration (± S.E) of the pre-oviposition, oviposition, post-oviposition periods, longevity and reproduction rate of *Tetranychus evansi* on five African nightshade species

Plant species	Pre-oviposition (d)	Oviposition (d)	Post-oviposition (d)	Female longevity (d)	Reproduction rate	
					Total fecundity (eggs/female)	Oviposition rate (eggs/female/day)
S. sarrachoides	2.4 ± 0.2a	4.1 ± 0.8b	1.8 ± 0.7a	8.5 ± 0.9b	5.0 ± 1.6b	0.5 ± 1.3b
S. americanum	2.3 ± 0.3a	7.6 ± 0.5a	0.9 ± 0.3ab	10.7± 0.5a	48.7 ± 5.3a	4.4 ± 0.4a
S. villosum	2.0 ± 0.2a	9.0 ± 0.6a	0.9 ± 0.3ab	11.9 ± 0.6a	61.7 ± 7.2a	4.8 ± 0.5a
S. tarderemotum	2.2 ± 0.3a	8.8 ± 0.7a	1.0 ± 0.2ab	12.0 ± 0.6a	62.3 ± 7.8a	4.9 ± 0.5a
S. scabrum	1.6 ± 0.1a	8.0 ± 0.6a	0.4 ± 0.1b	10.0 ± 0.6ab	58.7 ± 7.8a	5.2 ± 0.5a
F	2.04	6.13	2.76	4.25	10.82	15.47
P	0.0919	0.0001	0.0304	0.0029	<0.0001	<0.0001

For each phase, means followed by the same letter within columns are not significantly different (α = 0.05; SNK test)
d = days

4.3.3: Life tables

Daily egg production reached a peak on day six on *S. tarderemotum* (8.5), day seven on *S. americanum* (7.9), *S. sarrachoides* (1.9), *S. villosum* (10.5) respectively and day nine for *S. scabrum* (12.9) (Figure 4.1). Egg production decreased gradually thereafter on all species, although in comparison to other *Solanum* sp., oviposition on *S. sarrachoides* was very low. About 70% of *T. evansi* females survived longer than two days on *S. sarrachoides* and thereafter survival rate decreased tremendously with less than 20% surviving to the 14^{th} day (Figure 4.1). The intrinsic rate of natural increase (r_m), net reproductive rate (R_0), mean generation time (T), doubling time (DT) and finite rate of increase (λ) of *T. evansi* were significantly different on *S. sarrachoides* but not different for the other nightshade species. R_0 was more than 50 fold higher on the four *Solanum* sp. than on *S. sarrachoides*. The intrinsic rate (-0.06) and doubling time (-11.02) were both negative on *S. sarrachoides* (Table 4.3).

Table 4.3 Effects of various African nightshade species on life table parameters of *Tetranychus evansi*

†Parameters	African nightshade species				
	S. tarderemotum	*S. sarrachoides*	*S. americanum*	*S. villosum*	*S. scabrum*
R_0	15.70 ± 1.89a	0.25 ± 0.07b	15.01 ± 1.57a	16.84 ± 1.82a	12.65 ± 1.70a
r_m	0.188 ± 0.01a	-0.063 ± 0.01b	0.183 ± 0.01a	0.196 ± 0.01a	0.180 ± 0.01a
T	14.61 ± 0.28b	22.29 ± 0.68a	14.85 ± 0.31b	14.85 ± 0.21b	14.40 ± 0.23b
DT	3.68 ± 0.14a	-11.02 ± .34b	3.80 ± 0.13a	3.53 ± 0.12a	3.85 ± 0.17a
λ	1.21 ± 0.01a	0.94 ± 0.01b	1.20 ± 0.01a	1.20 ± 0.01a	1.22 ± 0.01a

For each parameter, means followed by the same letter within rows are not significantly different (SNK test; $\alpha = 0.05$).
†Jacknife estimates of R_0, Net reproductive rate; r_m, Intrinsic rate of increase; T, Mean generation time (day); DT, Doubling time (day) and λ, Finite rate of increase for the population.

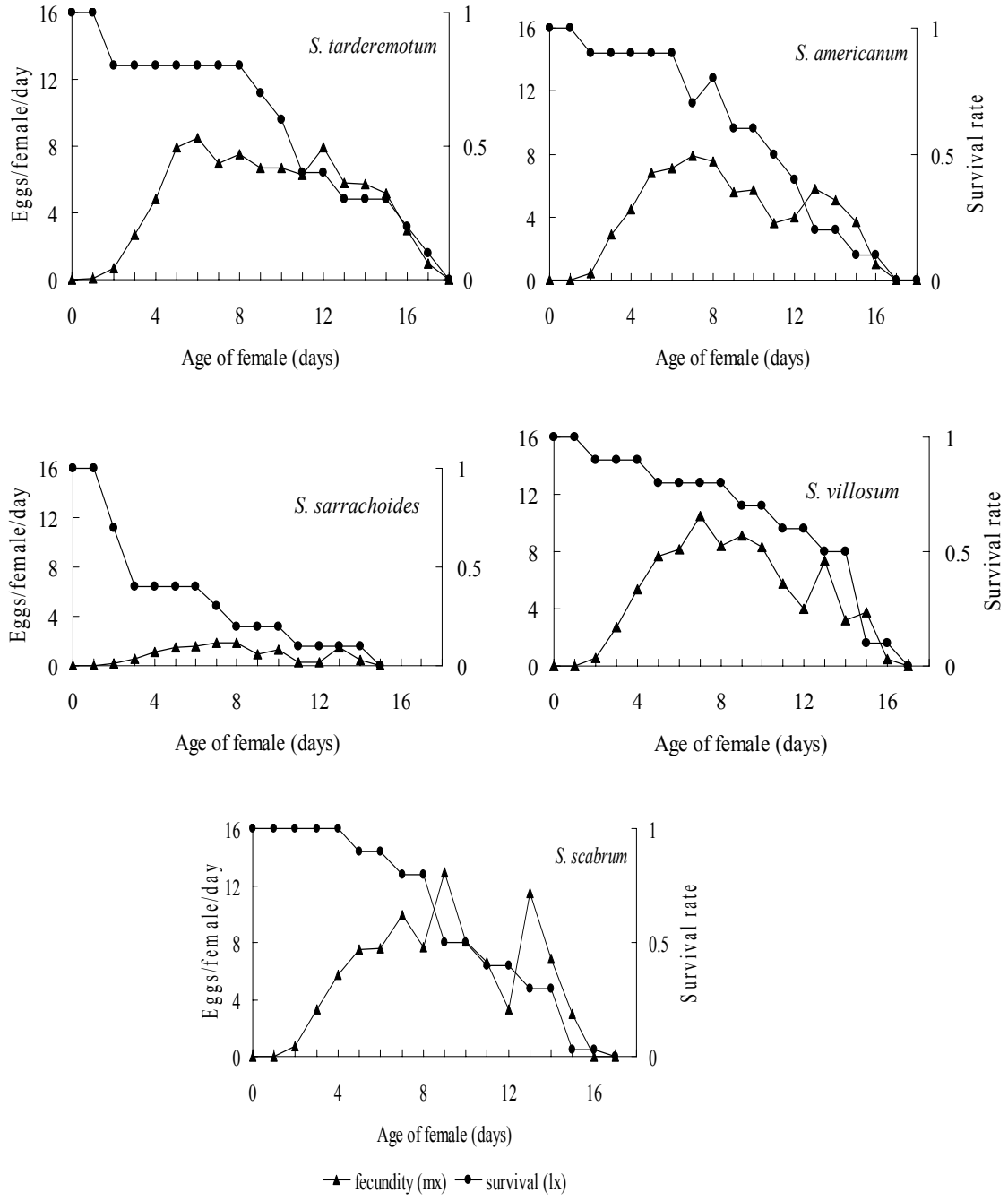

Figure 4.1 Age-specific fecundity (m_x) and age-specific survival rate (l_x) of *Tetranychus evansi* on five African nightshade species

4.4: Discussion

The study revealed that *T. evansi* performed differently on the five African nightshade species tested. Among the species tested, *S. sarrachoides* was the most unsuitable host whereas differences among the other nightshade species were not significant. The developmental time from egg to adult of *T. evansi* at 25 °C on all African nightshade species investigated was lower than reported by Moraes and McMurtry (1987) at the same temperature on leaves of nightshade, *Solanum douglasii* Dunal (13.1 days). However, Qureshi *et al*. (1969), also on *S. douglasii* reported a developmental time of 8.9 days at 23.3 °C and Bonato (1999) obtained 9.8 days on tomato at 26 °C.

The duration of the egg stage was the longest of all developmental stages in all studies of this mite which concurs with our present study. Bonato (1999) recorded developmental times from 6.3 – 13.6 days at temperatures ranging between 21 and 36 °C. The mean developmental time of the closely related mite species *Tetranychus ludeni* Zacher on nightshade *Solanum macrocarpon* L. was reported to be 10.1 days from egg to the adult stage (Adango *et al*., 2006) which relates very closely to those reported here. Fecundity and life table parameters of *T. evansi* were similar on *S. americanum*, *S. villosum*, *S. tarderemotum* and *S. scabrum* but significantly different on *S. sarrachoides*. The age specific survival rate (l_x) and age specific fecundity (m_x) show that *T. evansi* can successfully survive and reproduce on *S. tarderemotum*, *S. americanum*, *S. villosum* and *S. scabrum* but not on *S. sarrachoides*. The negative r_m shows that the *T. evansi* population will decrease on *S. sarrachoides*. This was caused by very low survival of

mite immatures, female fecundity and a short life span. The r_m value of between 0.180 to 0.196 females/female/day on the suitable nightshade species are relatively low compared to the published r_m of other *Tetranychus* species which range between 0.201 to 0.290 females/female/day at 25 ± 1 °C, except for *Tetranychus viennensis* Zacher (0.136) (Sabelis, 1985). Bonato (1999) reported an r_m value for *T. evansi* on tomato of 0.243 females/female/day at 26 °C, which is also higher than on the suitable nightshade species in this study.

Large differences in r_m values between host cultivars, probably due to different leaf nutrients and chemicals, were previously demonstrated in *T. urticae* (Van de Vrie *et al*. 1972; Leszczynski *et al*., 1988; Greco *et al*., 2006). Host plants as well as tomato plants used for mite rearing in this study belong to the family Solanaceae. Since mites suck plant contents, we cannot rule out the effect of constitutive secondary metabolites on population perfomance after transferring mites from tomato to *Solanum* sp. This presumably explains the higher immature mortality of *T. evansi* on *S. sarrachoides* compared with their counterparts on *S. americanum*, *S. villosum*, *S. tarderemotum* and *S. scabrum.* However, recent findings by Wosula *et al*. (2009) who reared *T. evansi* on tomato variety 'Cal J' and transferred them to tomato variety 'Money Maker' reported mite survival of 52.5%. Although host shift could have an effect in our results, mite mortality was also high in these recent findings. It is also widely agreed that although spider mites are highly polyphagous, they accept and perform differentially on diverse host plant species (Van den Boom *et al*., 2003; Greco *et al*., 2006). Further

investigations on the host shift by rearing mites for at least one generation on respective African nightshade species should be conducted to determine its effects on biological and demographic parameters of *T. evansi*.

4.5: Conclusion

This study demonstrates that *S. americanum*, *S. villosum*, *S. tarderemotum* and *S. scabrum* are suitable host plants for *T. evansi* and severe mite outbreaks are likely to occur under favorable conditions in the field. This differential suitability of nightshades to the mite is an important factor to consider while exploring IPM solutions for *T. evansi*. Moreover, a full knowledge of the morphological and chemical composition of these nightshade species as either potential host plants and/or non-host plants of the mite pest, and of how this can affect the growth of the mite might be an important key for developing resistant varieties for a cheaper and environmentally safer control of mite pest on vegetable farms in Africa.

CHAPTER FIVE

Effect of leaf trichomes and volatile chemicals of different African nightshade species on fecundity and behavioral response of *Tetranychus evansi* (Acari: Tetranychidae)

5.1: Introduction

Leaf trichomes are considered a mechanism of defense in many plants to prevent damage by herbivores (Marquis, 1992, Fernandes, 1994; Simmons and Gurr, 2005). Both physical and chemical effects on the incidence of trichomes on spider mites have been reported. Physically, trichomes may hinder or trap small arthropods such as spider mites (Simmons *et al*., 2003, 2004) and reduce fecundity (Handley *et al*., 2005). Gentile *et al*. (1969) and Rasmy (1985) reported that spider mites are killed when they get trapped in sticky exudates of trichomes of wild tomato. This exudate is released when the trichome cuticle breaks after touch by the mites (Van Haren *et al*., 1987).

Leaf trichomes may contain compounds that are toxic or repellent to spider mites and other arthropod herbivores attacking the plant (Williams *et al*., 1980; Kennedy and Dimock, 1983; Goffreda and Mutschler, 1989; van Dam and Hare, 1998). Volatile exudates of glandular trichomes of wild *Lycopersicon* which include the ketones; 2-tridecanone and 2-undecanone, and the terpenoids; zingiberene and γ-elemene, have been reported to be either toxic (Chatzivasileiadis and Sabelis, 1997) and/or repellent to *Tetranychus urticae* Koch (Weston *et al*., 1989). Various studies have also shown that

resistance of these *Lycopersicon* species to insects and mites depend upon type, density and exudate composition of glandular trichomes (Williams *et al*., 1980; Simmons *et al*., 2003). As such, spider mite repellence can be measured by quick, inexpensive techniques (Weston and Snyder, 1990) and can therefore be taken as indicative of the resistance level to other arthropod pests (Maluf *et al*., 2001).

Although research has shown the effects of trichomes of *Lycopersicon* species and their hybrids on pests including spider mites and their natural enemies (Simmons and Gurr, 2005), only limited information is available on the levels of resistance in African nightshades to spider mites. The objective of this study was to; (a) identify and quantify trichome types in different African nightshade species (b) assess the level of resistance of these nightshade species to *T. evansi* (c) estimate correlations between trichome densities and spider mite fecundity and movement and (d) and identify volatile chemicals associated with some of these nightshade species.

5.2: Materials and methods

5.2.1: Trichome identification and quantification

Plants of five different African nightshade species namely *Solanum villosum* (MW 13), *S. sarrachoides* (GBK 028726)*, S. scabrum* (SS 51), *S. tarderemotum* (MW 03) and *S. americanum* (SA) that were obtained from the Gene Bank of Kenya and AVRDC, Tanzania, were grown in the screenhouse following the procedures described in section 3.1.2. Plants that were 2, 4, 6 and 8 weeks old from the date of transplanting were used for these experiments. The sample contained 12 fully expanded leaves of plants of

respective ages that were collected from the 3rd – 5th portion of selected plants for each of the species and placed in beakers with water to prevent desiccation. Photographs of whole leaves on the abaxial surface were taken with a 25x light microscope (Leitz Orthoplan, Leitz GmbH, Wetzlar, Germany) along the midrib in order to establish the presence or absence of trichomes and their types. Classification of trichomes was made based on their morphology and the presence or absence of glands according to the criteria used by Luckwill (1943) to classify trichomes in *Lycopersicon* sp. Trichome counts were made under a 32x dissecting microscope (Leica MZ8; Leica Microsystems, Wetzlar, Germany) fitted with a square (each 0.11 mm^2) grid lens. Densities of each of the trichomes types were expressed as the number of trichomes per cm^2. Three replicates each with 36 leaves were carried out for respective nightshade species.

5.2.2: Spider mite fecundity and behavioral tests

5.2.2.1: Effect of trichomes on mite fecundity

Fecundity tests were carried out on leaf disks of the same age as the leaves on which number of trichomes was counted and representing each investigated species. One female deutonymph and two males were picked from the stock culture described in section 3.1.1 and placed on 25 mm leaf disks preserved on a Petri dish stacked with wet cotton wool. Four leaf disks per Petri dish constituted one replicate. Forty eight hours later, the female was checked as to whether it had emerged. The investigations on fecundity were evaluated during the first ten days of fecundate period. The experiments consisted of three replicates, each with 80 deutonymphs for respective plant species.

5.2.2.2: Thumbtack bioassays (no choice)

The resistance to *T. evansi* was quantified with the thumbtack bioassay as described by Weston and Snyder (1990) from leaves of respective species whose trichome density had previously been determined. One leaf of each of the five species was attached to a board of Styrofoam® through a metallic thumbtack (9 mm diameter) placed at the centre of its abaxial surface (Plate 5.1). Four leaves of each species were randomly placed on the Styrofoam® board, to comprise one replicate. Ten female spider mites were transferred with a fine paint brush to the head of each thumbtack. Distances traveled by each mite onto the leaf surface were measured as the shortest distance between the mite and the thumbtack edge, and were recorded after 15, 30, 45 and 60 min. Mites which stayed on the thumbtack were considered to have traveled a distance equal to zero. In order to differentiate leaf sizes among species, leaf length and width (in cm) were measured with a ruler. The trial was carried at 23 ± 1° C and 50 - 70% RH. Three replicates, each with 40 spider mites were carried out per species.

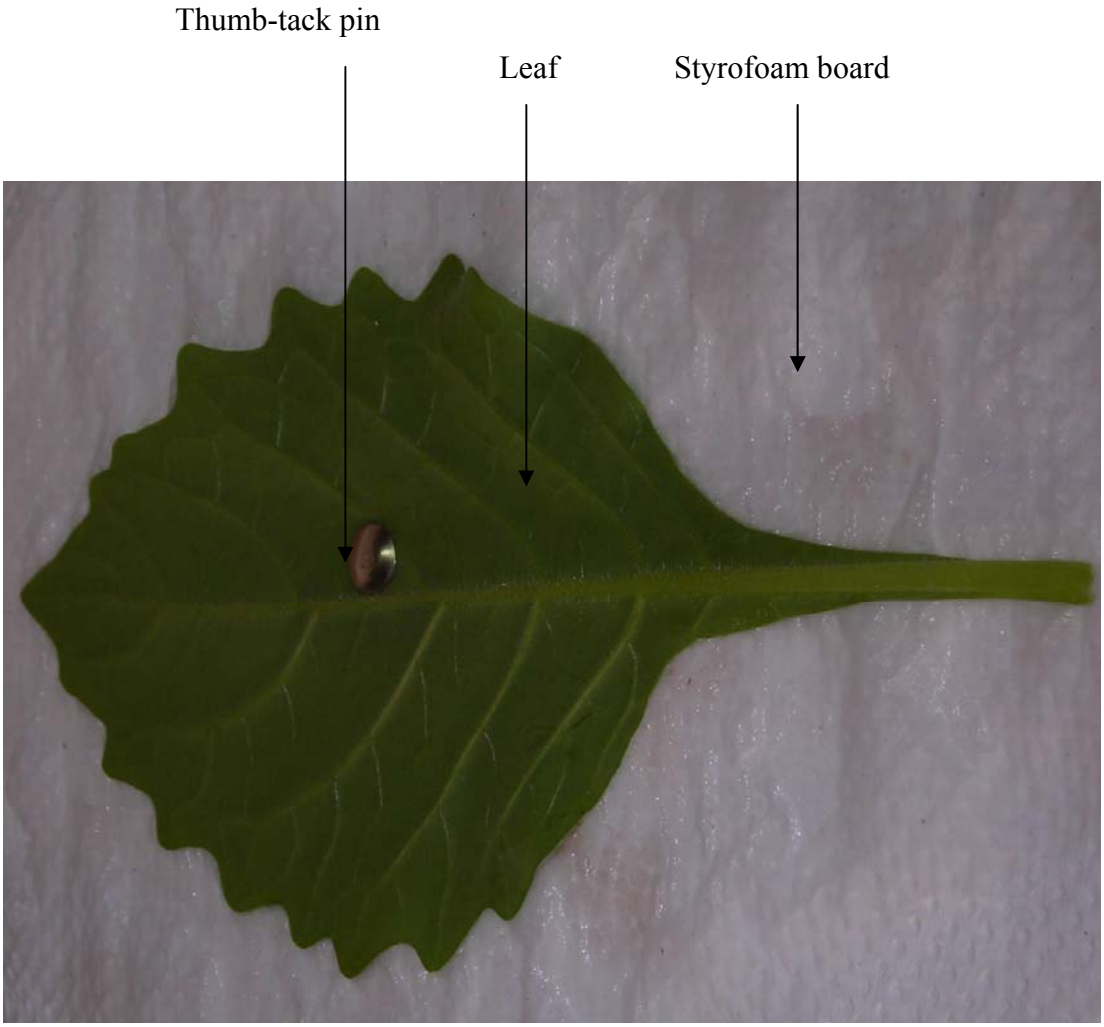

Plate 5.1 Thumb-tack (no-choice) bioassay set-up in the laboratory

5.2.2.3: Olfactometer bioassays

To quantify the olfactory response of female *T. evansi* to odor emitted by intact nightshade plants, a closed-system Y-tube olfactometer modified from that described by Sabelis and Van de Baan (1983) was used (Figure 5.1). A potted plant of the test accession was maintained in a glass chamber connected to one arm of the olfactometer while a blank chamber was connected to the other arm. Adult females were introduced singly at the entrance of the trunk and observed for a maximum of 10 min. A positive (+) response to the odor stimulus was recorded when the females that oriented themselves towards the odor source from the test accession reached the far end of the arm within 10 min from the start of the experiment.

Those mites that followed the control air stream in the opposite arm were recorded as showing a negative response (-). Mites that walked up and down without reaching any of the arms within 10 min were recorded as non-responders (0). After observing three individual spider mites, the Y-tube was removed and replaced with a clean one. The bioassay was performed at 23° ± 2°C, 50–70% R.H and during the day (0900 – 1400 hrs) under fluorescent lighting. The experiment was replicated four times, each with 10 spider mites per plant species.

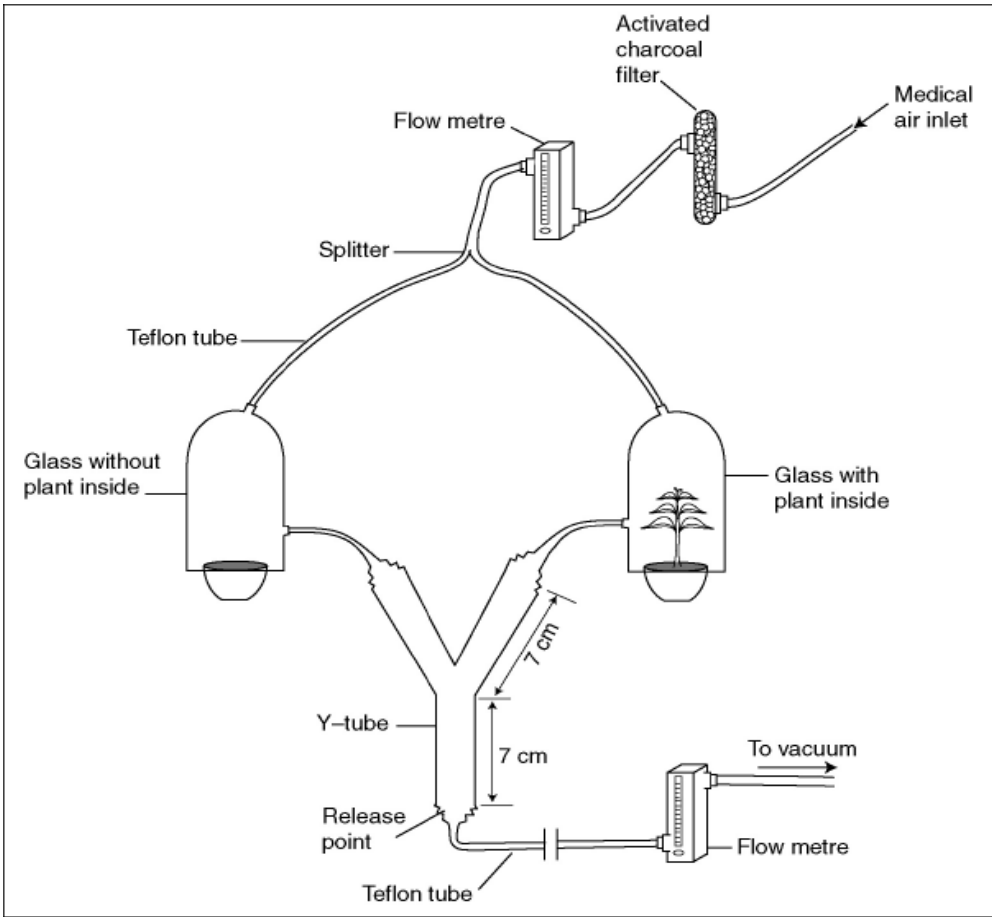

Figure 5.1 A schematic representation of Y-tube olfactometer set-up in the laboratory (Drawing courtesy of A. A. Yusuf, *icipe*, 2009)

5.2.3. Volatile collection and analysis

Based on the results from the mite behavioral studies, intact plants of two nightshade species; *S. sarrachoides and S. villosum*, were selected for volatile collection and analysis. Chemical odors emitted by intact potted plants of African nightshade were collected using a portable volatile collection system (Plate 5.2). The volatile collection system consisted of an air suction pump (Air Cadet vacuum/pressure station, Cole Palmer Instrument Co., USA), a flow meter, (Cole Palmer Instrument Co., USA), Reynolds® oven bag (turkey size 482 mm x 596 mm, Reynolds Kitchens, Richmond, VA) and Super Q adsorbent traps (Analytical Research Systems, Gainesville, FL).

The bag was cleaned by baking it for 12h in an oven at 120° C. The oven cleaned bag was placed over a branch with foliage and was closed up around the stem of the branch with a strong PVC thread. Airflow into the sampling bag was provided by two Teflon® tubes. One tube pushed air into the bag over the foliage while the other tube pulled air out of the bag through the super Q trap at the end and then through the flow meter at a rate of 265 ml/min. Volatiles were collected for 2h in the morning between 0930 and 1130 h. The adsorbent trap was removed, sealed with Teflon® tape and stored in a freezer (-18° C) until use. The experiment was replicated three times, each with 5 plants for each investigated species.

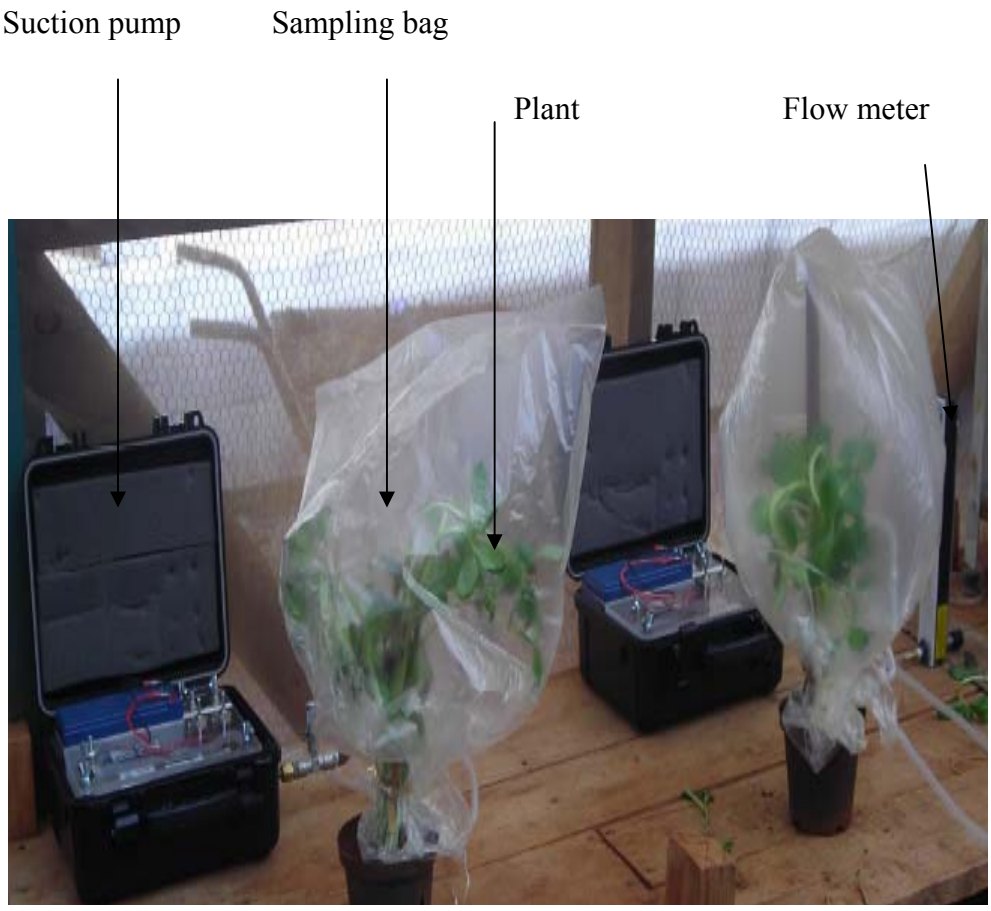

Plate 5.2 Volatile collection system demo placed on a bench in the greenhouse

All the adsorbent traps were eluted with 100 µl of GC/GC-MS–grade dichloromethane (Burdick and Jackson, Muskegon, Michigan, USA) and 1µl of Methyl Salicylate (Sigma Aldrich) was added to each respective 40 µl sample as the internal standard. One µl aliquot of the volatile extracts was analyzed by GC-MS as described in section 3.4. The temperature program was initialized at 3 min with the oven temperature at 35º C and then programmed at the rate of 10º C to 280º C where it was held for 12 min. Identification of constituent compounds in the volatiles was based on the interpretation of the mass spectral fragmentation obtained within the NIST MS data libraries (HP, USA) to obtain preliminary structural assignments followed by comparisons with spectral data via co-injections with authentic commercial standards.

Chemicals: Commercial standards such as hexanal, heptanal, decanal, nonanal, octanal, α-pinene, β-pinene and D-limonene were purchased from Aldrich (Milwaukee, Wisconsin, USA) while 3-methyl-2-butenal and β-ocimene were donated by Dr. Peter Teal of the USDA/ARS-CMAVE, Gainesville, Florida, USA. Their purities ranged from 95-99%.

5.2.4: Statistical analysis

Data on leaf trichome density and fecundity after the specified number of weeks was subjected to analysis of variance (Proc ANOVA, SAS Institute, 2000) and means separated by Student Newman Keuls test ($P \leq 0.05$). Trichome density was however square root transformed before analysis in order to normalize the error distribution. Back

transformed data is presented. Correlations (Proc CORR, SAS Institute, 2000) between trichome density and fecundity were carried out. Data on distances traveled by mites onto respective species and mites remaining on tack after the specified time duration were analyzed by general linear model analysis (Proc GLM, SAS Institute, 2000) and means separated by Student Newman Keuls test ($P \leq 0.05$), to confirm whether the bioassay detected differences in mite repellence amongst the nightshade species. Distances traveled by mites onto respective species were correlated with their trichome densities (Proc CORR, SAS Institute, 2000). The choices of the mites in olfactometer bioassays were analyzed with chi-square test (χ^2) test to determine significant differences from a 50:50 distribution. Concentration of each detected compound was computed based on the abundance of the internal standard injected into the GC-MS.

5.3: Results

5. 3.1: Trichome identification and quantification

A description of the morphological characteristics of identified trichomes is given in Table 5.1. The range in size of glandular trichomes (type i, ii) was <0.1-5 mm long while non-glandular trichomes (types iii, iv and v) were shorter in size with a range of <0.1-0.8 mm. The head of type i and ii trichomes was swollen in a glandular vesicle although larger in the latter (Table 5.1). Glandular trichomes, types i, were present only in *S. sarrachoides* while type ii were present but sparsely distributed in *S. sarrachoides* and *S. villosum*. Non-glandular trichomes, types iii, iv and v, were present in *S.*

tarderemotum, *S. scabrum* and *S. villosum* respectively. Leaves of *S. americanum* were glabrous (Figure 5.2).

Significantly differences were detected in the number of trichomes on an abaxial leaf surface of five African nightshade species. *S. sarrachoides* had the highest number of glandular trichomes whereas *S. villosum* had the highest number of non-glandular trichomes in comparison to other *Solanum* species examined (Table 5.2). Also significant differences were detected between the number of glandular and non-glandular trichomes and a time of taken measurements in *S. sarrachoides* (glandular: $F = 18.25$; $P<0.0001$), *S. villosum* (glandular: $F = 14.79$; $P<0.0001$; non-glandular: $F = 13.72$; $P<0.0001$), *S. scabrum* (glandular: $F = 4.24$; $P<0.0057$; non-glandular: $F = 7.49$; $P<0.0001$) and *S. tarderemotum* (glandular: $F = 10.78$; $P<0.0001$; non-glandular: $F = 13.48$; $P<0.0001$). Glandular trichomes increased from the 4^{th} to 8^{th} week of sampling on *S. sarrachoides* (> 30 trichomes/mm^2) whereas the density of non-glandular trichomes varied inconsistently in *S. villosum* (>10 trichomes/mm^2) from the 2^{nd} to the 8^{th} week of sampling. However, non-glandular trichomes were absent in *S. sarrachoides* and both trichome types were completely absent in *S. americanum* (Table 5.2).

Table 5.1 Description of trichome types identified in five examined African nightshade species

Nightshade species	Trichome type present †	Trichome description
S. sarrachoides	i	Slender, long, glandular hairs, 0.2-5 mm long, standing on a single stalk base, tip swollen in a small glandular vesicle.
S. Sarrachoides & *S. villosum*	ii	Short, glandular hairs, <0.1 mm long, standing on a single and thin stalk base, tip swollen in a large glandular vesicle.
S. tarderemotum	iii	Non-glandular appressed hairs, 0.1-0.5 mm long, tip pointed, standing on a single stalk base.
S. scabrum	iv	Non-glandular long appressed hairs, 0.6-0.8 mm long, standing on a single stalk base.
S. villosum	v	Non-glandular long appressed hairs, 0.4-0.8 mm long, tip pointed, standing on a large stalk base.

† i, ii, iii, iv and v refers to a randomly chosen roman number assigned to identified trichomes

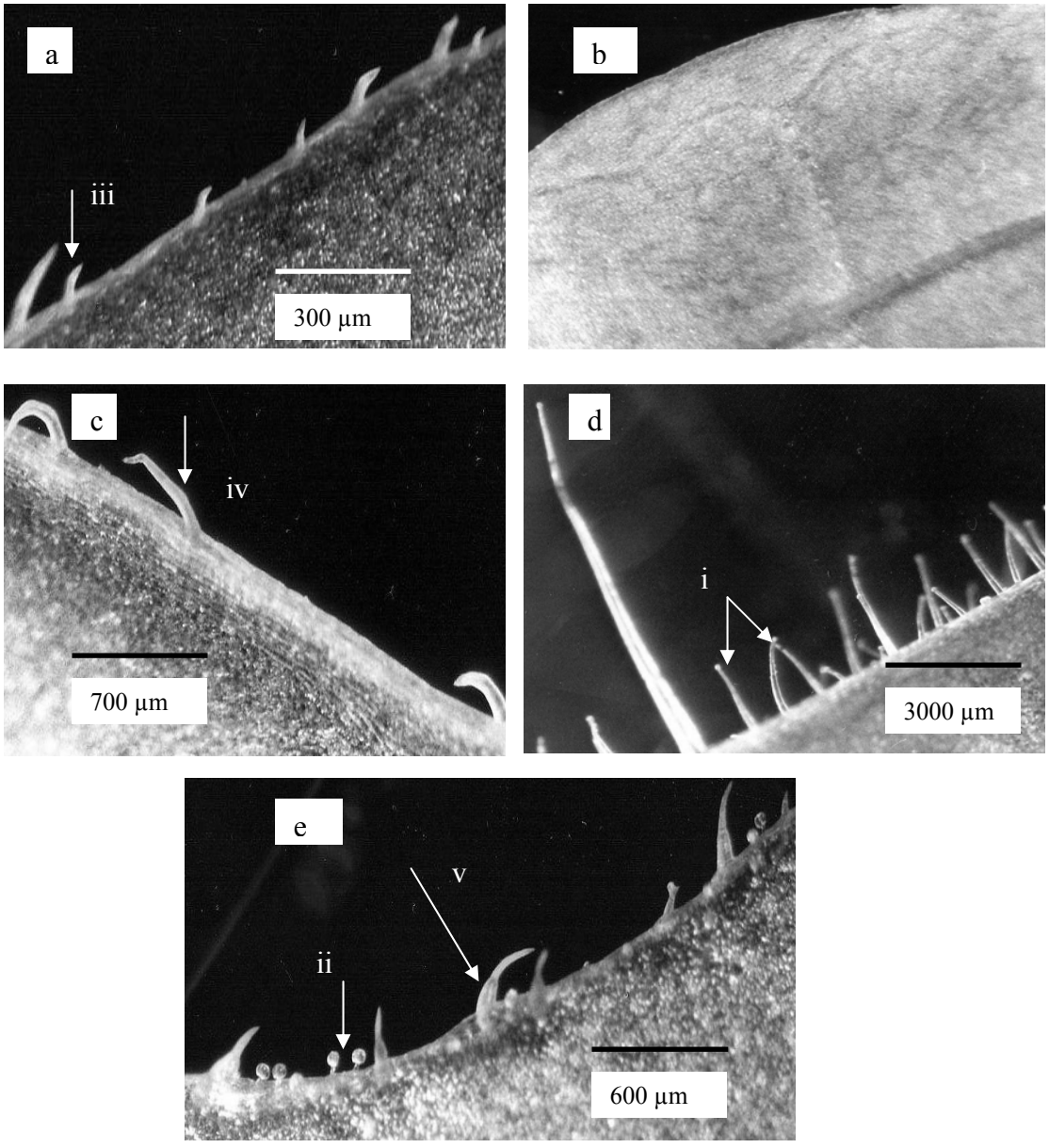

Figure 5.2 Trichome structures and types of five selected African nightshade species; a = *S. tarderemotum*; b = *S. americanum*; c = *S. scabrum*; d = *S. sarrachoides*; e = *S. villosum*.

Table 5.2 Trichome density (per mm^2) from leaves of examined African nightshade species

Nightshade species	Number of glandular trichomes† (i & ii)				Number of non-glandular trichomes‡ (iii, iv & v)			
	2 (wks)	4 (wks)	6 (wks)	8 (wks)	2 (wks)	4 (wks)	6 (wks)	8 (wks)
S. sarrachoides	31.8a^{B}	30.8a^{B}	33.6a^{B}	42.1a^{A}	0.0c^{A}	0.0c^{A}	0.0d^{A}	0.0d^{A}
S. villosum	2.7b^{B}	4.8b^{B}	5.1b^{B}	10.1b^{A}	19.4a^{A}	12.9a^{BC}	11.2a^{C}	14.5a^{B}
S. scabrum	3.1b^{B}	6.2b^{A}	4.1b^{AB}	5.6c^{A}	5.8b^{A}	2.7b^{C}	3.9c^{BC}	4.7c^{AB}
S. tarderemotum	1.4bc^{B}	4.5b^{A}	1.4c^{B}	4.4c^{A}	6.7b^{B}	3.9b^{C}	8.9b^{A}	7.2b^{B}
S. americanum	0.0c^{C}	0.0c^{C}	0.0c^{B}	0.0d^{A}	0.0c^{A}	0.0c^{A}	0.0d^{A}	0.0d^{A}
F	575.5	311.5	453.2	306.9	203.0	124.4	126.3	129.4
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means followed by the same letter within columns (showing differences among species) and within rows (in capital letters, superscript; showing differences among weeks) are not significantly different ($\alpha = 0.05$; SNK test) † = have 'heads'; ‡= have no 'heads'

5.3.2: Mite fecundity and behavioral responses

5.3.2.1: Effect of trichomes on mite fecundity

Significant differences were detected in number of eggs laid per female during the first 10d of fecundate period. Also, the number of eggs laid per female mite was significantly influenced by plant age when females were placed on *S. americanum*, *S. tarderemotum*, *S. villosum* and *S. scabrum* (Table 5.3). Mites laid significantly fewer eggs when placed on *S. sarrachoides* compared to other *Solanum* species. There was a significant negative correlation between the number of glandular hairs on the abaxial side and fecundity of *T. evansi* (Table 5.4).

Table 5.3 Fecundity of *Tetranychus evansi* on leaves of examined African nightshade species during the first 10 days of fecundate period of their life

Plant species	Sampling after			
	2 (wks)	4 (wks)	6 (wks)	8 (wks)
S. sarrachoides	$11.3b^{A}$	$9.4c^{A}$	$9.3c^{A}$	$3.8c^{A}$
S. villosum	$12.3b^{B}$	$35.2b^{A}$	$42.4a^{A}$	$29.8b^{A}$
S. scabrum	$24.3ab^{B}$	$57.2a^{A}$	$45.8a^{A}$	$44.5a^{A}$
S. tarderemotum	$31.1a^{B}$	$55.8a^{A}$	$35.4ab^{B}$	$37.2ab^{B}$
S. americanum	$16.8ab^{B}$	$48.1ab^{A}$	$27.2b^{B}$	$28.0b^{B}$
F	3.6	13.3	10.4	23.8
P	0.0094	<0.0001	<0.0001	<0.0001

Means followed by the same letter within columns (showing differences among species) and within rows (capital letter, superscript; showing differences among weeks) are not significantly different ($\alpha = 0.05$; SNK test)

Table 5.4 Coefficients of correlation (R) between trichome density and fecundity of *Tetranychus evansi* during the first 10 days of fecundate period of their life

	Fecundity (R)	P
Glandular	-0.649*	0.0019
Non-glandular	0.122ns	0.6095

*= significant; ns = not significant ($\alpha = 0.05$)

5.3.2.2: Thumb-tack bioassays (no choice)

Distances walked by *T. evansi* females onto the leaf surface after 15, 30, 45 and 60 min were significantly shorter on *S. sarrachoides* compared with other *Solanum* species tested (Table 5.5). *S. scabrum* had significantly broader and longer leaves and thus had the longest distance traveled by mites on the leaf surface (Table 5.5). However, the number of mites remaining on tack did not differ significantly with those of *S. tarderemotum*, *S. americanum* and *S. villosum* except at 60 min. Number of mites remaining on the tack after all the time durations were significantly more on *S. sarrachoides* (Table 5.6). A significant negative correlation between the densities of glandular trichomes and the distance traveled by mites after 15, 30, 45 and 60 min was detected (Table 5.7).

Table 5.5 Average distance (in cm) traveled by *Tetranychus evansi* females on the abaxial leaf surface of five selected African nightshade species; length and width of leaves of the five African nightshade species

Plant species	Distance traveled by mites on the abaxial leaf surface after				Leaf size	
	15 (min)	30 (min)	45 (min)	60 (min)	Length (cm)	Width (cm)
S. sarrachoides	0.2 ± 0.0d	0.2 ± 0.0c	0.3 ± 0.1d	0.3 ± 0.1c	19.3 ± 0.9b	9.1 ± 0.2cd
S. americanum	2.3 ± 0.2c	2.8 ± 0.2b	2.3 ± 0.2c	3.1 ± 0.2b	21.6 ± 1.0b	11.9 ± 0.4b
S. villosum	3.4 ± 0.2b	3.4 ± 0.2b	3.3 ± 0.2b	3.2 ± 0.1b	19.7 ± 1.1b	10.0 ± 0.4c
S. tarderemotum	3.0 ± 0.2bc	3.3 ± 0.2b	3.5 ± 0.2b	3.5 ± 0.2b	19.4 ± 0.8b	7.8 ± 0.2d
S. scabrum	4.6 ± 0.3a	5.1 ± 0.2a	5.1 ± 0.2a	5.1 ± 0.2a	32.8 ± 0.8a	17.3 ± 1.0a
F	68.3	100.8	100.3	100.3	40.9	45.5
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means followed by the same letter within columns are not significantly different ($\alpha = 0.05$; SNK test).

Table 5.6 Number of *Tetranychus evansi* females remaining on tack after 15, 30, 45 and 60 min on five African nightshade species

Plant species	Number of mites remaining on tack after			
	15 (min)	30 (min)	45 (min)	60 (min)
S. sarrachoides	4.7 ± 0.7a	3.9 ± 0.9a	4.0 ± 0.9a	4.1 ± 0.9a
S. americanum	0.7 ± 0.4b	0.6 ± 0.4b	0.3 ± 0.3b	0.3 ± 0.3b
S. villosum	0.8 ± 0.3b	0.6 ± 0.3b	0.5 ± 0.2b	0.6 ± 0.3b
S. tarderemotum	0.8 ± 0.4b	0.8 ± 0.3b	0.8 ± 0.4b	0.8 ± 0.4b
S. scabrum	0.8 ± 0.3b	0.2 ± 0.1b	0.1 ± 0.1b	0.0 ± 0.0b
F	15.2	10.9	13.7	12.1
P	<.0001	<.0001	<.0001	<.0001

Means followed by the same letter within columns are not significantly different (α = 0.05; SNK test; SAS Institute, 2000).

Table 5.7 Coefficients of correlation (R) between trichome densities and distance traveled by *Tetranychus evansi* females on the abaxial surface after 15, 30, 45 and 60 min of four week old African nightshade plants

Trichome types		Distance (in cm) traveled by mites onto the leaf surface after			
		15 min	30 min	45 min	60 min
Glandular	(R)	-0.412**	-0.427**	-0.390**	-0.436**
	(P)	<0.0001	<0.0001	<0.0001	<0.0001
Non-glandular	(R)	0.199	0.137	0.143	0.089
	(P)	<0.0001**	0.0008*	0.0005*	0.0302*

** = Highly significant; *= significant (α = 0.05; SAS Institute, 2000); glandular trichomes = i & ii; Non-glandular = iii, iv & v

5.3.2.3: Olfactometer bioassays

Significantly more spider mites chose the direction with *S. villosum* (27.5%) when comparisons were made against the control (5%), while significant differences were not observed between the control and the other nightshade species tested (Figure 5.3).

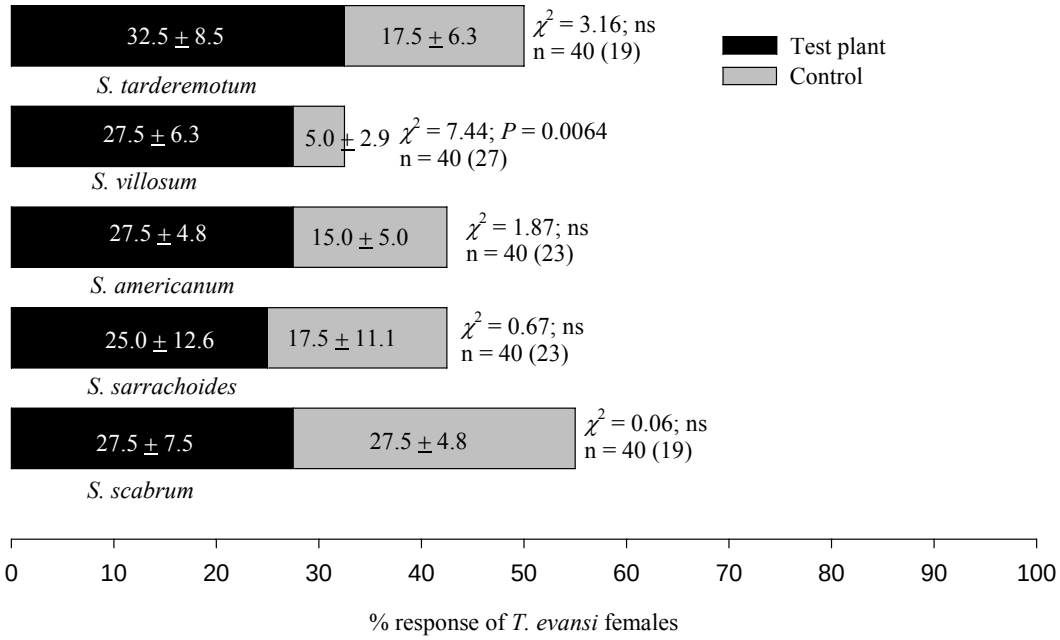

Figure 5.3 Response of *Tetranychus evansi* females to whole plant odors of African nightshades in a Y-tube olfactometer (mean % ± S.E)
ns indicates lack of significant differences between the control and the test accession
Chi-square test ($\alpha = 0.05$; χ^2); number of mites that did not make a choice is shown in brackets

5.3.3 Analysis of volatiles

Intact potted plants of *S. sarrachoides* and *S. villosum* released volatiles that varied quantitatively between the two species (Table 5.8). Compounds found in the volatiles were identified by comparison with authentic standards as belonging to the following classes; terpenoids, esters, aldehydes, ketones and green leaf alcohols. Quantification of

these compounds revealed that except for the ketones, other compounds were significantly higher in *S. sarrachoides* than in *S. villosum* (Figure 5.4).

Table 5.8 Amount of volatile compounds identified from 4-6 week old intact plants of two *Solanum spp*

Peak No.	Compounds	*S. sarrachoides* Conc. (ng/µl)	*S. villosum* Conc. (ng/µl)
1	2-Pentanone	0.07 ± 0.04	-
2	3,4-Dimethyl-1-nonen-4-ol	0.02 ± 0.02	-
3	4-Methyl-4-nonanol	0.01 ± 0.01	-
4	Hexanal	0.07 ±0.04	(tr)
5	(Z)-3-Hexen-1-ol	0.60 ± 0.20a	0.08 ± 0.04b
6	2-Nonen-4-one	0.03 ± 0.03	-
7	3,3-Dimethyl-2-butene	0.01 ± 0.01	-
8	3-Methyl-2-butenal	0.01 ± 0.01	-
9	(E)-3-Methyl-2-nonene	0.04 ± 0.04	-
10	α-Pinene	0.03 ± 0.03	(tr)
11	Benzaldehyde	0.03 ± 0.02	(tr)
12	β-Pinene	0.06 ± 0.03	(tr)
13	(Z)-3-Hexenyl acetate	0.11 ± 0.11a	0.34 ± 0.11a
14	D-Limonene	0.08 ± 0.05a	0.02 ± 0.02a
15	Nonanal	0.11 ± 0.04a	0.01 ± 0.01a
16	Decanal	0.19 ± 0.09	(tr)
17	2-Decanone	-	(tr)
18	2,2- Dimethyl-3-octene	-	0.01 ± 0.01
19	4-Octen-3-one	-	0.11 ± 0.08
20	(E)-2-Pentenal	-	0.03 ± 0.03
21	6-Methyl-5-hepten-2-one	-	0.04 ± 0.04
22	2-Tridecanone	-	(tr)

(tr) = trace concentration < 0.01 ng; - not detected; means followed by the same letter within rows are not significantly different (α = 0.05; SNK test; SAS Institute, 2000)

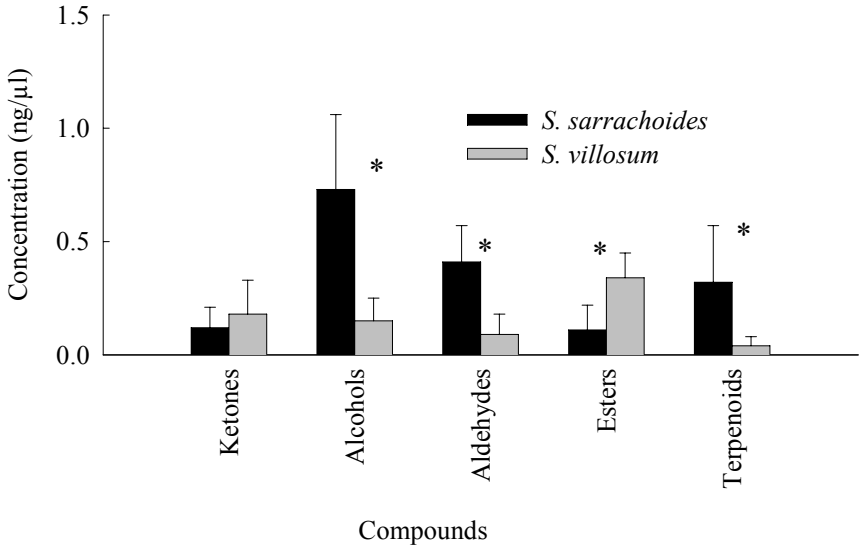

Figure 5.4 Variation in total volatile compounds extracted from leaves of intact plants of two different African nightshade species; * indicates significant differences; vertical bars indicate standard errors; (Student Newman Keuls test; $\alpha = 0.05$)

Representative total ion chromatograms of major volatile extracts from *S. sarrachoides* and *S. villosum* intact plants revealed quantitative and qualitative differences in the number of profiles with the green leaf volatiles as the most abundant in each respective species (Figure 5.5).

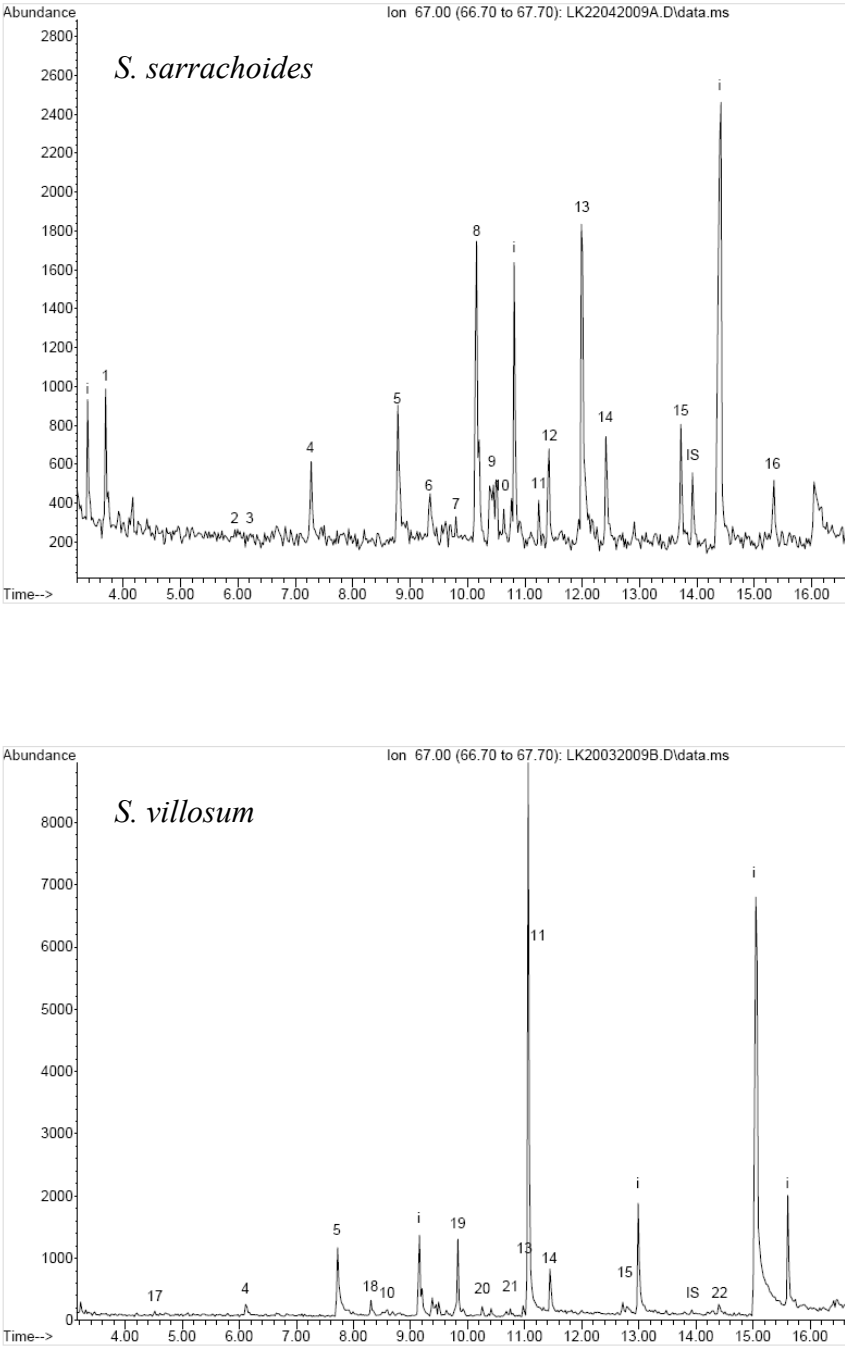

Figure 5.5 Representative ion chromatograms of volatiles adsorbed on Super Q from different samples of 4-6 week old *Solanum sarrachoides* and *S. villosum* plants. Peak number –compound: 1. 2-Pentanone; 2. 2. 3,4-Dimethyl-1-nonen-4-ol; 3. 4-Methyl-4-nonanol; 4. Hexanal; 5. (Z)-3-Hexen-1-ol; 6. 2-Nonen-4-one; 7. 3,3-Dimethyl-2-butene; 8. 3-Methyl-2-butenal; 9. (E)-3-Methyl-2-nonene; 10. α-Pinene; 11. Benzaldehyde; 12. β-Pinene; 13. (Z)-Hexenyl acetate; 14. D-Limonene; 15. Nonanal; 16. Decanal; 17. 2-Decanone; 18. 2,2-Dimethyl-3-octene; 19. 4-Octen-3-one; 20. (E)-2-Pentenal; 21. 5-Methyl-5-hepten-2-one; 22. 2-Tridecanone; i-impurity; IS-Internal standard

5.4: Discussion

The results from this study demonstrate that nightshade species may differentially influence fecundity, distance walked and olfactory responses of *T. evansi*. These differences were strongly negatively correlated to the density of glandular trichomes, suggesting that its association with resistance to *T. evansi* is both morphological and chemical. While morphological factors such as trichomes release exudates that entangle the small arthropod pests such as spider mites (Simmons *et al*., 2003, 2004), chemical compounds released may act as either toxins or repellents to the mites (Williams *et al*., 1980; Kennedy and Dimock, 1983; Goffreda and Mutschler, 1989).

The influence of the host plants on fecundity of *T. evansi* seems to vary with the age of the plant. Among the nightshade species, the density of glandular trichomes in *S. sarrachoides* increased with the age of the plant which negatively resulted into a decrease in mite fecundity. These results concur with those of Saeidi *et al*. (2007) who reported that the density of type IV glandular trichomes in *Lycopersicon* sp. increased with the age of the plant as well as the resistance to the two-spotted spider mite. The varied effects of trichome type and density in Solanaceous crops have been previously reported by several authors to confer resistance to spider mites and other arthropod pests (Thurston, 1970; Duffey and Isman, 1981; Kennedy and Sorenson, 1985; Goffreda *et al*., 1988; Wilkens *et al*., 1996; Elle *et al*., 1999; Simmons and Gurr, 2005).

Solanum scabrum, *S. villosum* and *S. tarderemotum* were characterized by high densities of non-glandular trichomes with a positive correlation with fecundity and distance traveled by mites. Although there are very few studies that have reported a positive relationship between non-glandular trichomes and survival of a pest (Simmons and Gurr, 2005), our results concur with those of Gurr and McGrath (2002) who reported that the density of type V non-glandular trichomes increased adult emergence and number of mines of potato tuber moth. In addition, Simmons and Gurr (2005) indicated that some pests prefer hirsute surfaces for oviposition and that pubescence may not always be favorable for pest suppression. Unlike the other *Solanum* species, in our case *S. americanum* is completely glabrous, and its resistance to *T. evansi* was low and was not significantly different in comparison to *S. scabrum*, *S. villosum* and *S. tarderemotum* in thumb-tack studies. Griesbach *et al*. (2002) screening for arthropod resistance in a petunia ecotype with glabrous leaves, also reported that the absence of trichomes was correlated with a lower resistance to spider mites.

Several differences observed in spider mite responses to fecundity, olfactory cues and distances walked on different nightshade species, could be attributed to the quantity and quality of volatile phytochemicals and other secondary metabolites among these plants. Glandular trichomes may permanently exude secretions soon after their production (Gregory *et al*., 1986) or secretions are discharged after the death of the trichome (Fahn, 1979), which results in an enrichment of the plant surface and the immediate vicinity with volatile and non-volatile repellents (Kellogg *et al*., 2002).

A number of volatile chemicals identified in the intact plants of *S. sarrachoides* and *S. villosum* ranged from green leaf volatiles, terpenes, aldehydes and ketones, but they differed qualitatively and quantitatively between the species. Although these differences in the volatile blend could be attributed to the type of plant tissue, abiotic factors such as light and moisture (Scutareanu *et al*., 1997; Gnanvossou *et al*., 2003) may also influence their emission. These volatile compounds represent an arsenal of defenses ranging from chemical toxins to feeding deterrents (Langenheim, 1994). For instance, although *S. sarrachoides* lacked volatile characteristics suitable to attract *T. evansi* in olfactometer studies, other responses such as fecundity might be influenced by the secretions that inhibit feeding (Levin, 1973; Stipanovic, 1983; Duffey, 1986), while mites walking on the leaf surface may get entrapped on the sticky exudates and subsequently dehydrated (Patterson *et al*., 1974; Rasmy, 1985).

Some of the volatiles identified in nightshades in this study have been previously reported to play a role in eliciting behavioral responses in other phytophagous arthropods. For instance, (*Z*)-3-hexen-1-ol was reported to elicit responses of the European spruce bark beetle *Ips typographies* (L.) released from intact birch leaves (Zhang *et al*., 1999) while (*Z*)-3-hexenyl acetate was found to be attractive to adult Colorado potato beetle, *Leptinotarsa decemlineata* (Visser *et al*., 1978). Hexanal was reported to increase trap catches of the adult carrot fly *Psila rosae* Fab (Guerin *et al*., 1983), reduce mite fecundity (Avdiushko *et al*., 1997) and decrease feeding of tomato horn worm, *Manduca sexta* L (Bolter *et al*., 1997). Limonene was reported to repel the

leek moth (Al Rouz and Thibout, 1988) and in combination with other terpenes, has been reported to prevent insect attack of pine cones (Dormont *et al*., 1997). Therefore, volatile phytochemicals are natural plant compounds that might be potential alternative pesticides (Lee *et al*., 2001; Ibrahim *et al*., 2004) that are not persistent in the environment and are safe to natural enemies, non target organisms and human beings for use in sustainable agriculture (Lacey and Shapiro-llan, 2003).

5.5: Conclusion

High densities of glandular trichomes particularly type i predominant in *S. sarrachoides* is highly associated with high levels of *T. evansi* resistance in this study. Therefore, trichome–based host plant resistance is a mechanism that should be taken into account when selecting plant cultivars for pest resistance. Selection and introgression of genes encoding for higher glandular trichome densities in susceptible African nightshade species is effective in order to obtain lines with increased level of resistance not only to *T. evansi* but to other spider mite species and other vegetable pests.

CHAPTER SIX

Population dynamics of *Tetranychus evansi* (Acari: Tetranychidae) on different African nightshade species grown under screenhouse conditions

6.1: Introduction

Spider mites have characteristic feeding symptoms on leaves causing damage to chlorophyll leaving stipples that become coherent with time (Nachman and Zemek, 2002). In severe cases, leaves become bronzed, dry out and even fall off the plant (Smith Meyer, 1996; ICIPE, 2004). Although *T. evansi* is not a serious pest of commercial greenhouse grown crops in Kenya which are mainly ornamentals (Wainwright, Pers. Comm.), it has been reported to have a high adaptation to various environmental conditions due to its ability to survive and multiply within a wide temperature range of between 10-36° C (Bonato, 1999), thus outbreaks in greenhouses may be expected. For instance, Migeon (2005) concluded that *T. evansi* has the potential to colonize both heated and unheated glass houses in most parts of France.

African nightshades are important indigenous vegetables that play an invaluable role in meeting the nutritional and economic needs of many rural households in Africa (Mwai *et al*., 2007). They are alleged to be tolerant to pests and diseases and there is no information regarding any plant resistance traits for genetic enhancement. Small scale farmers who are faced with the challenge of managing pests on indigenous vegetables (Schippers, 2001) have to rely heavily on pesticides which in the long-run raise both

consumer and environmental concerns (Hoy, 1998; Varela *et al*., 2003). Plants with resistance offer an efficient, economical and environmentally responsible alternative pest management approach.

Since screenhouse screening techniques are economical in time, space and labor, they have successfully been used previously to screen for resistance of several *Lycopersicon* species to *T. evansi* (Wosula, 2006). The number of life stages, if counted early at start up of infestation, for instance the number eggs laid per plant or motile individuals per leaf could provide a criterion for assessing non-preference (antixenosis) mechanism in indigenous vegetables (Sithanantham *et al*., 2003). Recent studies in the laboratory demonstrated that physical and chemical attributes of some nightshade species affect performance characters such as development, fecundity and survival of *T. evansi* that should influence population size.

Investigating further the population densities of *T. evansi* through screenhouse screening will give an indication of the nightshade species that are suitable for *T. evansi* population growth, and potentially expedite the germplasm screening process. Thus, the objective of this study was to establish (a) population densities of *T. evansi* on different African nightshade species grown under screenhouse conditions (b) the extent of damage caused by *T. evansi* infestation on these species

6.2: Materials and methods

6.2.1: Experimental site description

Screenhouse experiments were carried out at the Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya (latitude 1°10' 48' S, longitude 37° 07' 12' E, altitude 1525 m above sea level) between September, 2007 and December, 2008.

6.2.2: Spider mite culture and plant establishment

Spider mites were obtained from a stock culture maintained at *icipe* under experimental conditions described previously in section 3.1.1. Seedlings of five African nightshade species were raised in a screenhouse following the procedures also described in section 3.1.2. Four weeks after sowing (15 cm tall), they were transported to the experimental site at Jomo Kenyatta University of Agriculture and Technology.

6.2.3: Pots and planting medium

Seedlings were transplanted into ca. 29-cm inner diameter plastic pots and placed on raised wooden benches raised to a height of 73-80 cm above the ground. Each pot was thoroughly cleaned and dried before filling it with the potting medium. A mixture of soil, manure and sand in the ratios 3:2:1 (v/v) was prepared and used as the planting medium.

6.2.4: Cultural practices

Plants were watered with one liter of clean tap water on daily basis every morning and were top dressed with 3 g of CAN plant $^{-1}$ a week after transplanting. Since no pesticides were used throughout the experiment, scouting for insect pests such as aphids was done daily and if found, they were removed with a brush and killed while yellow sticky traps were placed in strategic positions within the screenhouse to mass trap all stages of flying insects.

6.2.5: Mite population density assessment

Four studies were carried out to assess the effect of five different African nightshade species on population density of *T. evansi*. The greenhouse temperature was monitored using a HOBO Pro Series Temp, RH monitor (version 32, 1998 ONSET, HOBO data loggers). The daily average temperature in the screenhouse was 21 ± 4° C and 60-80% RH. Number of spider mite motile individuals was counted under a 25x dissecting microscope. *T. evansi* leaf damage was based on a visual rating scale as described by Hussey and Parr, (1963). Leaf area (in cm^2) of sampled leaves was determined using a LI-COR, LI-3000 leaf area meter (LI-COR, Lincoln, NE).

6.2.5.1: Season 1 (Sep-Oct, 2007) and 2 (Nov-Dec, 2007)

For these studies, four leaves from 2-wk old plants were isolated between the 3rd and 8th leaf from the bottom of selected plants of respective species. Cotton wool was wound on the petioles and insect glue applied to restrict mites on specific leaves. Ten young (2 - 4

day old) female mites sourced from the stock culture at *icipe* were transferred to isolated leaves using an artists brush. The experiment was terminated 21d after *T. evansi* adult females were introduced. Isolated leaves per respective species were excised, placed in khaki paper bags and stored in a cool box and taken to the laboratory for assessment. The experiments were laid out on a completely randomized design with six replicates per season (n = 2400 spider mites; 240 leaves).

6.2.5.2: Season 3 (Apr-May, 2008) and 4 (Nov-Dec, 2008)

Further experiments were conducted to investigate the effects of African nightshades on motile populations of *T. evansi*. Two weeks after transplanting, ten young (2-4 day old) female mites sourced from the stock culture at *icipe* were transferred to each plant of each respective nightshade species using an artists brush. Fourteen days after infestation, spider mite populations were monitored and sampling done weekly for six and five consecutive weeks for season 3 and 4 respectively. Three plants per replicate per respective nightshade species were randomly selected at each sampling time. Three leaves were destructively sampled from the lower, middle and upper parts of the respective plant. The experiments were laid out on a completely randomized design (CRD) with four replicates per season (n = 4800 spider mites; 360 leaves).

6.2.6: Statistical analysis

General linear model analyses (PROC GLM, SAS Institute, 2000) were performed to detect treatment differences in the number of offspring produced; number of motile individuals. Spider mite counts were square root transformed ($\sqrt{\rho}$) before analysis to normalize the error distribution. Back-transformed data is presented. Percentage of leaf area damaged was computed based on leaf damage scored. When appropriate, means were separated with Student Newman Keuls test ($P \leq 0.05$).

6.3: Results

6.3.1: Mite population density in season 1 and 2

Significant differences were detected in leaf area among the five African nightshade species with *S. scabrum* showing a higher leaf area in both seasons compared to other *Solanum* sp. (Table 6.1). Significant differences were also detected in leaf area among species when comparisons were made between the two seasons in *S. americanum* ($F = 26.8$; $P<0.0001$), *S. tarderemotum* ($F = 55.5$; $P<0.0001$), *S. sarrachoides* ($F = 14.2$; $P = 0.0005$) and *S. scabrum* ($F = 29.6$; $P<0.0001$). No significant differences were detected between the two seasons in *S. villosum* ($F = 0.01$; $P = 0.9261$).

There were significant differences in number of *T. evansi* motile individuals per leaf among nightshade species. *S. scabrum* supported a significantly high population of *T. evansi* in comparison to the other four *Solanum* species examined in both seasons.

However, *S. sarrachoides* supported the least population in seasons 1 and 2 although *S. villosum* also did not support high mite populations in season 2 (Table 6.1). Significant differences in number of motile individuals were detected when comparisons were made between the two seasons in *S. americanum* ($F = 28.3$; $P<0.0001$), *S. sarrachoides* ($F = 42.7$; $P<0.0001$) and *S. scabrum* ($F = 5.0$; $P = 0.0314$). No significant differences were detected in *S. tarderemotum* ($F = 3.4$; $P = 0.0744$) and *S. villosum* ($F = 3.5$; $P = 0.0706$) (Table 6.1).

Table 6.1 Leaf area (cm^2) and number of motile individuals of *Tetranychus evansi* per leaf on five different African nightshade species

Plant species	Total leaf area (cm^2)		No. of motile individuals per leaf	
	Season 1	Season 2	Season 1	Season 2
S. americanum	$23.5 \pm 3.4b^{B}$	$70.8 \pm 6.0b^{A}$	$37.2 \pm 7.9bc^{B}$	$143.5 \pm 13.0a^{A}$
S. tarderemotum	$11.6 \pm 1.6b^{B}$	$35.7 \pm 2.2c^{A}$	$72.7 \pm 15.5b^{A}$	$99.6 \pm 7.0b^{A}$
S. sarrachoides	$14.2 \pm 1.0b^{B}$	$20.5 \pm 1.4d^{A}$	$19.5 \pm 4.9c^{B}$	$78.6 \pm 7.6b^{A}$
S. scabrum	$66.2 \pm 5.4a^{B}$	$128.1 \pm 9.7a^{A}$	$115.3 \pm 10.0a^{B}$	$166.3 \pm 20.0a^{A}$
S. villosum	$11.8 \pm 1.6b^{A}$	$12.0 \pm 1.3d^{A}$	$63.0 \pm 13.1b^{A}$	$37.4 \pm 7.0c^{A}$
F	57.2	81.8	15.7	18.6
P	<0.0001	<0.0001	0.0002	0.0023

Means within columns (in the same season) followed by the same letter are not significantly different. Means within rows (within same observation; capital letter in superscript) followed by the same letter are not significantly different ($\alpha = 0.05$; DF = 4; SNK test; SAS Institute, 2000)

Significant differences were also detected in leaf area (%) damaged by *T. evansi* in all the nightshade species in seasons 1 ($F = 13.77$; $P<0.001$) and season 2 ($F = 35.14$; $P<0.001$) (Figure 6.1). *S. tarderemotum* revealed the highest damage in season 1 and *S. villosum* in season 2. Although, *S. scabrum* supported high spider mite populations in both studies, leaf area damaged in season 2 was significantly low compared to the other species that supported significantly lower populations of *T. evansi* (Figure 6.1).

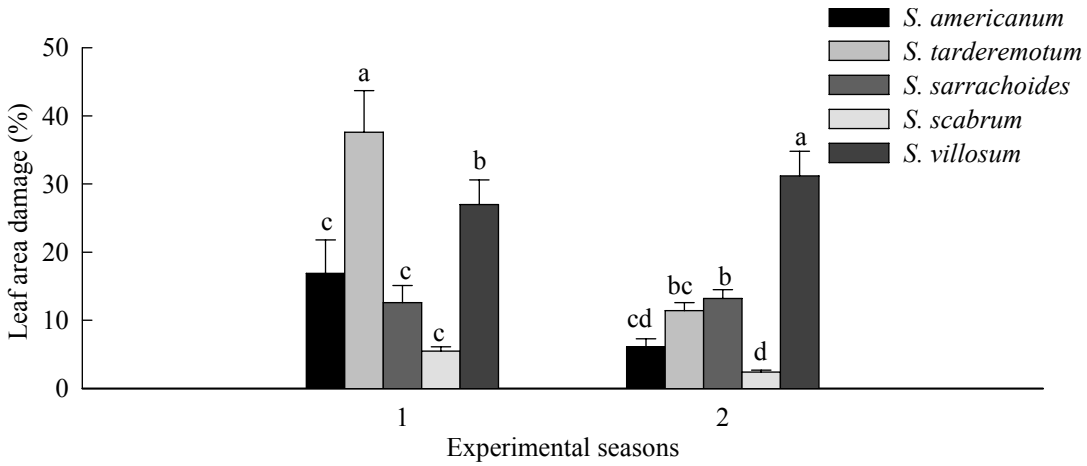

Figure 6.1 Leaf area (%) damaged by *Tetranychus evansi* feeding on different African nightshade species, season 1 and 2. Means (within same season) followed by the same letter are not significantly different; vertical bars indicate standard errors ($\alpha = 0.05$; DF = 4; SNK test; SAS Institute, 2000)

6.3.2: Mite population density in season 3 and 4

No significant differences among the nightshade species were detected in the number of *T. evansi* motile populations during the 1st (F = 1.3; P = 0.2909) and 2nd (F = 0.9; P = 0.4758) week in season 3 while in season 4, significant differences were not detected only during the 1st (F = 1.7; P = 0.1589) week (Figure 6.2). Significant differences were detected in *T. evansi* population densities during the 3rd (F = 2.5; P = 0.0463), 4th (F = 10.1; P<0.0001), 5th (F =13.7; P<0.0001) and 6th week (F = 33.3; P<0.0001) in season 3. In addition, significant differences were also detected in the 2nd (F =2.9; P = 0.0226), 3rd (F = 3.7; P = 0.0062), 4th (F = 15.2; P<0.0001) and 5th (F = 16.9; P<0.0001) week in season 4. Mite populations increased from the 3rd week in all *Solanum* species examined in season 3 and 4 except for *S. sarrachoides* (Figure 6.2).

Significant differences were also detected in leaf area (%) damaged during the 4th (F = 11.1; P<0.0001), 5th (F = 17.7; P<0.0001) and 6th week (F = 25.8; P<0.0001) in season 3 (Figure 10). However, no significant differences were detected during the 1st (F = 2.6; P = 0.0389), 2nd (F = 1.5; P = 0.2159) and 3rd (F = 1.6; P = 0.1787) week. Also, significant differences were detected in percentage leaf area damaged due to *T. evansi* during the 1st (F = 3.7; P = 0.0065), 2nd (F = 4.1; P = 0.0032), 3rd (F = 7.4; P<0.0001), 4th (F = 11.1; P<0.0001) and 5th (F = 10.2; P<0.0001) week in season 4 (Figure 6.3).

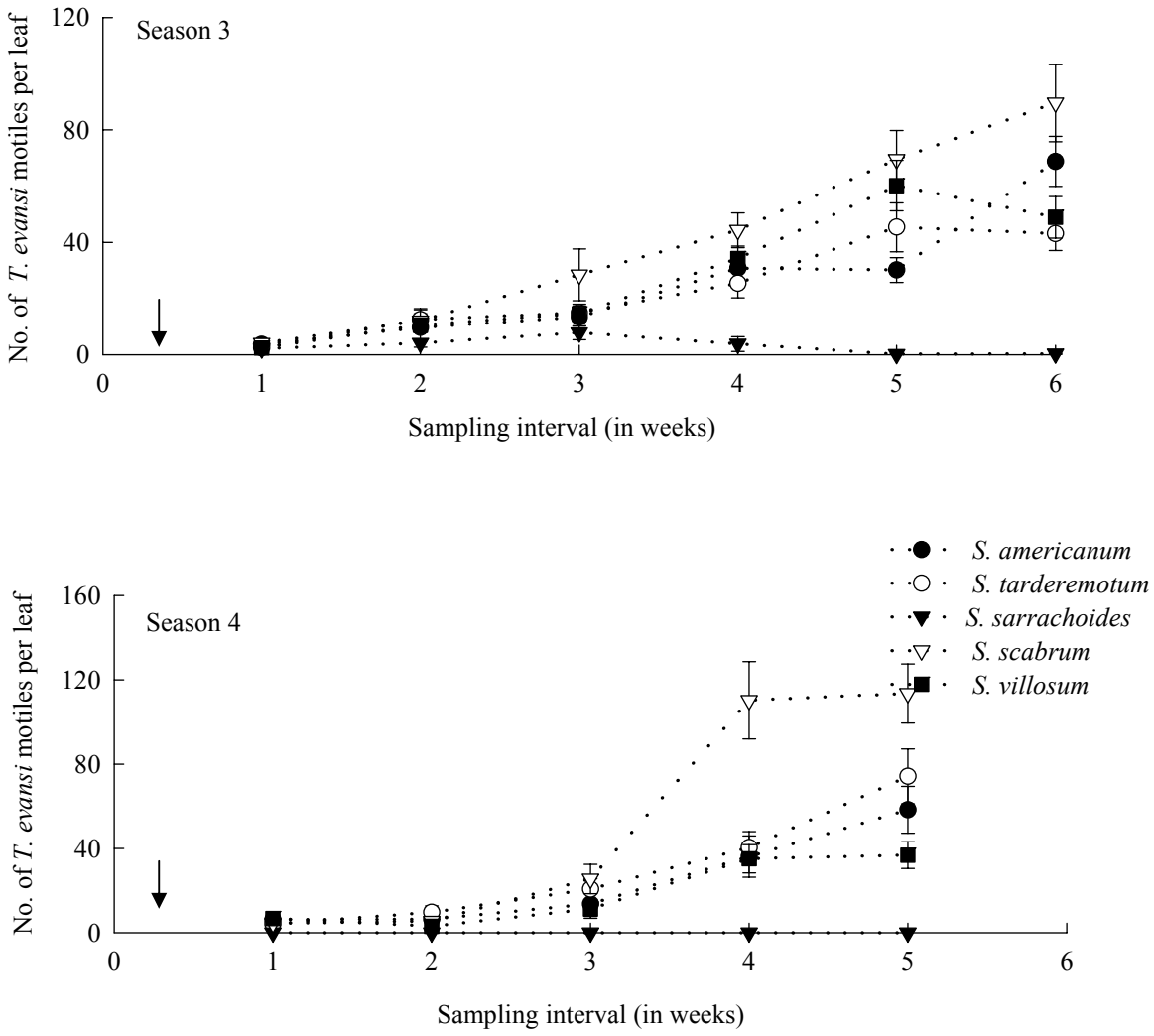

Figure 6.2 Mean number of *Tetranychus evansi* motile individuals per leaf on different African nightshade species in season 3 and 4; vertical bars indicate standard error bars ($\alpha = 0.05$; SNK test); Arrows indicate point of mite introduction.

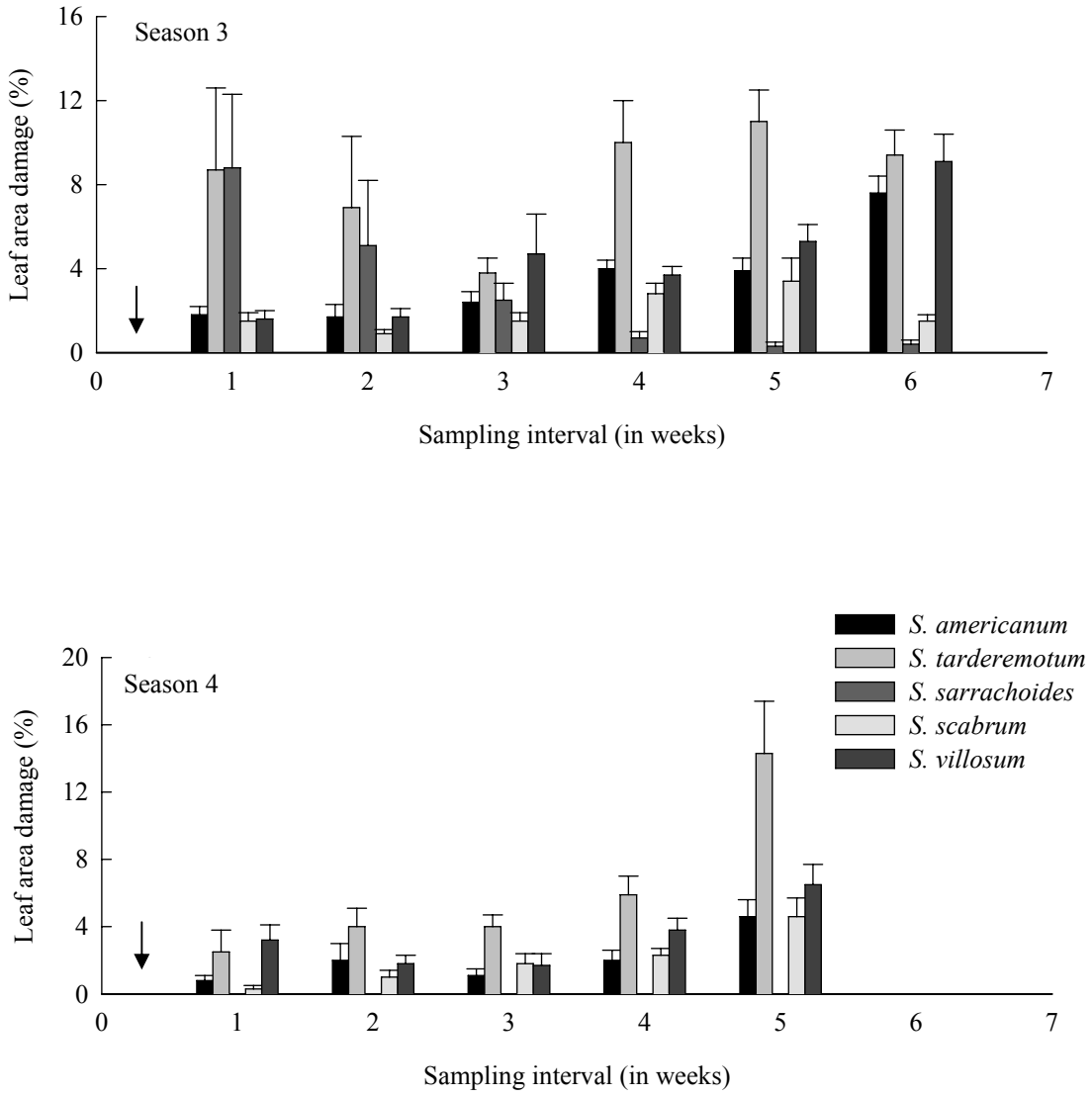

Figure 6.3 Leaf area (%) damaged by *Tetranychus evansi* feeding on different African nightshade species in season 3 and 4; means within the same sampling interval followed by the same letter are not significantly different; vertical bars indicate standard error bars (α = 0.05; DF = 4; SNK test; SAS Institute, 2000); arrows indicate point of mite introduction.

Significant differences were detected in total leaf area of the different nightshade species. *Solanum scabrum* had the highest total leaf area in both seasons (Table 6.2). Significant differences were also detected in leaf area among species when comparisons were made between the two seasons in *S. americanum* ($F = 24.8$; $P<0.0001$), *S. tarderemotum* ($F = 16.6$; $P<0.0001$), *S. scabrum* ($F = 5.7$; $P<0.0001$) and in *S. villosum* ($F = 41.5$; $P<0.0001$). No significant differences were detected between the two seasons in *S. sarrachoides* ($F = 3.7$; $P = 0.0550$) (Table 6.2).

The overall number of motile individuals differed significantly among species with the lower number observed in *S. sarrachoides*. Significant differences in number of motile individuals were detected when comparisons were made between the two seasons in *S. tarderemotum* ($F = 4.7$; $P = 0.0314$), *S. sarrachoides* ($F = 20.2$; $P<0.0001$) and *S. scabrum* ($F = 8.2$; $P = 0.0045$). No significant differences were detected in *S. americanum* ($F = 2.6$; $P = 0.1082$), and *S. villosum* ($F = 2.3$; $P = 0.1313$) (Table 6.2). The overall leaf area (%) damaged in both seasons differed significantly among species with *S. sarrachoides* depicting lower mite populations compared to other *Solanum* species examined (Figure 6.4).

Table 6.2 Total leaf area and overall number of *Tetranychus evansi* motile individuals on five different African nightshade species (season 3 and 4)

Plant species	Total leaf area (cm^2)		No. of *T. evansi* motile individuals per leaf	
	Season 3	Season 4	Season 3	Season 4
S. americanum	$74.5 \pm 2.6b^{A}$	$56.5 \pm 2.5b^{B}$	$26.1 \pm 1.9b^{A}$	$24.1 \pm 3.6b^{A}$
S. tarderemotum	$36.9 \pm 1.3d^{A}$	$29.2 \pm 1.2c^{B}$	$20.1 \pm 2.4b^{B}$	$29.8 \pm 3.7b^{A}$
S. sarrachoides	$30.8 \pm 1.3d^{A}$	$34.5 \pm 1.4c^{A}$	$3.6 \pm 0.8c^{A}$	$0.0 \pm 0.0c^{B}$
S. scabrum	$120.2 \pm 4.8a^{B}$	$142.9 \pm 8.2a^{A}$	$31.7 \pm 3.6a^{B}$	$51.8 \pm 6.1a^{A}$
S. villosum	$60.5 \pm 2.5c^{A}$	$40.5 \pm 1.9c^{B}$	$23.8 \pm 2.6b^{A}$	$18.6 \pm 2.3b^{A}$
F	223.20	138.20	27.48	25.45
P	<0.0001	<0.0001	<0.0001	<0.0001

Means within columns (in the same season) followed by the same letter are not significantly different. Means within rows (within same observation; capital letter in superscript) followed by the same letter are not significantly different ($\alpha = 0.05$; DF = 4; SNK test; SAS Institute, 2000)

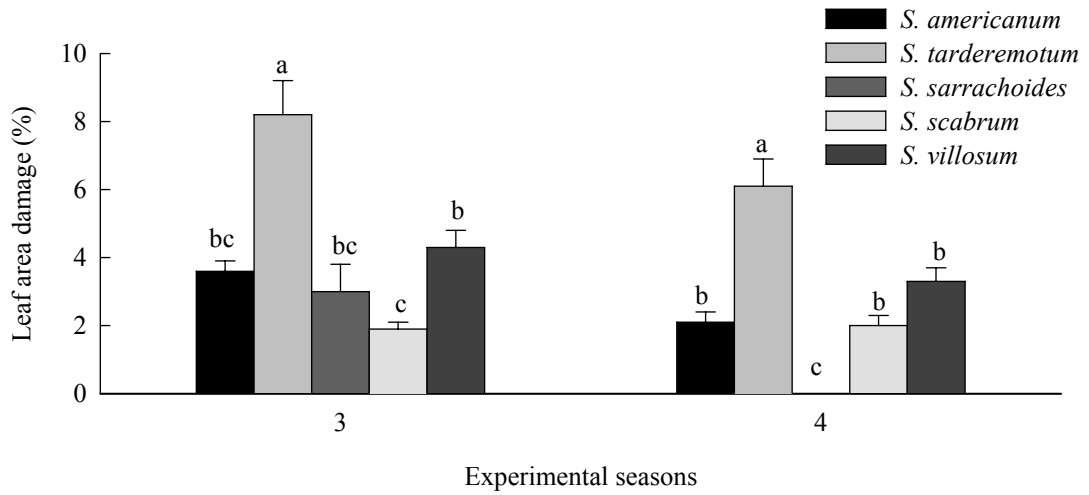

Figure 6.4 Overall leaf area (%) damaged by *Tetranychus evansi* feeding on different African nightshade species, season 3 and 4. Means (within same season) followed by the same letter are not significantly different; vertical bars indicate standard errors ($\alpha = 0.05$; DF = 4; SNK test).

6.4: Discussion

Results presented in this study suggest that *T. evansi* does not infest all African nightshades examined to the same degree although they belong to the same family. *Solanum scabrum* was highly preferred by *T. evansi* since the mite populations were significantly higher in all the four studies carried out. *S. tarderemotum*, *S. americanum* and *S. villosum* were also highly preferred hosts. However, the highest level of resistance was observed in *S. sarrachoides* where *T. evansi* populations remained low probably due to a high density of glandular trichomes as established in our laboratory studies (Chapter 5). This concurs with Rasmy (1985) who reported that *S. sarrachoides* was found resistant to *T. urticae*.

Mite populations decreased with the age of the plant (time of sampling) in *S. sarrachoides* probably indicating that the density of glandular trichomes increased as well compared to other *Solanum* species where glandular trichomes were either few or absent. However, when evaluating the density of trichomes in the laboratory, our studies revealed that glandular trichomes in *S. sarrachoides* increase with plant age and hence a simultaneous decrease in reproduction. The variation in quality of these nightshade species to *T. evansi* feeding is not only due to primary plant defense, but also the plants' response to herbivory which induces secondary metabolites (Karban and Baldwin, 1997; Tollrian and Harvell, 1999) as revealed in our laboratory studies.

These induced responses are herbivore specific (Stout *et al*., 1994) and can lead to reduced mite fecundity (Brown *et al*., 1991), hence reduced mite populations. For instance, Dabrowski (1973) reported the reproduction of spider mites to be almost zero when fed on gingko leaves which have toxic constituents. Previous studies by Wermelinger *et al*. (1990; 1991) indicate that various environmental factors such temperature and host plant nutrition influence the growth of a spider mite population. This finding concurs with the current study where differences mite populations among the nightshade species varied within study periods where temperature range was between 21-25° C indicating that these factors played a role. This also concurs with Bonato (1999) who reported that *T. evansi* populations can rise sharply since it can develop within a broad thermal range of 10-36° C.

Earlier reports by Brandenburg and Kennedy (1982) indicate that spider mites are able to choose potential host plants either by random walking or by passive dispersal by wind. Mites restricted on leaves of *S. sarrachoides* for 21 days in season 1 and 2 had higher reproduction; however, those placed on plants freely in season 3 and 4 escaped from this unfavorable species (Fry, 1989) and mite population was very low or zero. Yano *et al*. (1998) evaluating differences among plant species in acceptance by *T. urticae*, showed a positive correlation between host plant acceptance after one day and the mean number of eggs produced by these mites in 5 days. This study concurs also with findings by van den Boom *et al*. (2003) who reported that when more that 50% *T. urticae* females migrated from *Phaselous lunatus* L to *P. vulgaris* L after one day, reproduction was very low or zero on the former, hence lower population density.

Although mite populations were high on *S. scabrum*, the leaf area damaged was very low in comparison to other *Solanum* species except in one study where percentage leaf area damage was zero in *S. sarrachoides*. Despite the fact that leaf damage can be used for evaluation of host plant resistance (Giménez-Ferrer *et al*., 1994; Wilson *et al*., 1994), in our laboratory studies, *S. scabrum* did not reveal any invaluable physical or chemical factors resistant to *T. evansi*. These variations in percentage leaf area damaged can therefore be attributed to differences in leaf sizes with *S. scabrum* possessing significantly broad leaves in comparison to other *Solanum* species. However, this unique morphological characteristic in *S. scabrum* can be utilized and good yields obtained (Onyango, 2007) especially when mite populations are not very high. Therefore,

measuring the level of feeding damage is important in order to identify an economic threshold and set up a good IPM program (Bakr, 2005).

Differences in preference of *T. evansi* to African nightshade may have several implications on its population when feeding on different host plants. This is because the quality of the host plant determines choice made by the adult female for oviposition (Service, 1984; Thompson, 1988) which in turn influences mite populations. Therefore, a host plant that supports high mite fecundity creates the potential for a build-up of mite populations within a single generation.

6.5: Conclusion

High fecundity and high number of motile individuals of *T. evansi* in *S. americanum*, *S. tarderemotum, S. scabrum* and *S. villosum* would favor rapid development of mite populations and outbreaks in the field are expected. *Solanum sarrachoides* supports very low fecundity and/or zero populations of *T. evansi*. Therefore, it is important to consider exploring and utilizing the resistant mechanisms involved in *S. sarrachoides* for incorporation into breeding and IPM programs of *T. evansi* in African nightshade vegetable production.

CHAPTER SEVEN

Population dynamics of *Tetranychus evansi* (Acari: Tetranychidae) and its effect on growth and yield of different African nightshade species grown under field conditions

7.1: Introduction

Spider mites are considered as major pests of commercial crops and often require costly control measures due to development of resistance to most available acaricides (Cranham and Helle, 1985). *Tetranychus evansi* is a pest of numerous crops in the family Solanaceae and is also a web spinning spider mite that occurs during hot and dry periods and frequently reaches high population levels (Moraes *et al*., 1987; Knapp *et al*., 2003). Because of difficulties associated with spider mite control and huge economic losses thereof, there is much interest in the search for alternative control measures especially biological control and host plant resistance.

African nightshades are widely consumed indigenous vegetables as they are crucial to food security particularly during famines and other natural disasters (Chweya and Eyzaguirre, 1999). Although, they are often considered as weeds with global distribution, nightshades are very popular especially in the fresh vegetable markets in many African countries (Schippers, 2000). However, there is little scientific research hence limited availability of information on production methods, challenges, opportunities and utilization of these vegetables. This lack of attention has meant that

the potential value of nightshades is under-exploited which places them in danger of continued genetic erosion and ultimate disappearance, further restricting development options (Padulosi, 2000; Schippers, 2000; Thies, 2000; Padulosi *et al*., 2002).

Although most of the indigenous vegetables are alleged to withstand damage caused by pests and diseases (Maundu *et al*., 1999), many pests and diseases have been observed and others reported on African nightshades (Epenhuijsen, 1974; Fortuin and Omta, 1980). These pests and diseases are managed through regular sprays with 'appropriate' pesticides (Epenhuijsen, 1974; Fisher, 1977; Simms, 1997). This happens due to lack of knowledge on safer and affordable pest and disease control methods which is one of the major constraint to sustainable cultivation of these crops by African farmers (Schippers, 2001).

Research involving development and use of arthropod pest resistant cultivars has led to significantly improved food production, alleviation of poverty and hunger and improved nutrition in the major food producing areas of the world in the past (Khush, 1995; Smith, 2005). Although, spider mites are known to perform differentially on diverse host-plant species in terms of survival and fecundity (Agrawal, 2000), other factors such as temperature, humidity, light, level of predation, intra- and interspecific competition, quantity, quality and timing of pesticides, plant and soil nutrition are known to influence population parameters (Helle and Sabelis, 1985).

This has led to the investigations into the multi-trophic interactions involving host plants, spider mites and their natural enemies in an attempt to elucidate other biotic factors that may influence the efficiency of the natural enemies against these pests. Generally, most herbivorous arthropods are restricted to feeding on relatively few plant families, and it is believed that this host-range limitation may be due to fitness costs associated with alternative hosts (Fox and Morrow, 1981). However, the effects of many resistant crop cultivars have no detrimental effects, and in some cases, have additive or synergistic effects on the actions of pest arthropod predators and parasites (Eigenbrode and Trumble, 1994; Quisenberry and Schotzko, 1994).

The population dynamics of different spider mite species are often characterized by high fluctuations over time and space with mite outbreaks being associated with hot and dry weather conditions, absence of predators and good nutritional quality of host plants (Baumgärtner *et al*., 1988). The tolerance levels of various African nightshades consumed as vegetables to arthropod pests is largely unknown; although the population dynamics of *T. evansi* has been studied on American nightshade and tomato (Fiaboe, 2006). In order to assess tolerance levels in indigenous vegetables, the relative yields of leaves from pest infested and pest free (protected by a pesticide spray regime) plots could be compared; those accessions which are able to yield satisfactorily, even with normal levels of pest infestation, could be considered tolerant (Sithanantham *et al*., 2003).

Measuring the level of feeding damage can also be useful for evaluation of host-plant resistance (Giménez-Ferrer *et al*., 1994; Wilson, 1994), in studies of plant-mite interactions (Nachman and Zemek, 2002 a, b) and for fast estimation of pest infestation and predicting yield loss (Hussey and Parr, 1963; Tomkiewicz *et al*., 1993). Thus, accurate population estimates are vital for establishing the relationship between spider mite densities and plant injury, which in turn form decision making guidelines that determine action thresholds for chemical, biological, and other control measures (Binns and Nyrop, 1992).

This study determined whether different African nightshade species under *T. evansi* infestation influenced its population dynamics under field conditions. The effect of *T. evansi* damage on growth and yield of these African nightshade species was determined.

7.2: Materials and methods

7.2.1: Study site and experimental conditions

Two field studies were carried out at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) farm, Juja, Kenya (latitude 1°10' 48' S, longitude 37° 07' 12' E, altitude 1525 m above sea level); season 1 from August to October, 2008 and season 2 from January to March, 2009. The soils have been classified as eutric cambisols (FAO, UNESCO, 1974). However, amendments have been done involving adding new soils to horizon A; hence the current soils could be classified under Anthrosols (FAO, UNESCO, 1988; Muchena *et al*., 1978). The weather data (Appendix 1) were obtained

from the meteorology department at Thika, Kenya (latitude 0° 59' S, longitude 37° 04' E, altitude 1548 m above sea level). Soil samples were collected the experimental site, and analyzed at JKUAT, Department of Horticulture laboratory for nitrogen, phosphorus and potassium, electrical conductivity (Ec) and PH (Appendix 2).

7.2.2: Plant material and spider mite culture

Seeds of the five African nightshade species were sown and seedlings established following the procedure described previously in section 3.1.2. One month after sowing, well established seedlings were transplanted into polythene bags and hardened for a period of 3 - 4 weeks. After this hardening period, they were taken to the field and transplanted. Spider mites were obtained from the stock culture maintained at *icipe* under experimental conditions described previously in section 3.1.1.

7.2.3: Field layout

Each experiment was carried out as a split plot in a randomized complete block design with three replicates. The main plot factor comprised of a spraying regime at two levels, acaricide free and protected with acaricide; while the split plot factor consisted of the nightshade species, *Solanum americanum*, *S. tarderemotum*, *S. sarrachoides*, *S. scabrum* and *S. villosum*. The subplots were each measuring 2 x 2 m and a plant spacing of 30 x 30 cm was used. A 2-m empty strip was left between plots to prevent spray drift.

7.2.4: Acaricide used in protected plots

Protected plots were treated with Dynamec® [active ingredient 18 g/l (1.84% w/w) abamectin] purchased from local agrochemical stores in Nairobi, Kenya. Abamectin is a strong elmusifiable concentrate, with strong acaricidal properties.

7.2.5: Population dynamics of *Tetranychus evansi* on African nightshades

Scouting was done on weekly basis for three weeks after transplanting to establish the natural population of mites in the crop. Since no mites were found on the crop, plants were artificially infested with *T. evansi* and mites allowed two weeks to multiply before sampling began. Spraying was done in randomly selected plots a day after the first sampling. Sampling for *T. evansi* population densities in protected and acaricide free plots was done weekly for six consecutive weeks. Three leaves were picked from the upper, middle and lower parts of one randomly selected plant per plot, placed separately in labeled paper bags and put in a cooler box. Selected plants were thereof excised, placed in labeled polythene bags and put in a cooler box. All sampled materials were transported to the laboratory for mite population densities and yield assessment. The numbers of *T. evansi* motile individuals at each sampling interval were counted under a 25x dissecting microscope and recorded. *Tetranychus evansi* leaf damage visual rating scales developed for spider mites by Hussey and Parr (1963) were adopted for spider mite damage estimation. Leaf area (in cm^2) was measured with a LI-COR Li-3000 leaf area meter (LI-COR, Lincoln, NE) for calculating percentage leaf area damaged by *T. evansi*.

7.2.6: Effect of *Tetranychus evansi* on crop growth and yield

From the destructively sampled plants, leaves, flowers and fruits were separated from the stems and fresh weight determined using an electronic balance. All plant parts were then placed separately in envelopes and placed in an oven at 70° C for five days. The combined dry weight (in g) of all above ground plant parts was considered as the total plant dry matter (g/plant). Yield was evaluated by comparing fresh weight (g/plant) of leaves of plants from protected plots with those from unprotected ones. However, marketable and unmarketable leaves were not separated due to exclusive spider mite feeding since other factors in the field such as aphid infestation and birds contributed to loss in marketable leaf attributes.

7.2.7: Statistical analysis

The percentage values of *T. evansi* leaf damaged area were arcsine transformed before analysis was performed. Back-transformed data is presented. Single factor effects and their interactions were assessed using the Spit plot ANOVA, Proc GLM (SAS Institute, 2000). Whenever significant differences between factors were detected, levels of a factor were compared at each level of the other factor. If a significant effect was detected, means of the treatment were compared using Tukey's Studentized Range (HSD) multiple comparison procedure. To evaluate spider mite damage over the whole crop growth stages, data from different seasons were pooled on assumed homogeneity. The HOVTEST (levene) option of SAS was used to assess variance homogeneity. All tests were performed at $\alpha = 0.05$.

7.3: Results

7.3.1: Population dynamics of *Tetranychus evansi* on African nightshades

Season 1 (Aug-Oct, 2008)

Acaricide protected plots: Significant differences were not detected in population densities of *T. evansi* on the upper leaves during the 1st (F = 1.5; P = 0.2253), 2nd (F = 0.7; P = 0.5894), 3rd (F = 1.0; P = 0.4189), 4th (F = 1.6; P = 0.2076), 5th (F = 2.3; P = 0.0750) and 6th (F = 2.1; P = 0.0991) week (Figure 7.1). Similarly, significant differences were not detected on the middle leaves during the 1st week (F = 1.8; P = 0.1484), 2nd (F = 1.1; P = 0.3822), 3rd (F = 2.2; P = 0.0829) and 4th (F = 2.2; P = 0.0839) week except during the 6th week (F = 7.2; P<0.0002) where the population was high in *S. tarderemotum*. During the 5th week population densities remained at zero among all species (Figure 7.1). Significant differences were not also detected on the lower leaves during the 1st (F = 2.3; P = 0.0721), 2nd (F = 0.9; P = 0.4720), 3rd(F = 2.4; P = 0.0685), 4th (F = 0.9; P = 0.4764) and 5th (F = 2.3; P = 0.0811) week except during the 6th week (F = 2.9; P = 0.0356) where the population was high in *S. tarderemotum* and *S. villosum* for all leaf categories (Figure 7.1).

Acaricide free plots: Significant differences were not detected in population densities of *T. evansi* on the upper leaves during the 1st (F = 1.7; P = 0.1631), 2nd (F = 1.1; P = 0.3683), 4th (F = 1.1; P = 0.3829) and 5th week (F = 1.7; P = 0.1690) except during the 3rd (F = 2.8; P = 0.0383) and 6th week (F = 2.9; P = 0.0356) where the population was

higher in *S. villosum* and *S. tarderemotum* respectively in comparison to other species (Figure 7.1). Similarly, no significant differences were observed on the middle leaves during the 1st (F = 1.0; P = 0.4009), 2nd (F = 2.1; P = 0.0933), 4th (F = 1.8; P = 0.1478) and 5th (F = 1.6; P = 0.1927) week except during the 3rd (F = 5.4; P = 0.0014) and 6th week (F = 7.5; P = 0.0001) where mite population was higher in *S. villosum* and *S. scabrum* respectively in comparison to other species (Figure 7.1). Also significant differences were not detected on the lower leaves during the 1st (F = 1.4; P = 0.2527), 3rd (F = 1.5; P = 0.2174) and 4th (F = 2.6; P = 0.0506) week. However, significant differences among species were observed during the 2nd (F = 3.3; P = 0.0196), 5th (F = 2.9; P = 0.0358) and 6th (F = 3.0; P = 0.0299) week of sampling where mite populations were higher in *S. scabrum* and *S. villosum* in comparison to other species (Figure 7.1). In both spraying regimes, mite populations remained at zero level in *S. sarrachoides* throughout the sampling period (Figure 7.1).

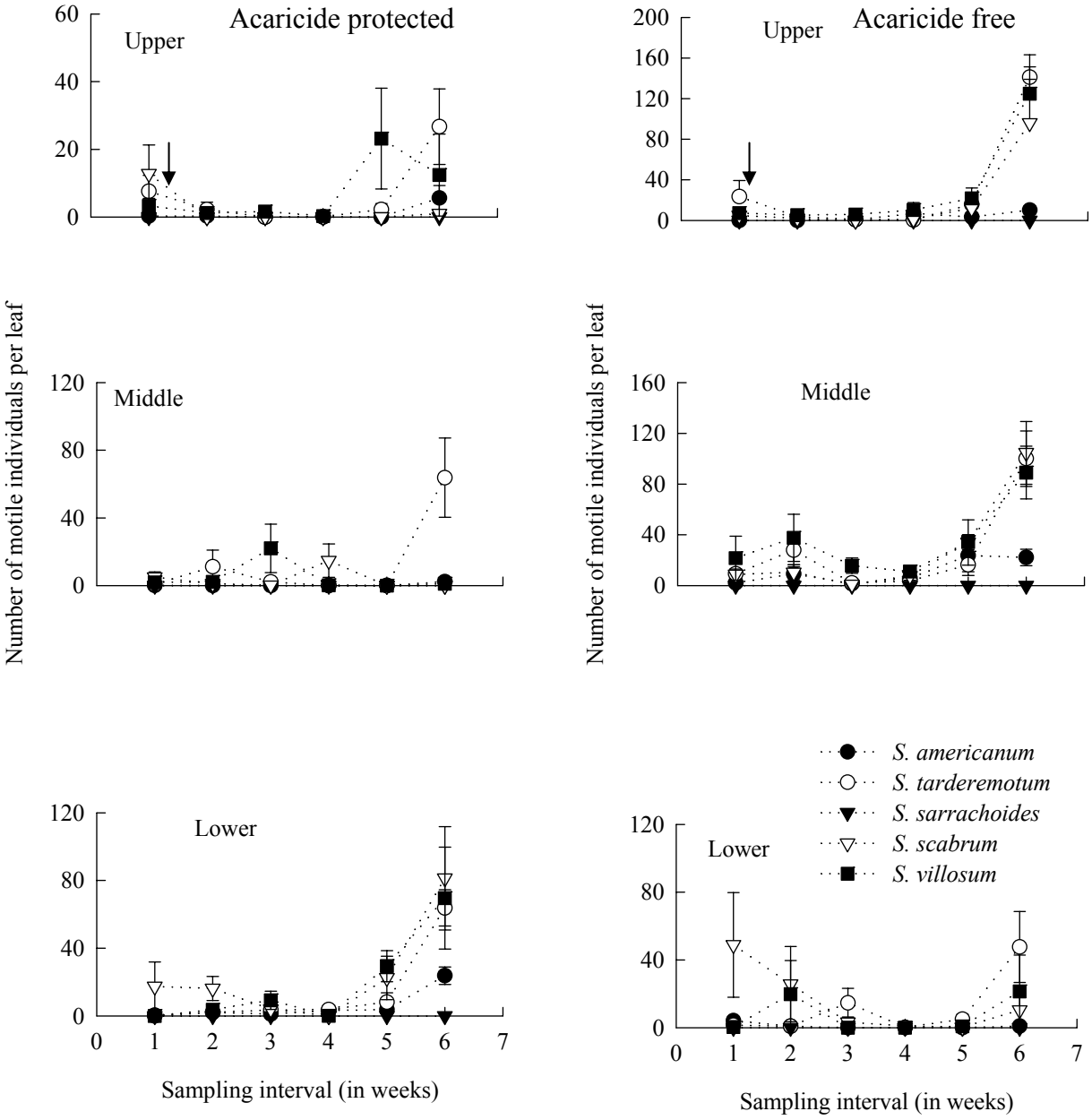

Figure 7.1 Population dynamics of *Tetranychus evansi* on upper, middle and lower leaves in acaricide protected and acaricide free plots on different African nightshade species during the first season; vertical bars indicate standard errors (α = 0.05; Tukey test); arrows indicate point of mite introduction

Total population mite densities in both spraying regimes

Significant differences were detected in acaricide protected plots during the 1st (F = 3.4; P = 0.0106) and 6th (F = 10.8; P<0.0001) week where mite population was higher in *S. scabrum* and *S. tarderemotum* respectively in comparison to other species. However, no significant differences were observed among species during the 2nd (F = 0.8; P = 0.5349), 3rd (F = 1.8; P = 0.1310), 4th (F = 2.3; P = 0.0654) and 5th week (F = 2.1; P = 0.0867) of sampling (Figure 7.2a).

Total mite populations in acaricide free plots varied significantly during the 3rd (F = 8.2; P<0.0001), 5th (F = 4.9; P = 0.0010) and 6th (F = 15.9; P<0.0001) week where mite population was higher in *S. villosum*, and *S. tarderemotum* in comparison to other species. However, no significant differences were observed during the 1st (F = 1.6; P = 0.1819), 2nd (F = 2.3; P = 0.0635) and 4th (F = 2.2; P = 0.0766) week of sampling (Figure 7.2b).

Percentage leaf area damage by *Tetranychus evansi* in both regimes

Significant differences were detected in acaricide protected plots at all levels throughout the sampling period; 1st (F = 3.0; P = 0.0212), 2nd (F = 4.1; P = 0.0035), 3rd (F = 3.8; P = 0.0055), 4th (F = 2.7; P = 0.0344), 5th (F = 9.3; P<0.0001) and 6th (F = 25.3; P<0.0001)

week (Figure 7.2c). A significant increase in mite population densities in the 6th week resulted in a higher percentage of leaf area damaged in *S. tarderemotum*.

Significant differences were detected in percentage leaf area damaged by *T. evansi* in acaricide free plots in the first season throughout the sampling period; 1st ($F = 5.05$; $P = 0.0008$), 2nd week ($F = 2.4$; $P = 0.0541$); 3rd ($F = 4.4$; $P = 0.0024$), 4th ($F = 5.1$; $P = 0.0007$), 5th ($F = 10.1$; $P<0.0001$) and 6th ($F = 16.4$; $P<0.0001$) (Figure 13d). A significant increase in mite population densities between the 5th and 6th week resulted in a higher percentage of leaf area damaged in *S. americanum*, *S. tarderemotum* and *S. villosum*. However, mite populations remained at zero level in *S. sarrachoides* throughout the sampling period, thus no leaf damage was observed (Figure 7.2).

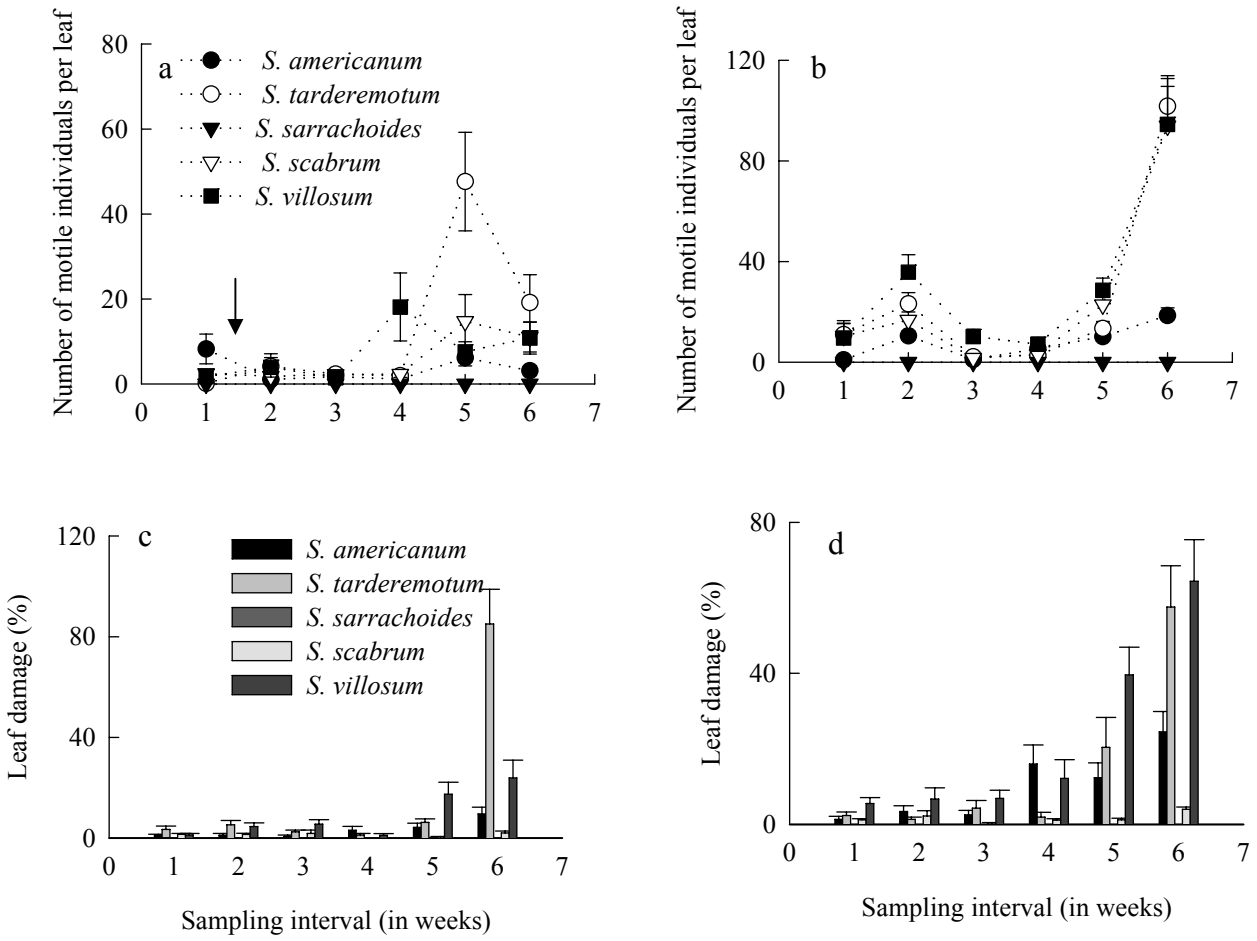

Figure 7.2 Total mite population densities in (a) acaricide protected and (b) acaricide free plots; percentage leaf area damage by *Tetranychus evansi* feeding on different African nightshade species in (c) acaricide protected and (d) acaricide free plots during the first season; vertical bars indicate standard errors ($\alpha = 0.05$; Tukey test); the arrow indicates point of mite infestation

Season 2 (Jan-Mar, 2009)

Acaricide protected plots: Significant differences were detected on the upper leaves during the 1st (F = 2.8; P = 0.0411) and the 2nd week (F = 3.5; P = 0.0152) where mite populations were higher in *S. americanum* and *S. tarderemotum* respectively in comparison to other *Solanum* species. No significant differences were observed during the 3rd (F = 1.0; P = 0.4189) and 5th (F = 1.0; P = 0.4427) week while during the 4th and 6th week, mite population densities remained at zero (Figure 7.3a). In the middle leaves, no significant differences were detected during the 1st (F = 2.2; P = 0.0927), 2nd (F = 1.1; P = 0.3703), 3rd (F = 1.2; P = 0.3173) and 4th (F = 2.1; P = 0.1044) week. However, significant differences were observed during the 5th (F = 5.8; P = 0.0009) and 6th (F = 4.6; P = 0.0037) week of sampling where mite populations were higher in *S. scabrum* in comparison to other species (Figure 7.3b). No significant differences were observed on the lower leaves during the 1st (F = 0.8; P = 0.5057), 2nd (F = 1.0; P = 0.4189), 3rd (F = 1.2; P = 0.3249), 5th (F = 0.9; P = 0.4571) and 6th (F = 1.0; P = 0.4189) week while during the 4th week population densities remained at zero (Figure 7.3c).

Acaricide free plots: No significant differences were detected on the upper leaves during the 1st (F = 2.5; P = 0.0604), 2nd (F = 1.0; P = 0.4385), 3rd (F = 2.0; P = 0.1183) and 4th (F = 1.5; P = 0.2193) week. However, significant differences were observed during the 5th (F = 2.9; P = 0.0342) and 6th (F = 2.6; P = 0.0479) week where mite populations were higher in *S. tarderemotum* and *S. villosum* respectively in comparison to other *Solanum* species (Figure 7.3d). In the middle leaves, no significant differences

were detected during the 1st (F = 1.3; P = 0.2736), 2nd (F = 2.2; P = 0.0854) and 3rd (F = 0.8; P = 0.5407) week of sampling. However, significant differences were observed during the 4th (F = 5.1; P = 0.0021), 5th (F = 17.7; P<0.0001) and 6th (F = 6.7; P = 0.0003) week where mite populations were higher in *S. villosum* and *S. tarderemotum* in comparison to other species (Figure 7.3e). No significant differences were detected on the lower leaves during the sampling period from the 1st (F = 2.4; P = 0.0650), 2nd (F = 1.0; P = 0.4189), 3rd (F = 2.2; P = 0.0832), 4th (F = 1.0; P = 0.4189), 5th (F = 1.0; P = 0.4270) and 6th (F = 1.0; P = 0.4189) week (Figure 7.3f). However, mite populations remained at zero in *S. sarrachoides* throughout the sampling period in both regimes (Figure 7.3).

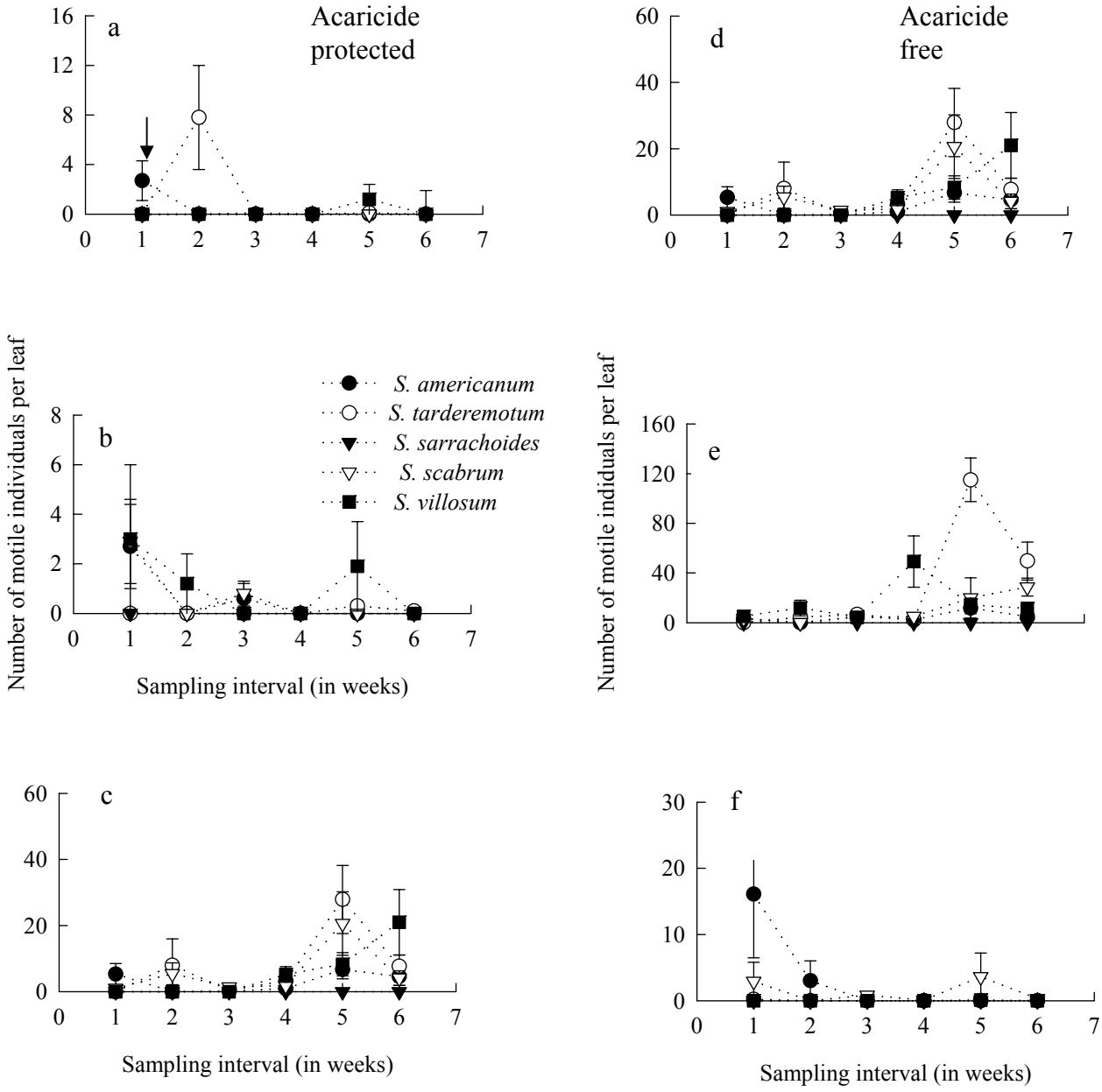

Figure 7.3 Population dynamics of *Tetranychus evansi* on (a) upper, (b) middle and (c) lower leaves of acaricide protected and on (d) upper, (e) middle and (f) lower leaves of acaricide free plots on different African nightshade species during the second season; vertical bars indicate standard errors ($\alpha = 0.05$; Tukey test); the arrow indicates point of infestation

Total mite population densities in both spraying regimes

Significant differences were detected in total mite population densities in the second season in acaricide protected plots at all sampling levels within the plant during the 1st (F = 3.2; P = 0.0147), 2nd (F = 3.6; P = 0.0077), 3rd (F = 2.6; P = 0.0386), 5th (F = 3.4; P = 0.0118) and 6th (F = 2.8; P = 0.0269) week although mite populations remained low in most of the species. However, during the 4th week no significant differences were detected among species (F = 1.7; P = 0.1443) (Figure 7.4a).

Significant differences were also detected in total mite population densities in the second season in acaricide free plots during the 1st (F = 3.8; P = 0.0057), 4th (F = 4.4; P = 0.0022), 5th (F = 9.7; P = <0.0001) and 6th (F = 3.9; P = 0.0047) week of sampling with mite populations increasing in the 5th and 6th week in *S. tarderemotum* in comparison to other *Solanum* species. However, no significant differences were observed during the 2nd (F = 1.0; P = 0.4150) and the 3rd (F = 0.9; P = 0.4610) week of sampling (Figure 7.4b).

Percentage leaf area damaged in both regimes

Significant differences were detected in percentage leaf area damaged by *T. evansi* in acaricide protected plots during the 2nd (F = 2.8; P = 0.0218), 5th (F = 6.2; P = 0.0001) and 6th (F = 5.3; P = 0.0005) week of sampling. However, no significant differences were observed during the 1st (F = 2.4; P = 0.0561), 3rd (F = 2.5; P = 0.0466), and the 4th (F = 2.4; P = 0.0574) week (Figure 7.4c). Percentage leaf area damaged increased from

the 4th week when spider mite populations were high in *S. villosum* and *S. tarderemotum*.

Percentage leaf area damaged in acaricide free plots in second season differed significantly during the 1st (F = 4.5; P = 0.0021), 3rd (F = 5.2; P = 0.0007), 4th (F = 6.6; P = <0.0001), 5th (F = 8.0; P<0.0001) and 6th (F = 9.0; P<0.0001) week except during the 2nd week (F = 4.1; P = 0.0035) where no significant differences were detected (Figure 7.4d). Percentage leaf area damage significantly increased in *S. tarderemotum* with increase in mite populations in the 5th and 6th week. Mite populations remained at zero in *S. sarrachoides* throughout the sampling period thus no leaf damage was observed (Figure 7.4).

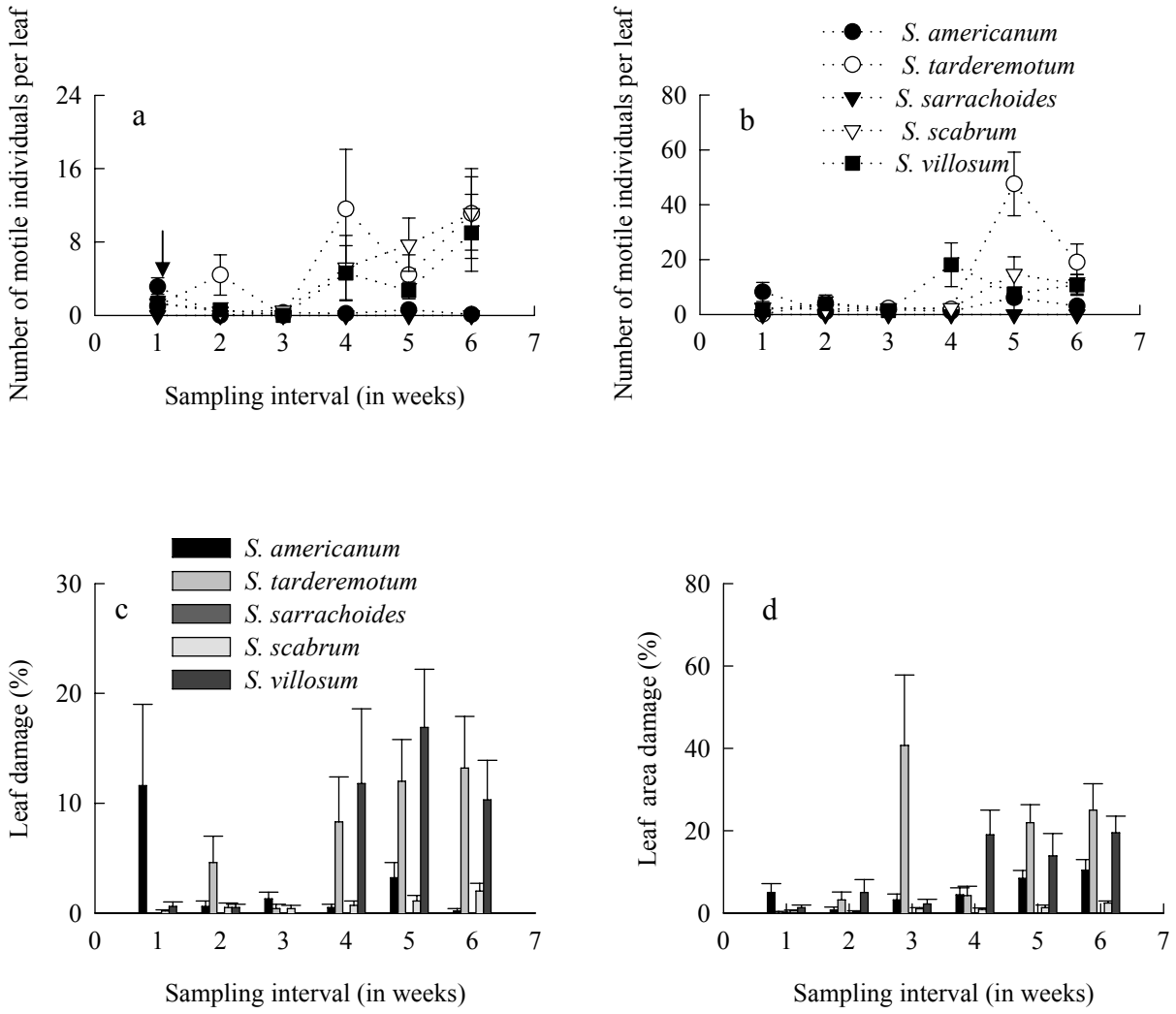

Figure 7.4 Total mite population densities in (a) acaricide protected and (b) acaricide free plots; percentage leaf area damage by *Tetranychus evansi* feeding on different African nightshade species in (c) acaricide protected and (d) acaricide free plots during the second season; vertical bars indicate standard errors ($\alpha = 0.05$; Tukey test); the arrow indicates point of mite introduction

Overall mite populations and leaf damage in both seasons and spraying regimes

Significant differences were detected when overall mite populations were compared among species in both seasons in acaricide protected (1st season; $F = 6.4$; $P<0.0001$; 2nd season; $F = 6.2$; $P<0.0001$) and acaricide free (1st season; $F = 15.1$; $P<0.0001$; 2nd season; $F = 9.1$; $P<0.0001$) plots. Mite populations were high in acaricide free plots in both seasons among all the species (Figure 7.5a and b). Significant differences were also detected when overall percentage leaf area damaged was compared among species in acaricide protected (1st season; $F = 18.0$; $P<0.0001$; 2nd season; $F = 7.9$; $P<0.0001$) and acaricide free (1st season; $F = 22.9$; $P<0.0001$; 2nd season; $F = 14.9$; $P<0.0001$) plots. Leaf damage was significantly high in *S. tarderemotum* and *S. villosum* in the two seasons and in both protected and acaricide free plots (Figure 7.5c and d).

Significant differences were not detected when overall mite populations were compared between the seasons in acaricide protected among the respective nightshade species (Figure 7.6a). Significant differences were detected in acaricide protected plots in *S. americanum* ($F = 4.3$; $P = 0.0394$), *S. tarderemotum* ($F = 6.1$; $P = 0.0137$), *S. scabrum* ($F = 15.5$; $P = 0.0001$) and *S. villosum* ($F = 22.9$; $P<0.0001$) where mite populations were higher during the first season (Figure 7.6b). Leaf area (%) damaged in *S. tarderemotum* was significantly higher in the first season compared to the second season ($F = 9.2$; $P = 0.0027$) in acaricide protected plots. No significant differences were detected among other *Solanum* species (Figure 7.6c). In acaricide free plots, leaf area (%) damaged was

significantly higher in the first season in *S. americanum* (F = 7.1; P = 0.0082) and *S. villosum* (F = 13.1; P = 0.0003) compared to the second season (Figure 7.6d).

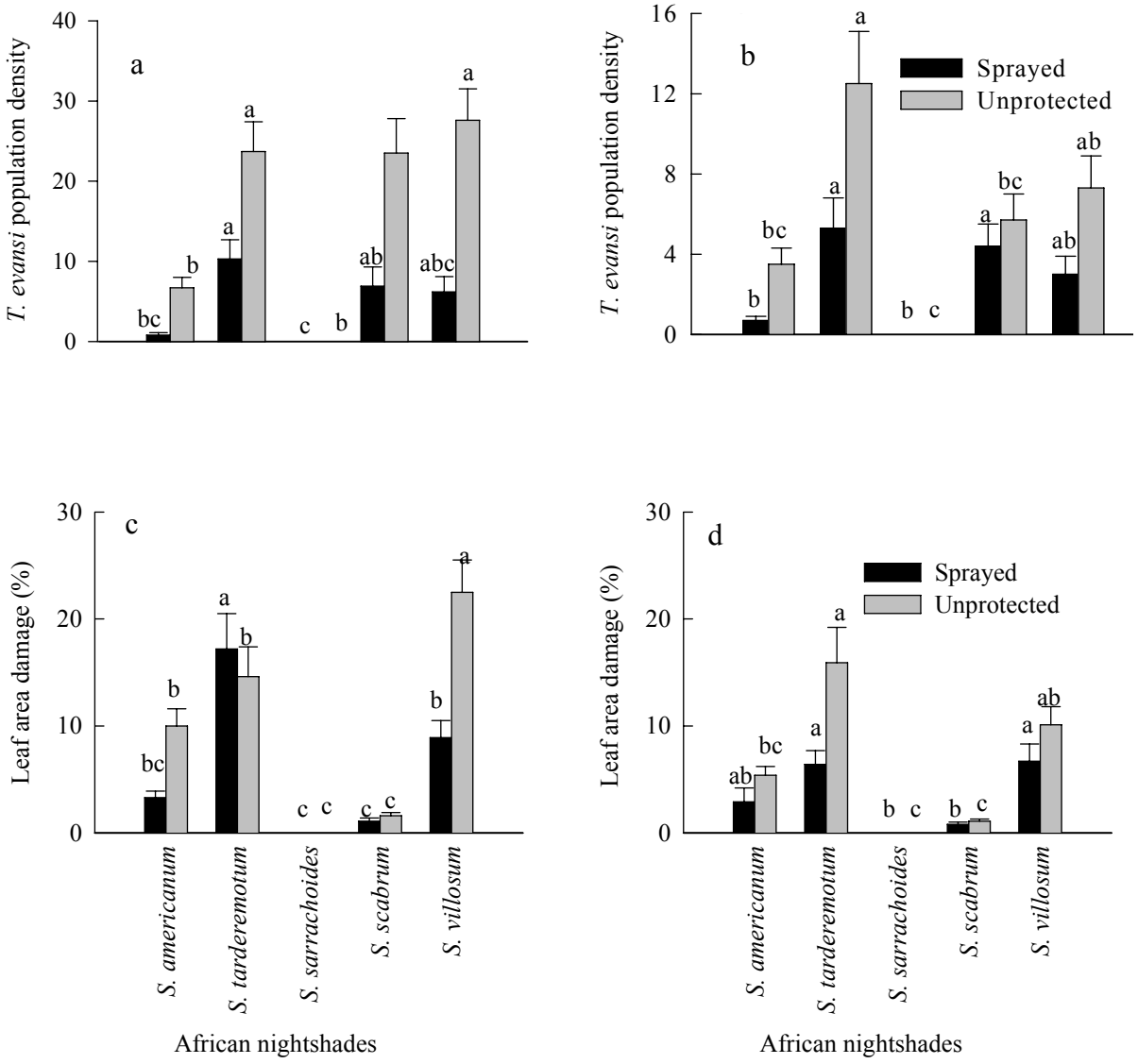

Figure 7.5 Overall *Tetranychus evansi* population density on acaricide protected and acaricide free plots during the (a) first and (b) second season; percentage leaf area damaged by *Tetranychus evansi* feeding on African nightshades on acaricide protected and acaricide free plots during the (c) first and (d) second season; vertical bars indicate standard errors; means followed by the same letter within the same spraying regime are not significantly different (α = 0.05; Tukey test)

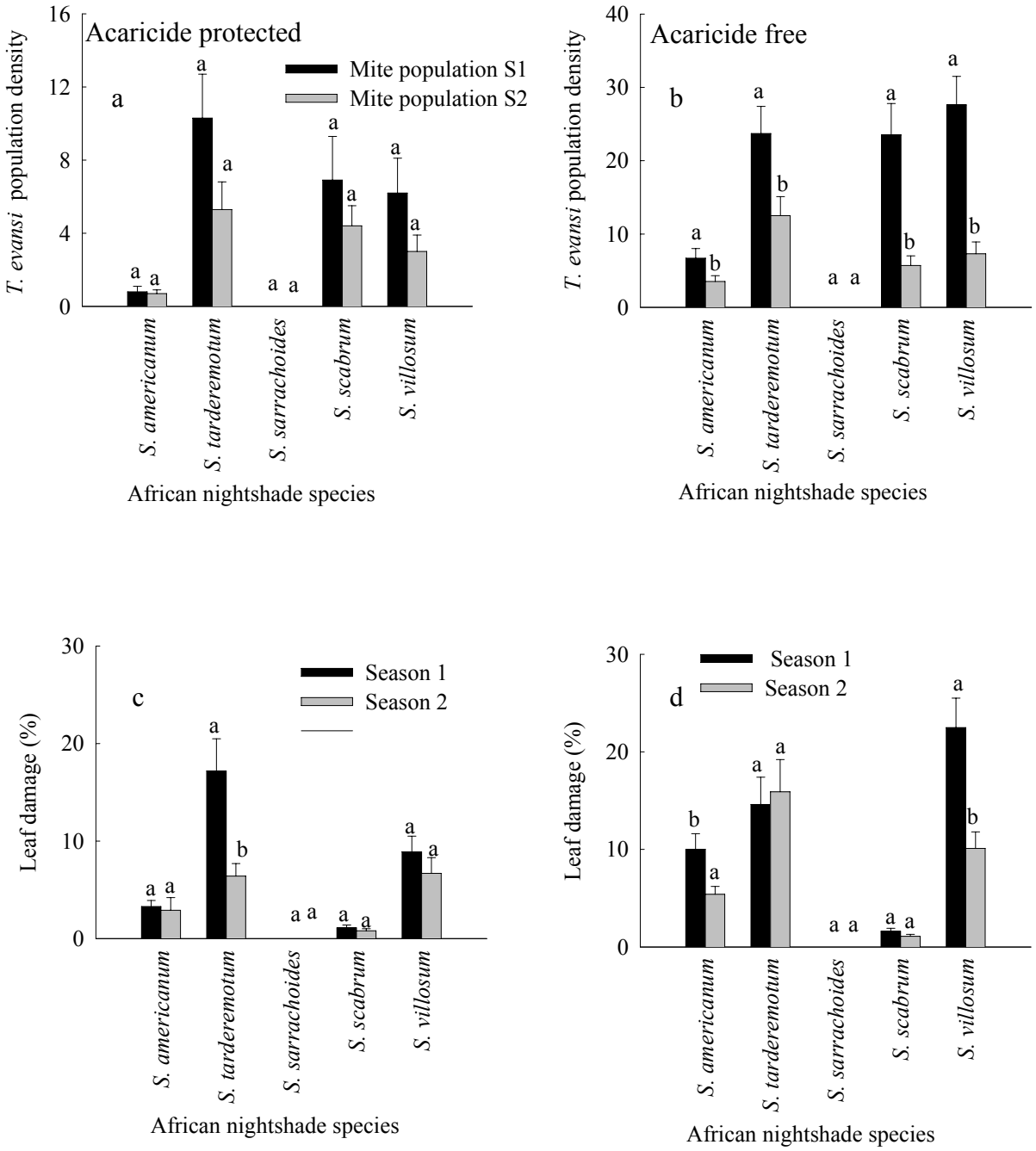

Figure 7.6 Comparison of overall *Tetranychus evansi* population density in (a) acaricide protected and (b) acaricide free plots during the first and second season; percentage leaf area damaged by *Tetranychus evansi* feeding on African nightshades on (c) acaricide protected and (d) acaricide free plots during the first and second season; vertical bars indicate standard errors; means followed by the same letter within the same nightshade species are not significantly different ($\alpha = 0.05$; Tukey test)

7.3.2: Effect of *T. evansi* on crop growth and yield

Season 1: Significant differences were not detected in plant dry matter partitioning among species in both acaricide protected plots during the 1st (F = 1.6; P = 0.2551), 2nd (F = 1.8; P = 0.2131), 3rd (F = 1.4; P = 0.3192), 4th (F = 2.1; P = 0.1536), 5th (F = 2.0; P = 0.1793) and 6th (F = 1.1; P = 0.4032) week (Figure 7.7a). Significant differences were also not detected in acaricide free plots among species during the 1st (F = 3.4; P = 0.0549), 2nd (F = 0.2; P = 0.5164), 3rd (F = 0.9; P = 0.5164), 4th (F = 0.5; P = 0.7734), 5th (F = 1.5; P = 0.2704) and 6th (F = 2.6; P = 0.1030) (Figure 7.7b).

Season 2: There were also no significant differences detected among species on acaricide protected plots during the 1st (F = 1.9; P = 0.1845), 2nd (F = 1.8; P = 0.2123), 3rd (F = 1.1; P = 0.4040), 4th (F = 0.9; P = 0.4774), 5th (F = 1.6; P = 0.2385) and 6th (F = 0.9; P = 0.4863) week (Figure 7.7c). However, in acaricide free plots significant differences were detected during the 1st (F = 3.6; P = 0.0445), 4th (F = 4.1; P = 0.0320) and 5th (F = 3.6; P = 0.0457) week where *S. scabrum* depicted the highest dry matter partitioned. Significant differences were not detected among species during the 2nd (F = 1.3; P = 0.3457), 3rd (F = 1.5; P = 0.2765) and 6th (F = 0.1; P = 0.9914) week (Figure 7.7d).

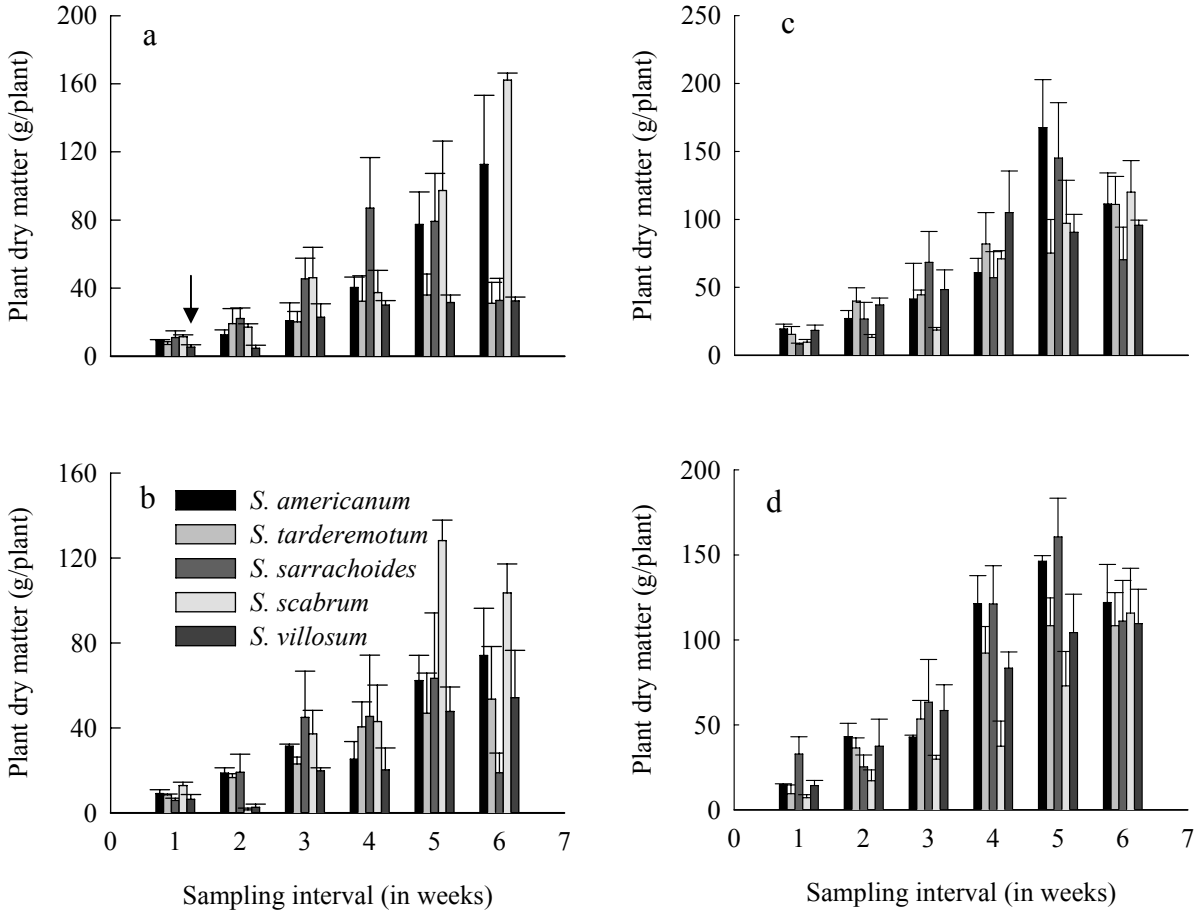

Figure 7.7 Mean dry matter (g/plant) of African nightshade plants under infestation by *Tetranychus evansi*. Bars are means (α = 0.05; Tukey test;) of plants assessed per week during the first season in (a) acaricide protected and (b) acaricide free plots and during the second season in (c) acaricide protected and (d) acaricide free plots; the arrow indicates point of mite introduction.

Significant differences were detected in acaricide protected plots (1st season: F = 5.2; P = 0.0009; 2nd season: F = 4.3; P = 0.0030) with *S. sarrachoides* and *S. scabrum* depicting higher yields in comparison to other *Solanum* species. No significant differences were detected in acaricide free plots in the first season (F = 3.3; P = 0.0157). However, significant differences were detected in the second season (F = 5.7; P = 0.0004) where *S. sarrachoides* depicted higher yields in comparison to other *Solanum* species (Table 7.1).

Significant differences were not detected when yield comparisons were made between the two spraying regimes during the first and second season in *S. americanum* (1st season: F = 0.6; P = 0.4280; 2nd season: F = 2.1; P = 0.1558), *S tarderemotum* (1st season: F = 0.0; P = 0.8842; 2nd season: F = 0.3; P = 0.6148), *S. sarrachoides* (1st season: F = 0.2 P = 0.6930; 2nd season: F = 1.4; P = 0.2403), *S. scabrum* (1st season: F = 0.5; P = 0.4682; 2nd season: F = 1.2; P = 0.2830) and *S. villosum* (1st season: F = 0.1; P = 0.8138; 2nd season: F = 0.1; P = 0.7299) (Table 7.1).

Table 7.1 Effect of *Tetranychus evansi* infestation on yield (in g/plant and metric tons/ha) of five African nightshade species examined under acaricide protected and acaricide free plots during season 1 and 2

Nightshade species	Mean yield of different African nightshade species							
	Season 1				Season 2			
	Acaricide protected plots		Acaricide free plots		Acaricide protected plots		Acaricide free plots	
	Weight (g /plant)	Weight (MT/ha)	Weight (g/plant)	Weight in (MT/ha)	Weight in (g/plant)	Weight in (MT/ha)	Weight (g/plant)	Weight in (MT/ha)
S. americanum	117.5ab	1.3ab^{A}	94.9a	1.1a^{A}	133.5b	1.5b^{A}	167.6b	1.9a^{A}
S. tarderemotum	83.5b	0.9b^{A}	86.9a	1.0a^{A}	173.2ab	1.9ab^{A}	162.1b	1.8b^{A}
S. sarrachoides	196.7a	2.2a^{A}	171.6a	1.9a^{A}	223.7a	2.5a^{A}	273.1a	3.0a^{A}
S. scabrum	210.3a	2.3a^{A}	175.5a	2.0a^{A}	225.5a	2.5a^{A}	192.0ab	2.1ab^{A}
S. villosum	69.3b	0.8b^{A}	72.7a	0.8a^{A}	158.2ab	1.8ab^{A}	151.6b	1.7b^{A}

Means followed by the same letter within columns (in the same season and spraying regime) are not significantly different; means followed by the same letter within rows (capital letter, superscript; in the same season and different spraying regimes) are not significantly different ($\alpha = 0.05$; DF =4; Tukey test; SAS Institute, 2000)

Significant differences were detected when comparisons were made between seasons. Yield was significantly higher during the second season in *S. americanum*, *S. tarderemotum*, *S. sarrachoides* and *S. villosum* in acaricide free plots (Table 7.2). In acaricide protected plots, significant differences were detected in *S. tarderemotum* and *S. villosum* with yields higher during the second season. No significant differences were detected between seasons in acaricide free and protected plots in *S. scabrum* (Table 7.2). Yields were negatively correlated to leaf area (%) damaged by *T. evansi* in acaricide free plots in both seasons (Table 7.3). However, in acaricide protected plots yield was negatively correlated to *T. evansi* damage only during the first season, but a rather weak positive correlation in the second season (Table 7.3).

Table 7.2 Seasonal variation in yield of five African nightshade species under *Tetranychus evansi* infestation grown under field conditions

Spraying regime	Seasons	Mean yield of different African nightshade species (MT/ha)				
		S. americanum	*S. tarderemotum*	*S. sarrachoides*	*S. scabrum*	*S. villosum*
Sprayed	1	1.3a	0.9b	2.2a	2.3a	0.8b
	2	1.5a	1.9a	2.5a	2.5a	1.8a
	F	0.3	9.2	0.3	0.1	28.2
	P	0.5976	0.0007	0.5927	0.7119	<0.0001
Unsprayed	1	1.1b	1.0b	1.9b	2.0a	0.8b
	2	1.9a	1.8a	3.0a	2.1a	1.7a
	F	12.0	12.4	4.3	0.2	22.4
	P	0.0015	0.0012	0.0030	0.6782	<0.0001

Means followed by the same letter within columns (within the same spraying regime) are not significantly different ($\alpha = 0.05$; DF = 4; Tukey test; SAS Institute); sprayed = acaricide protected; unsprayed = acaricide free.

Table 7.3 Coefficients of correlation (R) between leaf area damage and yield of African nightshades in the experimental seasons in acaricide free and protected plots

	Correlation coefficients (R)			
	Yield in acaricide free plots		Yield in acaricide protected plots	
	Season 1	Season 2	Season 1	Season 2
Leaf area damage	-0.054ns	-0.112ns	-0.095ns	0.002ns
	0.6104	0.2949	0.3729	0.9886

ns = not significant ($\alpha = 0.05$)

7.4: Discussion

Generally, *T. evansi* was more abundant during the first season which precedes the short rains than during the second season which precedes the long rains. During the first season, the daily average temperatures in the field were 21.1° C which is favorable for spider mite development and reproduction (Moraes and McMurtry, 1987; Bonato, 1999). This was also the case during the second season where temperatures were 21.6° C. Several studies already have been conducted regarding the effect of temperature on establishment and multiplication of spider mites (Wermelinger *et al*., 1990, 1992; Sarr, 2003; Knapp *et al*., 2006). Also studies by Campbell *et al*. (1974) indicated that peaks on aphid populations were positively correlated with moderate increases in temperature.

Peaks on mite populations in this study were found frequent on the middle and upper leaves than on the lower leaves in both seasons. Rondon *et al*. (2005) indicated that determining the location of the pest within the plant is important in order to establish the most effective control method. This study concurs with Leite *et al*. (2003) who reported

that spider mites prefer to feed on the tender upper and middle leaves than the lower ones since they are tougher for their piercing-sucking mouthparts. This characteristic feeding may encourage high spider mite populations especially on the younger leaves which may ultimately affect plant growth. Plant yield was negatively correlated to leaf area damaged by *T. evansi* in both seasons, except for one case in the second season, suggesting that huge losses can occur under heavy mite infestation. Mathews and Tunstall (1994) indicated that spider mite feeding leads to disturbances of the plant's metabolic processes such as photosynthesis and transpiration, which contribute to a decrease in plant growth, flowering and consequently, yield.

Chemical acaricide applications are the most common strategies used for control of spider mites (Jacobson *et al*., 2001; Herron *et al*., 2004) thus; mite populations were highest in unprotected plots than those plots protected with an acaricide spray. Croft (1991) warned that application of a single pesticide may accelerate the development of resistant populations via metabolic detoxification of the chemical (Fergusson-Kolmes *et al.*, 1991; Bynum *et al*., 1997). We observed that after spraying with abamectin mite populations remained at low levels but peaks began from the 4^{th} week.

Nevertheless, African nightshades differentially possess the ability to withstand or recover from damage caused by *T. evansi* populations. Although infestation was done inclusively in all plants, sampling in both protected and acaricide free plots revealed significant differences in the level of feeding damage among species. For instance, there

was no feeding damage observed on leaves of *S. sarrachoides* irrespective of the spraying regime or season. Plant dry matter and yields were significantly high in this species in comparison to those of other *Solanum* species, indicating that the levels of tolerance in this species are in combination with both antibiotic and antixenotic mechanisms of resistance (Smith, 2005). The presence of a high density of glandular trichomes reported in our laboratory studies could increase the levels of tolerance to damage by *T. evansi* in *S. sarrachoides*. This finding is supported by earlier reports by Meredith and Schuster, (1979) who indicated that some cultivars of cotton with high leaf pubescence were tolerant to the tarnished plant bug, *Lygus lineolaris* Palisot de Beauvois.

Differences in tolerance levels among species may also be attributed to the tolerant plants' increased net photosynthetic rate and the ability to shunt carbon from roots to shoots (Strauss and Agrawal, 1999). For instance, *S. sarrachoides* and *S. scabrum* produced the highest dry matter and yields in comparison to other *Solanum* sp which is a tolerance mechanism. Since *S. scabrum* produces broad leaves as revealed in our laboratory and screenhouse studies, the percentage leaf area damaged is low as reported earlier in our screenhouse studies, thus the tolerance levels increase. It is presumably able produce a greater biomass than those of a non-tolerant susceptible cultivar (Smith, 2005) with narrower leaves and hence a high percentage leaf area damaged. Kindler *et al*., (1971) reported tolerance levels of several cultivars of alfalfa to damage by the alfalfa aphid due to increased production of dry matter. This also concurs with Wiseman

(1972) and Hudon *et al*., (1979) who reported that some cultivars of maize produced greater yields although they were infested by the corn earworm and European corn borer due to their increased biomass.

Solanum sarrachoides was able to increase its biomass through multiple branching which was seen within a range of three weeks (personal observation). This is a tolerance mechanism in *S. sarrachoides* that concurs with findings by Sharma and Agrawal (1984) who reported that cotton cultivars tolerant to stem damage from feeding by the spotted bollworm, *Earias vitella* F. was due to the production of greater number of branches in response to *E. vitella* feeding. However, premature defoliation (personal observation) resulted in poor yields in *S. tarderemotum* which had the highest level of spider mite infestation irrespective of the spraying regime or season. This is because a high density of spider mites interferes with transpiration and photosynthesis (Sances *et al*., 1979) and therefore may cause premature defoliation (Mariethoz *et al*., 1994) hence poor yields.

7.5: Conclusion

Yields were high in *S. sarrachoides* and *S. scabrum* indicating some level of tolerance to *T. evansi* damage. Since tolerance involves only plant characteristics and is not part of an arthropod/plant interaction, selection of nightshade genotypes with increased growth and vigor for incorporation in IPM programs such as Push-Pull strategies or mixed cropping in order to survive *T. evansi* infestation is vital.

CHAPTER EIGHT

General discussion, conclusions and recommendations

8.1: General discussion

Previously, *T. evansi* has been reported to be one of the most destructive spider mite species on many Solanaceous crops worldwide (Escudero and Ferragut, 2005). African nightshades have been found severely infested by *T. evansi* and other tetranychid species in some countries in East and West Africa where they are grown for consumption (Oluoch Pers. Comm., AVRDC) although they are alleged to be resistant to common pests and diseases (Maundu *et al*., 1999). To manage spider mites in many vegetable crops, farmers have relied on application of heavy synthetic acaricides. However, this excessive and inappropriate use of acaricides has been related to environmental and health problems, affecting non-target organisms, and promoting the rapid development of spider mite resistance to these acaricides. There is therefore an increased demand for alternative pest control strategies that are sustainable and environmentally friendly.

Efforts to develop cultivars with increased levels of arthropod resistance is an important component for Integrated Pest Management (IPM) programs leading to reduced overdependence on acaricides for management of spider mites. The main objective of the studies presented here was therefore to provide information that will lead to management of *T. evansi* on current African nightshade species and further develop resistant genotypes based on the plant morphological and biochemical factors. To

achieve this objective, *T. evansi* biological parameters and responses to plant morphological and chemical factors were investigated in the laboratory. Further, *T. evansi* population densities and its effects on plant perfomance were investigated in both screenhouse and field studies.

The results presented in this study indicate that *T. evansi* performed differently on various African nightshades examined. This concurs with earlier authors who reported that although spider mites are highly polyphagous, they accept and perform differentially on diverse host plant species (van den Boom *et al*., 2003; Greco *et al*., 2006). One nightshade species, *S. sarrachoides*, was found to be the most resistant species in laboratory, screenhouse and field studies. In laboratory studies, the intrinsic rate of natural increase, r_m, of *T. evansi* in *S. sarrachoides* was negative (-0.063 females/female/day) indicating that the mite populations will decrease on this host even under favorable conditions. However, the r_m values of between 0.180 – 0.196 females/female/day on the suitable nightshade species were relatively low compared to the published r_m of other *Tetranychus* species which range between 0.201 – 0.290 females/female/day at 25 ± 1°C, except for *Tetranychus viennensis* Zacher (0.136) (Sabelis, 1985). A high density of glandular trichomes in *S. sarrachoides* reported in this study is presumably associated with the high levels of resistance to *T. evansi*.

Varied effects of trichome type and density in Solanaceous crops have been previously reported by several authors to confer resistance to spider mites and other arthropod pests (Thurston, 1970; Duffey and Isman, 1981; Kennedy and Sorenson, 1985; Goffreda *et al*.,

1988; Wilkens *et al*., 1996; Elle *et al*., 1999; Simmons and Gurr, 2005). However, other factors such as temperature and plant mineral nutrition may influence the establishment and multiplication of spider mites (Wermelinger *et al*., 1990, 1992; Sarr, 2003; Knapp *et al*., 2006).

Volatile plant compounds have been reported to represent an arsenal of defenses ranging from chemical toxins to feeding deterrents (Langenheim, 1994). In this study, *S. sarrachoides*, found to be most resistant, lacked volatile characteristics suitable to attract *T. evansi* in olfactometer studies. However, other mite responses such as reduced fecundity might have been influenced by the secretions that inhibit feeding (Levin, 1973; Stipanovic, 1983; Duffey, 1986), while mites walking on the leaf surface may get entrapped on the sticky exudates of glandular trichomes and subsequently dehydrated (Patterson *et al*., 1974; Rasmy, 1985).

Variations in percentage leaf area damaged among the nightshade species may be attributed to differences in leaf sizes with *S. scabrum*, commonly known as the broad-leafed nightshade, possessing significantly wider leaves in comparison to other *Solanum* species. Thus, it is presumably able produce a greater biomass than those of a non-tolerant susceptible cultivar (Smith, 2005) with narrower leaves. This unique morphological characteristic in *S. scabrum* can be utilized and good yields obtained especially when pest populations are not very high. This study also concurs with Strauss and Agrawal (1999) who indicated that a tolerant plant's ability to increase the net

photosynthetic rate and to shunt carbon from roots to shoots contributes to high dry matter and hence yield even under heavy pest infestation. Selection of nightshade genotypes with increased growth and vigor in order to survive *T. evansi* infestation is vital.

8.2: Conclusions

1. *Solanum americanum*, *S. villosum*, *S. tarderemotum* and *S. scabrum* are susceptible host plants for *T. evansi* due to the shorter adult developmental period, longer adult longevity, higher reproduction and intrinsic rate of natural increase and severe mite outbreaks are likely to occur under favorable conditions in the field.
2. *Solanum sarrachoides* seems resistant to *T. evansi* as the intrinsic rate of natural increase was negative on this host species in laboratory studies. This indicates that mite populations will presumably always decrease in this host even under favorable conditions.
3. Intact potted plants of *S. sarrachoides* and *S. villosum* release volatiles that vary quantitatively and qualitatively with the former producing significantly higher amounts than the latter. Indications are that these compounds presumably play a role in host plant selection by *T. evansi* for either feeding or oviposition, hence influencing its population densities.
4. High levels of resistance to *T. evansi* found in *S. sarrachoides* in both screenhouse and field studies are associated with a high density of glandular trichomes found predominant in our laboratory studies. This indicates that both

morphological and chemical factors associated with the glandular trichomes exist in this species and confer different levels of resistance.

5. Although mite population densities were high in *S. scabrum*, percentage of leaf area damaged and hence yield was not affected in this host in field studies. This demonstrates that some level of tolerance to *T. evansi* damage exists in this nightshade species.

8.3: Recommendations

1. Life history characteristics of *T. evansi* should be carried out on other species and/or accessions of nightshade in order to give a wide range of selection for resistant genotypes.
2. Other phytochemicals such as alkaloids, essential oils from various nightshade species and/or accessions could be sampled and toxicity tests to *T. evansi* carried out.
3. Further work should be done to establish ways of developing Integrated Pest Management systems based on resistant African nightshade germplasm. These could include Push-Pull strategies, biological control and mixed cropping
4. Effects of abiotic factors under several field studies could be carried out in order to give a clear indication of multiseasonal population dynamics of spider mites on African nightshade.
5. Breeding for resistant African nightshade genotypes to *T. evansi* and other spider mites species could be initiated.

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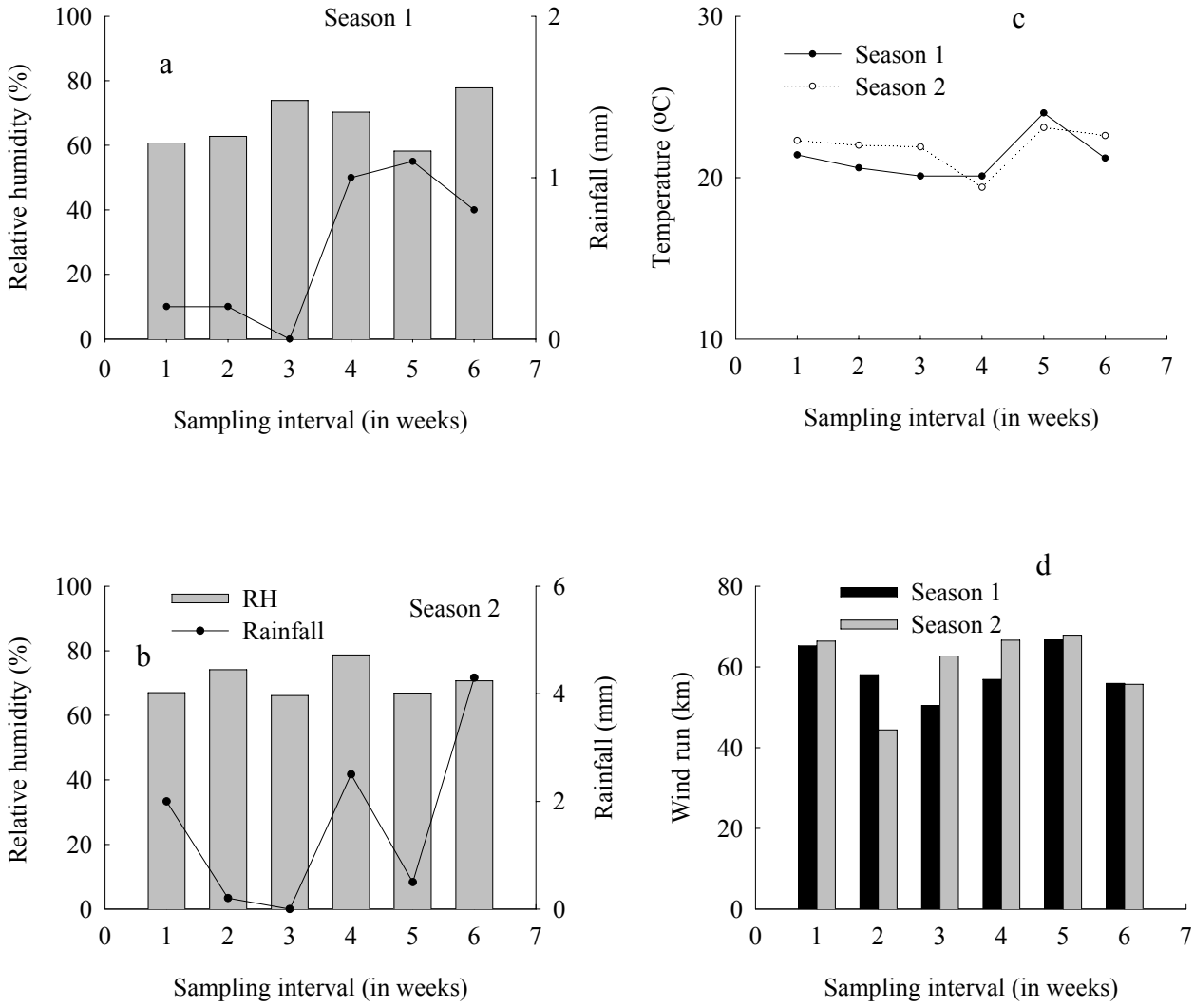
Appendices



Appendix 1 Weekly weather conditions for rainfall and relative humidity during seasons (a) 1 and (b) 2; (c) temperature and (d) wind run during the experimental period (2006-2009)

Appendix 2 Soil PH, electrical conductivity and essential minerals collected from different locations within the field experimental site used during the two seasons

Seasons	PH	Ec	N (%)	P (ppm)	K (meq/100g of soil)
1	6.4 ± 0.05b	0.2 ± 0.01a	0.4 ± 0.01a	23.0 ± 4.6b	2.7 ± 0.2b
2	6.8 ± 0.12a	0.2 ± 0.01a	0.4 ± 0.01a	61.0 ± 5.9a	3.7 ± 0.2a
F	10.57	0.20	0.02	25.91	14.04
P	0.0174	0.6704	0.8965	0.0022	0.0096

Key: Ec = Electrical conductivity; N = Nitrogen; P = Phosphorus; K = Potassium; Meq = Milliequivalent; ppm = parts per million