Promotor:

Prof. dr. M. Dicke

Hoogleraar in de Insect-Plant Relaties

in het bijzonder Tritrofe Interacties

Wageningen Universiteit

Co-Promotor:

Dr. R. Hanna

Biological Control Center for Africa

International Institute of Tropical Agriculture

Cotonou, Republic of Benin

Promotiecommissie:

Prof. dr. J.S. Yaninek, Purdue University, West Lafayette, Indiana, USA

Prof. dr. M.W. Sabelis, Universiteit van Amsterdam

Prof. dr. J.C. van Lenteren, Wageningen Universiteit

Prof. dr. L.E.M. Vet, Nederlands Instituut voor Oecologisch Onderzoek en Wageningen Universiteit

Infochemical use by predatory mites of the cassava green mite in a multitrophic context

Désiré Gnanvossou

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Propositions

1. Herbivore-induced cassava plant volatiles play an important role in distant prey location by the predatory mites *Typhlodromalus manihoti* and *T. aripo.* (*this thesis*)

2. *Typhlodromalus aripo*'s foraging strategy limits the magnitude of interspecific interactions, thereby facilitating coexistence with its competitor *T. manihoti* in the same habitat. (*this thesis*)

3. All organisms engender chemical signals, and all, in their respective ways, respond to the chemical emissions of others. *[Eisner, T. & Meinwald, J., 1995 (eds.). Chemical ecology: the chemistry of biotic interaction. National Academy Press, Washington, D.C., pp 214]*

4. Herbivorous insects most generally come to human notice when they compete with us, e.g. by eating our crops. *(Schoonhoven, L.M., Jermy, T. & van Loon J.J.A., 1998. Insect-plant biology: from physiology to evolution. Chapman & Hall, London, pp 409)*

5. Insects are the chief competitors of man for the domination of this planet. *(Wigglesworth, V.B., 1976. Insects and the life of man: collected essays on pure science and applied biology. Chapman & Hall, London. John Wiley & Sons, Inc., New York, pp 217)*

6. Entomologists need to know the ecology of the insects they study in order to understand them and to orient efforts to manage populations of either harmful or beneficial species. *(Huffaker, C.B. & Rabb, R.L., 1984, Ecological Entomology, second edition, John Wiley & Sons, New York, pp 756)*

7. Predatory arthropods may protect other organisms from their natural enemies and may get something in return from the organism that gains the protection, such as shelter, nutritious substances or perhaps nothing more than a signal betraying the presence of prey. *(Dicke & Sabelis, 1988. How plants obtain predatory mites as bodyguards. Netherlands Journal of Zoology 38: 148-156)*

8. The business, and the fun, of science is to notice that phenomena of the natural world require explanation, and then to find the explanations. *(Colinvaux, P.A., 1973. Introduction to Ecology. John Wiley & Sons, Inc., pp 621)*

9.   Chemicals are not the only, or indeed the best solution to the problem.   Biological control has permanently solved many pest problems throughout the world. *[Huffaker, C.B. & Messenger, P.S., 1976 (eds.).   Theory and Practice of Biological Control, Academic Press, Inc., London LTD, pp 788]*

10. If you have an idea and I have an idea and we exchange these ideas, then each of us will have two ideas. *(George Bernard Shaw)*

11. As far as cheese production is concerned, no country in the world can compete with The Netherlands.

Propositions belonging to the thesis entitled *"Infochemical use by predatory mites of the cassava green mite in a multitrophic context"*.

Désiré Gnanvossou

Wageningen, 21 January 2002.

Contents

Abstract

This thesis describes research on multitrophic interactions in a system consisting of (1) cassava plants (*Manihot esculenta*), (2) three herbivorous mites, i.e. the cassava green mite *Mononychellus tanajoa,* the red spider mite *Oligonychus gossypii* and the two-spotted spider mite *Tetranychus urticae* and (3) two exotic predatory mites *Typhlodromalus manihoti* and *T. aripo,* in Africa. The objectives are to understand how the two exotic predators (i) exploit chemical information to locate the target prey in pure and mixed odors conditions with the alternative prey mites, (ii) perform when feeding on different prey mite species and (iii) interact with each other.

The predatory mites, *T. manihoti* and *T. aripo* were attracted to cassava leaves infested by *M. tanajoa* compared with non-infested leaves, when the predators were starved for 2, 6 or 10 hours. They were not attracted to 400 female *M. tanajoa* removed from infested plants nor to mechanically wounded leaves. In a choice situation, *T. manihoti* and *T. aripo* preferred odors from leaves infested by *M. tanajoa* to odors from leaves infested by *O. gossypii* regardless of the ratio *M. tanajoa:O. gossypii.* When *M. tanajoa*-infested leaves and *T. urticae*-infested leaves were offered in a choice situation, the response of the two predator species depended on the density of *T. urticae.* *Typhlodromalus manihoti* and *T. aripo* were attracted to odors from cassava leaves infested with both *M. tanajoa* and *O. gossypii* or to a mixture of odors from leaves infested with *M. tanajoa* and odors from leaves infested with *O. gossypii,* when compared to odors from non-infested leaves. In contrast, mixed odors from *M. tanajoa*-infested leaves and *T. urticae*-infested leaves did not yield a preference over odors from non-infested leaves.

Typhlodromalus manihoti and *T. aripo* had a higher intrinsic rate of population increase (r_m) and net reproduction (R_o), and a shorter generation time and doubling time when they were feeding on *M. tanajoa* than on *O. gossypii* or *T. urticae.* Prey-related odor preference matches predator performance if the key prey is compared to the two inferior prey mite species.

Typhlodromalus aripo displayed a marked preference for odors emitted from either infested cassava apices or infested young cassava leaves over infested old cassava leaves but showed equal preference for odors from apices and young leaves both infested with *M. tanajoa.* *Typhlodromalus manihoti* did not discriminate between volatiles from the three infested cassava plant parts. This mirrors the differential distribution of the two predators on cassava plant foliage.

Carnivorous arthropods when searching for adequate food and habitat for themselves and their progeny should in the meantime avoid becoming food for other organisms. Intraguild interactions have been investigated for the predatory mite species *T. manihoti, T. aripo* and the native species *Euseius fustis.* *Typhlodromalus manihoti* is able to discriminate between odors from patches with con- and heterospecific competitors and prefers to visit patches with heterospecifics. *Typhlodromalus aripo* preferred to move away from patches with heterospecifics or conspecifics. *Euseius fustis* avoided odors from patches with conspecifics as well as odors from patches with the heterospecifics *T. manihoti* and *T. aripo.*

In conclusion, this thesis shows that the distribution and diversity of prey species, intraguild predation and competition are likely to play an important role in infochemical use by *T. manihoti* and *T. aripo*. In addition to predator-prey interactions, interactions between predators can also be considered as important factors affecting population dynamics of both prey and predators.

1

General introduction

1. Cassava as major food crop and its associated pests

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae), a ligneous shrub from the Neotropics, was introduced into Africa by the Portuguese in the late sixteenth century (Jones, 1959). This crop is grown generally between latitudes 30° N and 30° S, often in marginal areas of lowland tropical regions with 750 to 3000 mm rainfall (Cock, 1985). Cassava is propagated from vegetative cuttings planted during the wet season and it is harvested from 8 to more than 36 months after planting (Cock, 1985). Cassava is grown mainly for its storage roots. This crop is one of the four major sources of calories for over a half billion people in tropical Africa, Asia and Latin America (El-Sharkawy, 1993). It provides over half of the energy for several hundred million people in Africa alone (IITA, 1992). In addition to storage roots being a source of carbohydrates, young cassava leaves are consumed as a vegetable providing consumers with proteins, vitamins and minerals (Hahn, 1988).

The major constraints to stable production of cassava in Africa are diseases, insects, mites, weeds, unsuitable soil, agronomic limitations and socioeconomic factors. The exotic cassava green mite *Mononychellus tanajoa* (Bondar) and the exotic cassava mealybug *Phenacoccus manihoti* Mat. Ferr. (Homoptera: Pseudococcidae) are by far the most economically important arthropod pests of cassava in Africa (Yaninek *et al.*, 1989a). Except for patchy outbreaks of the grasshopper *Zonocerus variegatus* L. and the red spider mite *Oligonychus gossypii* (Zacher) are considered minor pests of cassava in Africa. A third spider mite species, the two-spotted spider mite *Tetranychus urticae* (Koch) is widely spread (Bellotti *et al.*, 1987) but only occasionally infests cassava in Africa (Yaninek *et al.*, 1989b). The whitefly *Bemisia tabaci* (Genn) although does not cause much physical damage to

cassava, it is a very important pest of cassava because of its ability to vector geminiviruses that cause cassava mosaic disease which has been traditionally most important in West Africa. Recent novel strain of the virus has cause an epidemic of unusually severe CMD and has decimated cassava production in the Lake Victoria Region (Legg, 2000). In addition to CMD, Bacterial Blight (*Xanthomonas campestris* pv. *Cassavae* and pv. *manihotis*) and anthracnose *Colletrotrichum gloeosporioides* (*Gloromella cingulata*) are also economically most important diseases (Mahungu *et al.*, 1994).

2. Invasion, spread and damage of *Mononychellus tanajoa* in Africa

2.1. Invasion and spread

An exotic mite pest was discovered in November 1971 attacking cassava in Uganda (Nyiira, 1972). The pest was identified as the neotropical spider mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) commonly known as the cassava green mite. It spread within 14 years to 27 countries in the cassava belt of Africa (Yaninek *et al.*, 1989b).

2.2. Damage

Mononychellus tanajoa feeds on young leaves by inserting its chelicerae into the abaxial surface and sucking out the fluid content of individual palisade and the spongy mesophyll cells. This causes chlorosis, which increases from a few spots to complete loss of chlorophyll (Ayanru and Sharma, 1984). It threatens production in many marginal areas where cassava is often the last crop available for harvest when all other crops have failed (Dorosh, 1988; Nweke, 1992). Large mite populations can cause leaf loss and persistent mite attack can lead to total defoliation, but cassava plants are rarely killed. Damage by the cassava green mite affects the plant's photosynthetic capacity and growth rate, especially in highly susceptible cultivars, leading to 30 to 50% reduction in storage root yield (Nyiira, 1976).

3. Life history of *Mononychellus tanajoa*

The life history of *M. tanajoa* is similar to that of most species of the subfamily Tetranychinae. *Mononychellus tanajoa* develops through the following stages: egg, larva,

two nymphal stages (proto- and deutonymph), and adult. The reproduction is arrhenotokous (Yaninek *et al.*, 1989b).

Temperature and host-plant characteristics are important factors affecting the growth rate and development of *M. tanajoa* (Yaninek *et al.*, 1989b). It has been shown that egg production by *M. tanajoa* was highest on young leaves of young plants during the wet season (Yaninek *et al.*, 1989b). The net reproduction rates (R_o) and the intrinsic rates of increase (r_m) generally were lower on dry season than that on wet season host-plants. These life history parameters (R_o and r_m) declined with plant age and leaf age regardless of season. The unsuitability of senescent leaves as food source may explain the decline in *M. tanajoa* numbers with increasing leaf and plant age, especially in Africa, where relatively few and inefficient natural enemies were present (Yaninek *et al.*, 1989b).

4. Previous measures to control *Mononychellus tanajoa* in Africa

Before the implementation of classical biological control in Africa, different strategies such as chemical control, cultural control and host-plant resistance were tested in attempts to reduce cassava green mite populations.

4.1. Chemical control

When *M. tanajoa* was discovered in Uganda in 1971, several chemicals were screened as possible acaricides, but none were sufficiently effective. Chemical control of *M. tanajoa* is presently discouraged because of the long growing cycle of the crop. Chemical control is also expensive relative to the cash value of cassava, poses health hazards to humans and animals, and usually causes secondary pest outbreaks (Yaninek *et al.*, 1989b).

4.2. Cultural control

Cassava plants of less than 9 months old are especially vulnerable to cassava green mite attack (Yaninek *et al.*, 1989b). Therefore, adjusting planting times has been the sustained form of plant protection available. Planting cassava early in the wet season promotes good plant establishment and increases the plant's tolerance to cassava green mite. Other potentially effective cultural practices such as mixed cropping which may also help to reduce mite populations have not been tested for *M. tanajoa* control in cassava production in Africa.

Cultural control methods are very effective and have been successfully used in closely managed agricultural systems, but their application to subsistence farming presented a number of difficulties such as availability of labor, which is often a serious limiting factor at planting time. Priority was likely to be given to cash crops, a source of high income, with short growing and maturation periods (Yaninek *et al.*, 1989b).

4.3. Host-plant resistance

Cassava pests have been studied for many years in attempts to develop host plant resistance. Although most of the early breeding efforts focused on developing early maturing cultivars, a number of cassava cultivars have been developed for resistance against several important pests including *M. tanajoa* in Africa (Mahungu *et al.*, 1994). The process of breeding including the identification of sources of resistance, incorporating these sources into productive and acceptable plant types, testing them and finally distributing them to the farmers may take several years (Yaninek *et al.*, 1989b). Leaf pubescence and drought tolerance as characteristics that may reduce mite damage were also investigated (Yaninek *et al.*, 1989b).

5. Biological control of *Mononychellus tanajoa* in Africa

The exotic nature of cassava green mite and its host-plant in Africa, and the absence of adequately adapted natural enemies, resulted in cassava green mite reaching a pest status in Africa. The impact of the natural enemies of *M. tanajoa* has been assessed in the Neotropics where it was shown that they preserve at least 30% of the potential cassava production (Braun *et al.*, 1989; de Moraes, unpublished data). This evidence pointed to the Neotropics as a source of effective predators that were introduced into Africa to control the pest, the basis of action for classical biological control in Africa. In this perspective, the International Institute of Tropical Agriculture (IITA) initiated a classical biological control campaign against *M. tanajoa* in the mid-1980s (Herren and Bennett, 1984; Yaninek, 1985; Mégevand *et al.*, 1987; Yaninek *et al.*, 1987). The initial experimental release campaign focused exclusively on phytoseiids of Colombian origin and began in Nigeria with *Neoseiulus idaeus* Denmark and Muma (Acari: Phytoseiidae) in 1983 (Yaninek *et al.*, 1993). None of these became established over a five years release campaign (Rogg and Yaninek, 1990). The recovery period of Colombian phytoseiids released in more than 10 sites varied from 1

month to 6 months for any release site (Yaninek *et al.*, 1993). Since 1989, natural enemies from Northeastern Brazil have been shipped to Africa for experimental releases. Among the several species and populations of phytoseiids introduced against *M. tanajoa* in Africa, the Brazilian populations of *Typhlodromalus manihoti* Moraes (formerly *T. limonicus sensu lato* Garman and McGregor, see de Moraes *et al.*, 1994) (Yaninek *et al.*, 1998) and *Typhlodromalus aripo* DeLeon (Acari: Phytoseiidae) (Yaninek *et al.*, 2001) have been the most effective.

6. Biology, establishment and spread of *Typhlodromalus manihoti* and *Typhlodromalus aripo*

6.1. Food requirements, effect of temperature and relative humidity

Typhlodromalus manihoti and *T. aripo* as many other phytoseiids, develop through 5 developmental stages which are: egg, larva, protonymph and deutonymph, before becoming adult. The reproduction is pseudo-arrhenotokous (Schulten, 1985). Laboratory studies showed that both *T. manihoti* and *T. aripo* could develop to maturity on a range of food sources commonly found in the cassava ecosystem including other tetranychid prey, pollen, nymphs of the whitefly *B. tabaci* (Yaninek *et al.*, 2001) and cassava plant exudate (Bakker, 1993; Toko *et al.*, 1994; Yaninek *et al.*, 2001). However, both species require either *M. tanajoa* or the inferior prey *O. gossypii* for oviposition. *Typhlodromalus aripo* (Yaninek *et al.*, 2001; chapter 4) but not *T. manihoti*, can also reproduce on *Tetranychus urticae* Koch and maize pollen (Yaninek *et al.*, 2001).

Laboratory results of relative humidity effects on egg hatching success revealed that egg hatchability in both *T. aripo* (Bakker *et al.*, 1993) *and T. manihoti* (Bakker *et al.*, 1993; Mégevand, 1997) decreased from 100% to 0% when the Saturation Deficit (SD) increased from 0 to 10 mm Hg. Fifty percent of *T. manihoti* and *T. aripo* eggs hatched at 3 and 7 mm Hg SD respectively (Bakker *et al.*, 1993). These results suggest that a Saturation Deficit above 10 mm Hg is not suitable for *T. manihoti* and *T. aripo* population growth.

6.2. Distribution in the cassava plant

Previous studies on within-cassava plant movement of *T. aripo* showed that *T. aripo* was found in the apices of the plants only, awaiting the cassava green mite to move up from the young infested leaves whereas *T. manihoti* inhabited and consumed prey on the leaves only (Bakker, 1993). A recent study has demonstrated that *T. aripo* showed temporal partitioning of resources on young leaves: They are mostly active during the first part of the night in foraging on the upper young leaves of cassava foliage and move to the apex where they aggregate (Onzo *et al.*, in prep.). Which life stages disperse from the apices to feed is currently under investigation. It has been argued from small-scale field experiments that *T. aripo* disappeared from cassava plants when the apex was removed, however the apex in itself is not a sufficient condition for this predator species to persist in the field (Bakker, 1993).

6.3. Establishment and spread

The Brazilian population of *T. manihoti* was first released in Africa in 1989. Yaninek *et al.* (1998) showed that the phenology of this population in cassava fields varied with season and prey density. It was more abundant during the dry season when *M. tanajoa* densities were high. *Typhlodromalus manihoti* probably disperses from cassava plants when *M. tanajoa* densities can no longer be exploited, to the surrounding vegetation, particularly Euphorbiaceae, but no observations were made to see whether these habitats supported *T. manihoti* development and reproduction. Establishments are now found in 3 countries within 8 years (Benin, Ghana, and Nigeria) where the phytoseiids cover an area estimated at 4,300 km^2 (Yaninek *et al.*, 1998). *Typhlodromalus manihoti* dispersed at a rate of 3.3 km per year, spreading five times faster during the dry compared to the wet season in proportion to the frequency of surrounding cassava fields. This is probably due to the fact that during the wet season, some fields were harvested and replanted or used as fallow, thus reducing the frequency of cassava fields. Established *T. manihoti* populations comprised a quarter of the total phytoseiid fauna found on cassava during the wet season and often double this amount or more during the dry season (Yaninek *et al.*, 1998). The impact of *T. manihoti* on *M. tanajoa* has been difficult to assess in the field because of its patchy distribution during the dry season (Yaninek *et al.*, 1998) but that should not discourage further investigations.

Typhlodromalus aripo was first released in Africa in late 1993. With natural spread and further releases, *T. aripo* readily established and spread to cover 12 countries in 4 years where it has been shown to have a big impact on the population dynamics of *M. tanajoa* (Yaninek *et al.*, 2001). The phenology of *T. aripo* shows the same trend as that of *T. manihoti*, but does not occur on surrounding vegetation. It presently covers an estimated 900,000 km^2. It spreads at a rate of ca 12.5 km in the first season, and up to 200 km per season thereafter (Yaninek *et al.*, 2001). Results from production trials conducted in Benin and Nigeria, showed an increase in crop yield of between 35% and 40% when *T. aripo* is present or ca $60 added value per hectare (Yaninek *et al.*, 2001).

7. Herbivore-induced synomones and prey location

Volatile chemicals are among the main information-conveying agents available to entomophagous arthropods (Vinson, 1976). Many terms have been proposed to describe different classes of semiochemicals or infochemicals. Semiochemical terminology according to Nordlund and Lewis (1976) is based largely on the origin of the compounds in specific interactions. Dicke and Sabelis (1988) proposed an adaptation of this terminology that abandons the origin criterion of Nordlund and Lewis. They classify infochemicals on the basis of the costs and benefits of the interaction for both interactants. Infochemicals or semiochemicals comprise two different groups of chemicals: pheromones and allelochemicals. Three sub-groups belong to the latter: kairomones, allomones and synomones (see definitions by Dicke and Sabelis, 1988).

When searching for prey, carnivorous arthropods may exploit infochemicals from their prey and from their prey's food plant. Chemical stimuli from plants are most detectable because of the plant's relatively larger biomass but they are less reliable indicators of herbivore presence. In contrast, chemical stimuli derived from the herbivore itself are generally the most reliable source of information on herbivore presence and identity, but they are less detectable (Vet and Dicke, 1992). This means that natural enemies should combine the advantageous characteristics of information from both trophic levels to locate their prey (Vet and Dicke, 1992). The infochemicals ideally should inform the natural enemies whether an herbivore is present, which species it belongs to, how many they are, whether it can be parasitized or eaten.

Plant chemicals that are emitted upon damage by the herbivore and result in the attraction of predators, are favorable to the plant and the predators; therefore, they are called herbivore-induced synomones (Vet and Dicke, 1992). Damage by herbivores increases the emission of plant volatiles and natural enemies attacking herbivore feeding stages, exploit this information. The value of plant information depends on the degree of herbivore infestation. Predators can differentiate between herbivore-infested and non-infested plants and in many cases the attractive chemicals have been shown to be released by the plant (Vet and Dicke, 1992; Dicke and Vet, 1999; Sabelis *et al.*, 1999). Plant volatiles that are released upon damage by herbivores can be specific indicators of the herbivore's identity (Dicke *et al.*, 1990b). Corn seedlings release large amounts of volatile terpenoids after damage inflicted by *Spodoptera* caterpillars, which attract the larval parasitoid *Cotesia marginiventris*. Induction of these synomones can be simulated by treating leaves with caterpillar oral secretions (Turlings *et al.*, 1990). Similarly, the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) is attracted by spider mite-induced synomones emitted by bean or cucumber plants (Dicke *et al.*, 1990b). Emission of these synomones is not restricted to the damaged site, but they can be released systemically by the whole plant under attack (Dicke *et al.*, 1990b). Once the carnivores arrive at the infested plant, odor gradients within the plant can be used to locate the infested leaves and the prey colony (Dicke, 1995). In phytoseiid mites, Jagers Op Akkerhuis *et al.* (1985), and De Bruyne *et al.* (1991), reported that the front legs are not used for walking but are equipped with olfactory chemoreceptors on the dorsal side of the tarsi which allow them to detect and recognize odors in the air. In *P. persimilis*, the front legs are moved up and down at high frequency and amplitude, which may facilitate the perception of herbivore-induced synomone (Dicke *et al.*, 1991).

8. Variation in the production of herbivore-induced synomones

Leaf growth stage, cultivars, plant species, attacking herbivore species, time of the day, light intensity, season and water stress constitute the main factors affecting the production of herbivore-induced synomone (Takabayashi *et al.*, 1994b; Turlings *et al.*, 1995).

8.1. Effects of leaf age

To a plant, young upper leaves are more important than old lower leaves with respect to plant growth and reproduction. In an investigation of the production of herbivore-induced

synomone in cucumber leaves of different stages, Takabayashi *et al.* (1994a) reported that the predatory mite *P. persimilis* responded to young *T. urticae*-infested, but not to old *T. urticae*-infested leaves in a Y-tube olfactometer. Qualitatively, the total blends of volatiles from infested old and young leaves appeared to be similar. However, several differences have been recorded when comparing compounds between the blends of old and young leaves (Takabayashi *et al.*, 1994a). The most striking differences between the blends of volatiles from infested young and infested old cucumber leaves were the relative amount of the two oximes, 3-methylbutanal O-methyloxime and one unidentified oxime. Infested young leaves produced less oximes than infested old leaves (Takabayashi *et al.*, 1994a). In contrast, the infested young leaves produced slightly more nitriles than infested old leaves did. The relative amounts of two of the main herbivore-induced plant chemicals, (*E*)-ß-ocimene and (3*E*)-4,8-dimethyl-1,3,7-nonatriene, differ according to the age of the cucumber leaf. (*E*)-ß-ocimene was produced less in infested young leaves whereas (3*E*)-4,8-dimethyl-1,3,7-nonatriene was produced more in infested young leaves (Takabayashi *et al.*, 1994a).

8.2. Effects of plant cultivars

Plant genotypes or cultivars can affect the composition of herbivore-induced plant volatiles (Takabayashi *et al.*, 1991; Loughrin *et al.*, 1995). Loughrin *et al.* (1995) reported that the average volatile emission from beet armyworm, *Spodoptera exigua* larvae-infested leaves of a naturalized cotton variety was sevenfold higher and the relative percentages of (*E*)-ß-ocimene and (3*E*)-4,8-dimethyl-1,3,7-nonatriene were also much higher than that of damaged leaves of commercial cultivars. Takabayashi *et al.* (1991) investigated the blends of volatiles emitted by leaves of two different apple cultivars infested by spider mites and reported differences in blend composition. The relative percentages of (*E*)-ß-ocimene and (3*E*)-4,8-dimethyl-1,3,7-nonatriene were much higher in *T. urticae* infested leaves of apple cv. Summer Red than when this spider mite infested apple leaves of cv. Cox Orange Pippin. Differences were also observed for several other volatile components (Takabayashi *et al.*, 1994b). The results of the response of *P. persimilis* to the herbivore-induced synomone emitted by leaves from two different bean plant cultivars (*Phaseolus vulgaris*) with the same level of *T. urticae* infestation also showed a significant difference in attractiveness to *P. persimilis* (Dicke *et al.*, 1990b). It remains unknown whether this was due to differences in the amount or composition of herbivore-induced synomone. Recently, cultivar differences in

the emission of herbivore-induced plant volatiles have been reported for gerbera (Krips *et al.*, 2001) and corn (Gouinguené *et al.*, 2001).

8.3. Effects of herbivore species

Several reports revealed that different herbivore-induced synomone blends are induced by different herbivore species. The predatory mites *Amblyseius andersoni* (= *A. potentillae*) and *A. finlandicus* responded to apple foliage infested by the European red spider mite *Panonychus ulmi* (Sabelis and van de Baan, 1983), which is reported by Dicke (1988a) to be a suitable prey species for both phytoseiid species. However, they did not respond to apple foliage infested by the two-spotted spider mite *T. urticae*, which is not a suitable prey species for both predatory mites (Sabelis and van de Baan, 1983). In contrast, satiated females of *P. persimilis* responded to volatiles emitted from apple leaves (Cox Orange Pippin) infested by *T. urticae*, but not to the volatiles emitted from apple leaves infested by *P. ulmi* (Sabelis and van de Baan, 1983; Sabelis and Dicke, 1985). When apple leaves infested by *T. urticae* and those infested by *P. ulmi* were offered simultaneously in a Y-tube olfactometer, starved females preferred *T. urticae*-infested apple leaves (Sabelis and Dicke, 1985). The composition of the volatile blends emitted by apple leaves of cv. Summer Red when infested by *T. urticae* or *P. ulmi* showed many quantitative differences (Takabayashi *et al.*, 1991). The four chemicals that are known to attract *P. persimilis*, (*E*)-ß-ocimene, (3 *E*)-4,8,-dimethyl-1,3,7-nonatriene, linalool and methyl salicylate, were found in the headspace of apple leaves infested by both spider mite species during chemical analysis. However, the relative percentages differed between the two blends (Takabayashi *et al.*, 1994b). That predatory mites are differentially attracted by volatiles emitted by plants of the same species infested by different herbivore species, has also been reported by Dicke and Groeneveld (1986) and Dicke (1988a,b). Different herbivore instars may even result in different plant responses (Takabayashi *et al.*, 1995). However, so far almost no studies have been conducted to elucidate how plants respond to simultaneous infestation by two or more herbivore species (Dicke and Vet, 1999; Vos *et al.*, 2001; Shiojiri *et al.*, 2001) and to what extent predatory mites are attracted to plants infested by more than one herbivore.

8.4. Effects of abiotic factors

Environmental conditions such as light intensity, season and water stress greatly influence plant physiology and therefore potentially the production of herbivore-induced synomone

(Takabayashi *et al.*, 1994b). Non-infested leaves of lima bean plants placed under high light intensity were more attractive than non-infested leaves of lima bean plants placed under low light intensity (Takabayashi *et al.*, 1990). Chemical analysis revealed that the headspace of leaves under high light intensity emitted relatively higher amounts of the synomone components than leaves under low light intensity (Takabayashi *et al.*, 1994b). These authors concluded that the presence of higher amounts of (*E*)-ß-ocimene in the volatiles of the leaves under high light intensity may be due to: (1) plants under high light intensity receiving more photolytic energy input used to produce plant synomone and/or (2) plants under high light intensity suffering water stress that induces the production of plant synomone.

Water stress may affect the production of herbivore-induced synomone by plants. A Y-tube olfactometer bioassay conducted with leaves of non-infested lima bean plants reared under water stress and non-water stress conditions showed that *P. persimilis* preferred the odor of water stressed lima bean plants to odors from non-stressed plants (Takabayashi *et al.*, 1994b). The headspace of leaves from water-stressed lima bean plants was characterized by relatively higher amounts of synomones [linalool, (*E*)-ß-ocimene, (3 *E*)-4,8-dimethyl-1,3,7-nonatriene and methyl salicylate] than leaves from non-water-stressed lima bean plants (Takabayashi *et al.*, 1994b).

9. Infochemicals and competition among carnivores

In addition to using infochemicals in detecting prey patches in an early phase of the searching process (Vinson, 1981; Vinson, 1984; Vet and Dicke, 1992), natural enemies also recognize volatile infochemicals associated with con- or heterospecifics. These odors affect the distribution of predators or parasitoids over prey patches. Instead of visiting patches to determine the presence of conspecific or heterospecific competitors, predators and parasitoids can use this mechanism to determine competitor presence from a distance, and decide whether to avoid or to visit this patch.

Avoidance behavior in arthropod predators and parasitoids can have important ecological implications. For instance, predators and parasitoids that are able to distinguish between odors from food patches with and without competitors can save time and energy in determining which patches they should visit (Janssen *et al.*, 1995a). Refraining from visiting patches occupied by conspecifics prevents the formation of predator and parasitoid

aggregations on prey patches, which has important consequences for the stability and dynamics of predator-prey and parasitoid-host interactions (Janssen *et al.*, 1997). Adult females of *Leptopilina heterotoma* (Thompson) (Hym., Eucoilidae) refrain from visiting patches where females of another, closely related parasitoid species are present (Janssen *et al.*, 1995a,b). There is evidence that avoidance of patches with the competitor may lead to changes in the distribution of parasitoids over patches in the field (Janssen *et al.*, 1995a). Janssen *et al.* (1997) showed that *P. persimilis* females distinguish between odors emanating from prey patches with and without conspecifics. The infochemical responsible for the decreased attractiveness of prey patches with predators is produced by adult prey. Removal of adult prey resulted in a rapid disappearance in avoidance of predator-invaded prey patches (Janssen *et al.*, 1997). Hislop and Prokopy (1981) reported that the predatory mites *Amblyseius fallacis* and *Phytoseiulus macropilis* deposit marking pheromones during searching in prey patches leading to a reduced searching time by subsequent predators.

10. Infochemicals in plant-spider mite-phytoseiid interactions

Anyone who aims to understand plant-herbivore interactions should include the third trophic level, consisting of natural enemies of herbivores, since they play an important role in plant defense against the herbivores. To protect themselves against herbivores, two types of defenses are used by plants (Price *et al.*, 1980; Dicke and Sabelis, 1988). Firstly, the direct defense acts on herbivores directly and negatively affects herbivore performance. It can be mediated by plant chemicals such as toxins, repellents or antifeedants, or by structures such as trichomes. Secondly, the indirect defense promotes the effectiveness of natural enemies of herbivores and can be mediated by infochemicals produced from interactions between plants and herbivores (Price *et al.*, 1980; Dicke and Sabelis, 1988). In this section, I will concentrate on the interactions between plant, spider mite and phytoseiid with emphasis on the importance of infochemicals for the interactants.

10.1. Importance for the plant

It is known that plants constitute a high potential food source for herbivores such as spider mites. One should then keep in mind that spider mites are ravenous feeders and may kill the plant if it is insufficiently protected. A plant infested by spider mites would, therefore, benefit from increasing the influx of predators (Sabelis and Dicke, 1985). Since the

beginning of the 1980s, evidence from numerous studies has shown that plants may also respond to herbivore damage by emitting herbivore-induced synomone that affects the biology of both carnivores and herbivores as well as plants (Dicke *et al.*, 1993). In the system consisting of plants-spider mites and predatory mites, induced indirect defense is essential to plant growth: it may determine the balance between life and death. Predatory mites recruited as "bodyguards" may exterminate spider mite populations leading to improved plant fitness. In the absence of predators, the food plant resource is overexploited by spider mites and can result in plant death (Sabelis, 1981; Helle and Sabelis, 1985).

10.2. Importance for the phytoseiid mites

To predatory mites, allelochemicals play an essential role in affecting prey-searching and prey-selection behavior in several ways. First, predatory mites disperse largely on wind currents and, after landing, they use synomones in making foraging decisions such as whether to stay and where or how long to search (Sabelis and Van de Baan, 1983; Dicke and Groeneveld, 1986; Dicke *et al.*, 1989). Second, once predatory mites are in a prey patch, their behavior is affected by volatile and nonvolatile infochemicals (Sabelis, 1981; Sabelis and Dicke, 1985). Therefore, the predators stay in the patch as long as prey-related allelochemicals are present. Third, predatory mites can distinguish between herbivore-induced synomones produced by the same plant attacked by different prey species (Sabelis and van de Baan, 1983; Dicke and Groeneveld, 1986; Dicke, 1988b). This suggests that predatory mites can detect suitable prey species and feed on them by responding to herbivore-induced synomones. Later on, the reproductive success may lead to a rapidly growing predator population able to disperse and search for new prey patches (Sabelis and Dicke, 1985). Chemical analysis of the volatiles from *T. urticae*-infested lima bean plants revealed that four of these infochemicals attracted the predator *P. persimilis*: the terpenes (E)-ß-ocimene and linalool, the homoterpene 4,8-dimethyl-1,3(E),7-nonatriene and the phenolic methyl salicylate (Dicke *et al.*, 1990a).

10.3. Importance for the spider mites

The infochemicals that attract phytoseiids to spider mite-infested plants may also be exploited by spider mites to locate suitable host plants. For example the spider mite, *T. urticae* is attracted by a volatile kairomone of undamaged lima bean plants, but disperses from an odor plume of lima bean plants heavily infested by conspecifics (Bernstein, 1984;

Dicke, 1986). This does not lead to a complete avoidance of the infested plants. The spider mites only use the volatile chemicals to move away from the infested leaves and stay on other leaves (non-infested) of the same plant (Dicke *et al.*, 1993) or disperse to other non-infested neighbouring plants. In the context of intraspecific spider mite interactions, the volatile infochemical emitted from *T. urticae*-infested plants is a pheromone, in this case, a (+, +)-dispersing pheromone (Dicke and Sabelis, 1988). The behavioral reaction of the spider mites depends on the ratio of kairomone to pheromone. At a low ratio (low degree of infestation), the spider mites are attracted; at a high ratio (high degree of infestation), they are repelled. Dicke (1986) argued from biological evidence that the pheromone shares components with the herbivore-induced synomone. From the combined data of Dabrowski and Rodriguez (1971), Dicke (1986), and Dicke *et al.* (1990a), it can be deduced that at least one component of the spider mite pheromone is also a component of the predatory-prey synomone (i.e. linalool). However, in another study with a different plant species and different bioassay, *T. urticae* was slightly attracted to volatiles from spider mite-infested plants (Pallini *et al.*, 1997). More studies are needed on the responses of spider mites to herbivore-induced plant volatiles and variation in responses among systems.

11. Importance of infochemicals for biological control and plant breeding

The criteria for selecting predators for biological control of *M. tanajoa* have been: (i) the spatial and temporal association of the predator and *M. tanajoa* in the field (Yaninek and Herren, 1988), (ii) the predator's rate of reproduction when fed with *M. tanajoa* (Yaninek and Herren, 1988), and (iii) agrometeorological comparison of areas of origin and release (Yaninek and Bellotti, 1987). McMurtry (1982) showed that most of these criteria successfully used for biological control varied considerably between species. For example, successful predators were not always the ones with high reproductive potential (McMurtry, 1982; Yaninek *et al.*, 2001), and the correlation between the distribution of predator and prey was high for some species and low for others. This necessitates the use of additional selection criteria, such as prey preference, to improve the reliability of the selection procedure (Janssen *et al.*, 1990).

An important factor in the biological control of *M. tanajoa* in Africa is that candidate natural enemies must be capable of permanent establishment and will have to search for new

prey patches when they have eradicated the colonies in which they were released. Moreover, it is impossible to release natural enemies on each infested plant. For these reasons, predators need to be as efficient as possible in locating colonies of the target pest. Ever since the first discovery of infochemical use by natural enemies in the early 1970s, investigators have speculated on possibilities for applying these infochemicals in pest management (Gross, 1981; Dicke *et al.*, 1990a). The involvement of chemicals in interactions between individual organisms has been recognized for many years (Dicke and Sabelis, 1988). The role of herbivore-induced plant volatiles in spider mite extermination seems to be essential (Sabelis and van der Meer, 1986; Dicke *et al.*, 1990b). Herbivore-induced plant volatiles combine several advantages: their spatial distribution is linked to that of the herbivores and they are produced in large amounts. In addition, the application of these infochemicals does not need any action by man. It can be seen, therefore, as a recruitment of biological control agents by the plant itself after being attacked by herbivores. The use of the response to the allelochemicals as a criterion in selection of natural enemies for biological control is a valuable idea that can be used to develop biological control of the cassava green mite in Africa (Janssen *et al.*, 1990). Thus, integrating infochemical use in selecting natural enemies is recommended for a successful development of a biological control program.

Current plant breeding practices do not consider effects of plants on the third trophic level. Plant characteristics not only affect herbivores but they may also affect the plant-inhabiting or visiting natural enemies of herbivores (Dicke, 1995). Data on mite-plant systems show that plants are involved in production of volatile allelochemicals that attract predatory mites and retain them in spider-mite patches. Because differences in attraction have been found for different bean or gerbera cultivars infested by *T. urticae*, plant breeders may enhance predator efficiency by breeding plants with higher rates of synomone emission (Dicke *et al.*, 1990b; Krips *et al.*, 1999). For these possibilities to become reality, it is necessary to convince plant breeders of the importance of an indirect component of plant defense, through the action of the third trophic level (Price *et al.*, 1980). The elimination of indirect defense in a plant breeding program for selection of cultivars may reduce the net resistance under field conditions, mainly in cases where natural enemies play a significant role in reduction of herbivore populations below the Economic Injury Level. In selecting a cultivar, plant breeders should aim at improving conditions that affect predator-searching behavior with the aim of increasing the effectiveness of herbivore population control (Dicke

et al., 1990b). Therefore, a better understanding by plant breeders of the interaction between plants and biocontrol agents should be aimed for.

12. Research justification and objectives

Ten predatory mite species have so far been introduced for biological control of the cassava green mite in Africa, after selection based on the criteria listed at the beginning of the previous section. Of these 10 species, only *T. manihoti* and *T. aripo* have been successfully established and have spread in the cassava agroecosystem in Africa. *Typhlodromalus manihoti* was known to survive periods of low prey densities by moving to alternative prey on associated plants and reinvading cassava plants when *M. tanajoa* populations began to increase. *Typhlodromalus aripo* uses a wide range of food resources on which it can develop and reproduce, and its presence in cassava shoot tips may protect it against hard conditions. However, the selection criteria used and characteristics of the phytoseiid species *T. manihoti* and *T. aripo* have not been sufficient to understand the success of *T. manihoti* and *T. aripo* and their impact on cassava green mite in the African cassava agroecosystem. The broad objective of this thesis is to improve our understanding of the biology and ecology of these two predators. The information gained through this research will enable us to understand the following biological and ecological interactions: (1) how the two predators use infochemicals to locate prey, (2) the relationship between predator response to prey-specific infochemicals and the intrinsic developmental and reproductive value of the prey for the predator, (3) how infochemicals mediate interspecific and intraspecific interactions, including aggregation, avoidance, and competition, and (4) the relationship between their use of infochemicals and their relative success in persisting in the environment and in regulating prey abundance.

Outline of the thesis

I conducted a series of studies on attraction of the two exotic predatory mite species *T. aripo* and *T. manihoti* to herbivore-induced cassava plant volatiles and to cues related to the presence of conspecifics or heterospecifics in the presence and absence of prey. In Chapter 2; I investigated how the two predators use cassava plant volatiles in distant prey (*M.*

tanajoa) location and whether volatiles from *M. tanajoa*-infested cassava plants are the most important cues in distant prey location. To understand how the two predators cope with mixtures of herbivore-induced cassava plant volatiles, I tested their prey-related odor preference for odors emitted from cassava leaves infested with the key prey *M. tanajoa*, or the inferior prey species *O. gossypii* and *T. urticae*, and for odors from mixtures of two prey species on cassava leaves. This study is relevant since these inferior prey mites co-occur with the target prey *M. tanajoa* in the cassava agroecosystem (Chapter 3). In Chapter 4, I report on the intrinsic developmental and reproductive value, for *T. manihoti* and *T. aripo* when feeding upon *M. tanajoa*, *O. gossypii* and *T. urticae* in no choice experiments. The data from these experiments are compared with those from prey-related odor preference. Chapter 5 describes habitat use by the two predators in the context of coexistence on cassava plant foliage. Chapter 6 investigates strategies displayed by the two exotic predator species and the native predator *Euseius* (= *Amblyseius*) *fustis* (Pritchard and Baker) when sharing the same prey mite *M. tanajoa* in the same habitat, in relation to the use of infochemicals. I added *E. fustis* to the studies to determine if, when in the same prey patch, this native predator affects prey location behavior by *T. manihoti* and *T. aripo* and consequently, through competitive interactions, their impact on *M. tanajoa* populations. I end this thesis with a general discussion (chapter 7) that summarizes and synthesizes all the findings of the preceding chapters.

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2

Attraction of the predatory mites *Typhlodromalus manihoti* Moraes and *Typhlodromalus aripo* DeLeon (Acari: Phytoseiidae) to cassava plants infested by cassava green mite

Désiré Gnanvossou, Rachid Hanna, Marcel Dicke & J. Steve Yaninek

Abstract

The attraction of the predatory mites, *Typhlodromalus manihoti* and *Typhlodromalus aripo*, to the host plant-spider mite complex, *Manihot esculenta* – *Mononychellus tanajoa*, was investigated with a Y-tube olfactometer. Factors examined included predator starvation period, several combinations of cassava leaf biomass and initial *M. tanajoa* infestations, *M. tanajoa*-damaged leaves with mites and/or their residues removed, *M. tanajoa* alone, and mechanically damaged cassava leaves. We found that females of *T. manihoti* and *T. aripo* were significantly attracted to *M. tanajoa*-infested cassava leaves when the predators were starved for 2, 6 or 10 hours. Satiated *T. aripo* was significantly attracted to infested cassava leaves whereas satiated *T. manihoti* did not discriminate between infested and non-infested leaves. When a choice was given between either two or four leaves infested with 200 female *M. tanajoa* and an equivalent number of non-infested leaves, 2 h-starved *T. manihoti* and *T. aripo* were significantly attracted to each of the infested groups of cassava leaves. At a density of 12 female *M. tanajoa* per leaf on four leaves, 2 h-starved *T. manihoti* was still attracted to *M. tanajoa*-infested leaves whereas 2 h-starved *T. aripo* was not attracted. When a choice was given between non-infested cassava leaves and either infested leaves from which only *M. tanajoa* females had been removed, or infested leaves from which all *M. tanajoa* and their visible products (web, feces) had been wiped off, *T. aripo* preferred odors from both types of previously infested leaves. *Typhlodromalus manihoti* was only attracted to infested leaves from which the *M. tanajoa* females only had been removed. Finally, the two predators were not attracted to 400 female *M. tanajoa* on clean cotton wool or to mechanically wounded leaves. This supports the hypothesis that *M. tanajoa* damage induces volatile cues in cassava leaves that attract *T. manihoti* and *T. aripo* to *M. tanajoa*- infested leaves.

Introduction

Prey location by predatory mites has long been considered a matter of chance (Jackson and Ford, 1973; Rabbinge, 1976). However, since the 1980s new discoveries have revealed that upon spider mite damage, plants emit volatile infochemicals that attract the natural enemies of herbivores (Sabelis and van de Baan, 1983; Sabelis *et al.*, 1984; Dicke, 1988a,b; Dicke and Sabelis, 1988). This phenomenon has been studied for two decades and a wealth of knowledge has been accumulated on several plant-herbivore-predator (Dicke and Sabelis, 1988; Shimoda *et al.*, 1997; Takabayashi *et al.*, 1998) and plant-herbivore-parasitoid (Turlings *et al.*, 1990, 1995) systems. For example, when infested by the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), lima bean plants, *Phaseolus lunatus* L., emit herbivore-induced plant volatiles that attract the phytoseiid mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (Sabelis and van de Baan, 1983; Dicke *et al.*, 1990; Takabayashi *et al.*, 1994). The spider mites themselves were not attractive (Sabelis *et al.*, 1984). Plants have been also shown to emit an attractive odor when attacked by caterpillars. Turlings *et al.*, (1990, 1991) showed that females of the larval parasitoid, *Cotesia marginiventris* (Cresson) were strongly attracted to such volatiles rather than responding to odor cues coming directly from their hosts. Behavioral data also showed that predators and parasitoids discriminated between plants with herbivores and plants without herbivores based on blends of volatiles (Vinson, 1975; Nordlund *et al.*, 1981; Vet and Dicke, 1992). They can also discriminate odors from plants of the same species that are infested by different herbivore species (Sabelis and van de Baan, 1983; Hofstee *et al.*, 1993; Geervliet *et al.*, 1998; De Moraes *et al.*, 1998; Dicke, 1999). Predators and parasitoids are also attracted to herbivore products such as feces, but this attraction is generally much weaker than that of herbivore-induced plant volatiles (Sabelis *et al.*, 1984; Vet and Dicke, 1992; Steinberg *et al.*, 1993).

Two predatory mites *Typhlodromalus manihoti* Moraes (formerly *T. limonicus* (Garman and McGregor) sensu lato; see De Moraes *et al.*, 1994, and *T. aripo* DeLeon, were introduced from Brazil into Africa as biological control agents of the cassava green mite *Mononychellus tanajoa* (Bondar), which was accidentally introduced into Africa from South America in the 1970 (Lyon, 1973; Yaninek *et al.*, 1998). *Typhlodromalus manihoti* was shown to be effective predators of the *M. tanajoa* in the Neotropics (Bellotti *et al.*, 1987; Bakker, 1993) whereas *T. aripo* was shown to be efficient predator of *M. tanajoa* only after its introduction

into Africa (Yaninek *et al.*, 2001). While *M. tanajoa* is their preferred and most profitable prey, they also make use of other food sources such as the tetranychid mites *Oligonychus gossypii* Zacher and *Tetranychus urticae* Koch (chapter 4), and plant-based foods such as pollen from *Zea mays* L. and *Leucaena leucocephala* L. (IITA, 1997). Little is known, however, about the details of their prey location behavior.

In this study, we report on several experiments that were conducted to determine whether the two phytoseiid species *T. aripo* and *T. manihoti* are attracted by *M. tanajoa*-infested cassava plants. These data are important for understanding the differences observed in the ability of *T. manihoti* and *T. aripo* to establish and locate prey patches and how that relates to the predators' potential impact on *M. tanajoa* populations in the cassava agroecosystem in Africa. In two-choice experiments using a Y-tube olfactometer, we attempted to answer the following questions: (1) Are *M. tanajoa*-induced odors from cassava leaves detectable cues for the predators *T. manihoti* and *T. aripo*? (2) Is starvation needed for attraction of *T. manihoti* and *T. aripo* to *M. tanajoa*-induced cassava leaf odors? (3) Do *T. manihoti* and *T. aripo* have similar degrees of attraction to volatile infochemicals from *M. tanajoa*-infested cassava plants?

Materials and Methods

Spider mites. The *M. tanajoa* used in the experiment originated from mites renewed periodically from cassava fields in southern Benin. The culture was maintained indoors on potted cassava plants at 26 ± 1 °C; 65-80% RH. Ground coconut husks were used as medium in the pots to grow the plants.

Predatory mites. The populations of *T. manihoti* and *T. aripo* used in this study were collected in July 1998 from fields near Sè (78 km N-W. of Cotonou, Benin), and maintained in the laboratory at 25 ± 1°C and 80 ± 10% RH on detached cassava leaves infested with *M. tanajoa* as described by Mégevand *et al.* (1993). The petioles of these leaves were placed in water-filled vials sealed with parafilm to keep them hydrated. The vials with leaves were placed on a plastic tray (35 x 35 x 6 cm) and covered with a similar tray. New infested leaves were added daily to the rearing units. The predatory mites had been maintained 4 to 5

months under the aforementioned laboratory conditions prior to their use in the olfactometer experiments.

Plants.   Cassava plants (*Manihot esculenta* Crantz, variety "Agric") used in the experiments were grown in 2.5 litre plastic pots with top soil and maintained in a greenhouse (30 ± 5 °C; 70 ± 10% RH) at the Benin research station of the International Institute of tropical Agriculture (IITA-Benin).   Cassava plants were allowed to grow for 4 to 5 weeks before their use in the experiments.

Y-tube olfactometer.   Prey location behavior of *T. manihoti* and *T. aripo* was investigated in two-choice tests using a closed system Y-tube olfactometer at IITA-Benin, at a range of predator starvation periods and prey densities.   The system, except for the compressor (SERBATOI® AUTOCLAVI; Type ELTO; Vol. 50) used to generate an air stream through the olfactometer, was identical to that described by Takabayashi and Dicke (1992).   This olfactometer setup has been extensively used for studying the olfactory responses of predatory mites (Sabelis and van de Baan, 1983; Takabayashi and Dicke, 1992) and the conclusions from these olfactometer experiments have been supported by greenhouse studies (Janssen, 1999).

Adult female *T. manihoti* and *T. aripo* collected from cultures with ample food were considered satiated (Sabelis, 1981).   Individuals were enclosed in a plastic tube (10 mm diam. 40 mm long) and kept without food for 2, 6 or 10 hours at 25-28°C; 65-90% RH prior to the olfactometer bioassays.   At the start of the olfactometer tests, female predators were placed individually at the base of an iron wire positioned in the middle of the Y-shaped glass tube, and parallel to the tube walls.   Predators were observed until they reached the end of one of the arms, or for a maximum of five minutes.   The tested predators were removed at the end of each observation.   After a series of five mites, the odor sources were connected to the opposite arm of the olfactometer to correct for any unforeseen asymmetry in the experimental set-up.   Individuals of both species were tested alternately with the same odor sources.   The number of females reaching the end of either arm was recorded for each predator species. Thirteen different tests were carried out and each test was repeated 3-10 times (on 3-10 consecutive days) with 20 predators per species per repetition.   New odor sources were used for each repetition.

Preparation of odor sources. To investigate the olfactory response of the two predator species, air passing through the arms of the Y-tube olfactometer was provided by different odor sources prepared as follows:

1. Effect of starvation

Female predators satiated or starved for 2, 6, or 10 hours were used to test the effect of starvation on olfactometer response by *T. manihoti* and *T. aripo.* Early in the morning, the petioles of eight newly collected young leaves from 4-5 week old cassava plants were immediately placed in water-filled glass vials sealed with parafilm. The third young leaf from the apex was chosen. In the afternoon of the same day, four leaves were infested with 100 adult female *M. tanajoa* per leaf. All leaves were incubated for three days in plastic cages (length: 70 cm; width: 40 cm; height: 40 cm), with two fluorescent light sources above each cage at $26 \pm 1°C$. The same number of non-infested leaves was kept under the same conditions. All *M. tanajoa*-infested leaves were incubated in one cage and non-infested leaves in another cage.

2. Effect of number of leaves and prey density

To investigate the effect of number of leaf and prey density on the olfactory response by *T. manihoti* and *T. aripo,* we varied the number of leaves from two to four and *M. tanajoa* densities from 12 to 100 mites per leaf. Early in the morning, the petioles of either four or eight newly collected young leaves from 4-5 weeks old cassava plants were immediately placed in water-filled glass vials sealed with parafilm. The third young leaf from the apex was chosen as in the previous test. In the afternoon of the same day, the following *M. tanajoa* infestation levels were established: two leaves were each infested with 100 adult *M. tanajoa* females, or four leaves were each infested with 100, 50, 25 or 12 adult *M. tanajoa* females. Mite-infested and non-infested leaves were incubated in separate cages for three days under the same conditions as described above (section 1). Two hour-starved predators were used in this test.

3. Effect of previous infestation by M. tanajoa

Two types of experiments tested the effect of prey removal on the response of *T. manihoti* and *T. aripo.* In one experiment, three days after the infestation of leaves in the plastic cages and immediately prior to the start of the olfactometer tests, *M. tanajoa* adult females were

removed from the leaves with a fine brush (all other spider mite stages and their products were left on the leaf). In a second experiment, all spider mites and their products (feces, silk, etc.) were removed with a cotton wool pad soaked in distilled water. Leaves were checked under binocular microscope (Wild M3B; 25x) to ensure that all items were sufficiently removed. Two hour-starved predators were used.

4. Effect of volatile cues produced by M. tanajoa removed from infested plants

Four hundred adult female *M. tanajoa* were individually collected with a fine brush from infested leaves of potted cassava plants kept indoors at 25-28°C; 65-90% RH. These mites were placed on cotton wool and three hours later, they were collectively used as an odor source in the Y-tube olfactometer. The three hours delay was needed to ensure that possible odors from infested plants that may be absorbed to the mites had volatilized before their use in the Y-tube olfactometer. The objective was to determine the relative contribution of volatile cues produced by *M. tanajoa* only in the attraction of predators. The odor sources were each kept in a 10 cm-glass tube during the experiments. Clean cotton wool was used as control in the opposite arm of the olfactometer. We also determined the predators' response to infested leaves versus clean air and compared it to their response to *M. tanajoa* removed from infested plants. Two hour-starved predators were used during the two tests.

5. Effect of mechanical damage on cassava leaves

To test the response of *T. manihoti* and *T. aripo* to mechanically damaged cassava leaves, we placed carborundum powder on a wet cotton pad and rubbed it gently on the lower surface of detached cassava leaves. Approximately 0.50 ± 0.04g of carborundum powder was used to rub four leaves. These leaves were tested in the olfactometer immediately after they were rubbed with carborundum powder. In the other arm of the olfactometer, we placed the same number of undamaged leaves. Two hour-starved predators were used during the test.

Statistics. To analyze the response of each predator species, a one-tailed binomial test was evaluated (see Zar, 1984). The difference between predator species was statistically tested with a Chi-square analysis with Yates correction; data were arranged in a 2 x 2 contingency table (see Zar, 1984).

Results

1. Effect of starvation

Satiated adult female *T. aripo* were significantly attracted to *M. tanajoa*-infested leaves compared with non-infested leaves whereas *T. manihoti* did not show a significant preference for either odor source (Figure 1). When starved for 2, 6 or 10 hours, both predator species were significantly attracted to *M. tanajoa*-infested leaves. No significant difference in response was found between the two predator species ($P>0.05$).
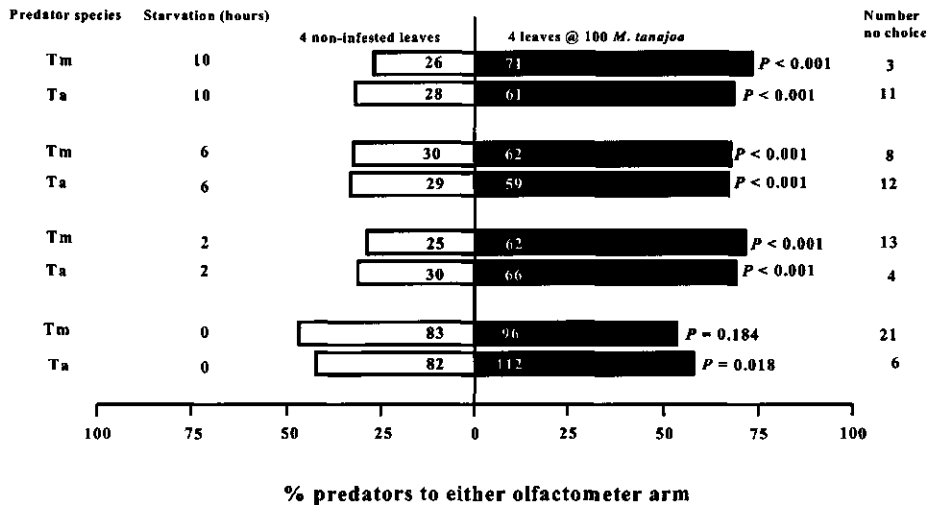

Figure 1. Effect of starvation on the response of the predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta) to volatiles from *Mononychellus tanajoa*-infested cassava leaves in a Y-tube olfactometer. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test. [Open bars = 4 non-infested cassava leaves; filled bars = 4 cassava leaves, each with 100-*M. tanajoa*].

2. Effect of number of leaves and prey density

Adult female *T. manihoti* and *T. aripo* discriminated between two leaves each infested with 100 adult female *M. tanajoa* and two non-infested leaves. The same result was obtained when the two predators were allowed to choose between four leaves each infested with either 50 or 25 adult female *M. tanajoa* and four non-infested leaves, but a significant difference was only found between the two predators at the 50 *M. tanajoa* infestation level (P=0.002). Moreover, at a density of 12 *M. tanajoa* adult females per leaf, *T. aripo* did not show a preference to either odor source, whereas *T. manihoti* was significantly attracted to *M. tanajoa*-infested leaves (Figure 2).
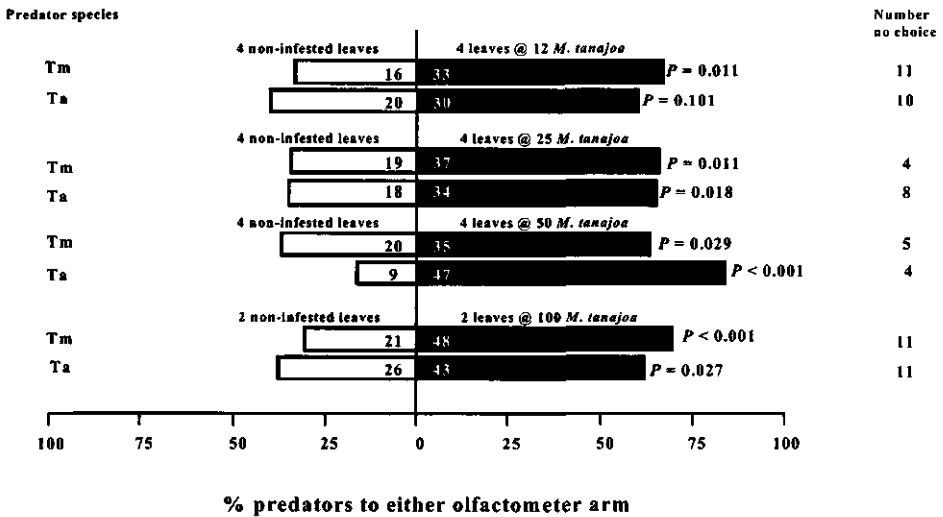

Figure 2. Response of 2 h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta) to volatiles from cassava leaves infested with various densities of *Mononychellus tanajoa* in a Y-tube olfactometer. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

3. Effect of previous infestation by M. tanajoa

Both *T. manihoti* and *T. aripo* similarly preferred the odors from infested leaves from which only living *M. tanajoa* females had been removed, over non-infested leaves (*P*< 0.001) (Figure 3). When all *M. tanajoa* life stages and their residues were removed, *T. aripo* were still strongly attracted to previously infested leaves but *T. manihoti* did not discriminate between previously infested and non-infested leaves (Figure 3).
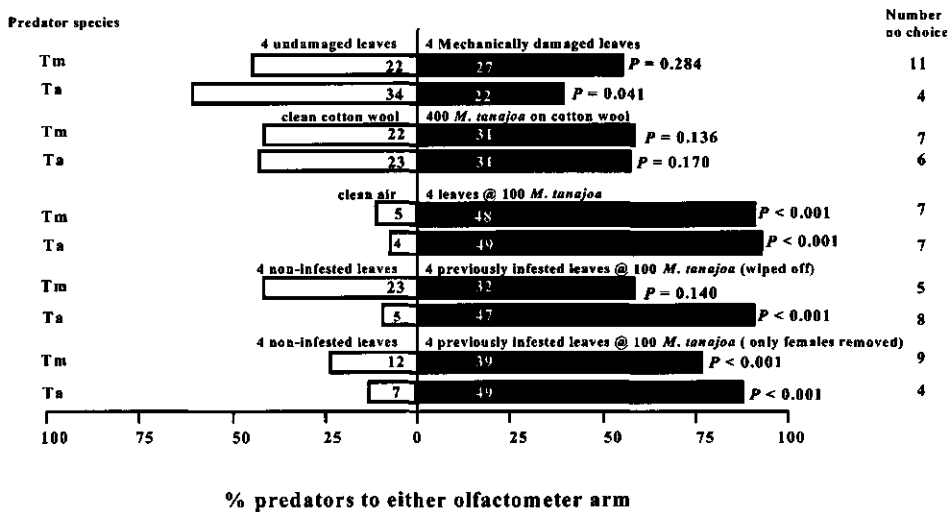

Figure 3. Response of 2 h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta) in a Y-tube olfactometer. A choice was given between undamaged cassava leaves and mechanically damaged leaves; between clean cotton wool and isolated spider mites on cotton wool; between clean air and cassava leaves with spider mite-damage; between non-infested cassava leaves and either leaves with *Mononychellus tanajoa* colonies from which females were removed or from which all mite stages and their residues were removed. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

4. Effect of volatile cues produced by M. tanajoa removed from infested plants

Typhlodromalus aripo and *T. manihoti* were strongly attracted to *M. tanajoa*-infested leaves compared with clean air. However, when they were allowed to choose between *M. tanajoa* females isolated on cotton wool and cotton wool without mites, neither of the two predator species showed a preference to *M. tanajoa* (Figure 3).

5. Effect of mechanical damage on cassava leaves

When given a choice between mechanically damaged and undamaged leaves, 60.7% of *T. aripo* adult females chose the arm with undamaged leaves, but only at a marginally significant level ($P=0.041$), whereas *T. manihoti* did not distinguish between the two odor sources as the distribution of its response was not significantly different from 50:50 (Figure 3).

Discussion

In this study we show that the predatory mites *T. manihoti* and *T. aripo* are attracted to *M. tanajoa*-infested cassava leaves, and we assume that such attraction is determined by volatiles emitted from the infested leaves, as attractive volatiles caused by *M. tanajoa* damage to cassava leaves have been previously collected and identified but not directly implicated in predator attraction (A. Janssen and M.A. Posthumus, unpubl. data). Moreover, we were able to show that the predators' attraction to *M. tanajoa*-infested cassava depended on several factors, including predator starvation period, initial mite infestation levels, number of cassava leaves, and whether leaves are damaged by mites or by mechanical means.

Our results reveal that satiated *T. aripo* are significantly attracted to cassava leaves infested by *M. tanajoa* whereas satiated *T. manihoti* are not significantly attracted. However, when *T. manihoti* and *T. aripo* were starved for 2, 6 or 10 hours they are both attracted to *M. tanajoa*-infested leaves. For 6 or 10 h-starved predators, the response was similar to that of predators starved for 2 hours but stronger than that of satiated predators. These findings show that food deprivation contributes to an increased olfactory response in *T. aripo* and *T. manihoti* and that the two predator species can detect nearby *M. tanajoa* patches when they are hungry. Responses of predatory mites to herbivore-induced plant volatiles have been found to vary

considerably with internal and external factors (reviewed in Takabayashi *et al.*, 1994; Dicke *et al.*, 1998 and Dicke, 1999), and one of the factors affecting the response of predators is starvation (Sabelis and van de Baan 1983, Dicke, 1988b). Janssen *et al.* (1990) investigated the response of several predatory mites species, including *T. manihoti,* in satiated condition to *M. tanajoa*-infested cassava plants. They reported that when satiated, 71% of the *T. manihoti* were attracted to plants infested by its key prey *M. tanajoa* compared to the 54% found in this study. Differences in the testing and/or rearing methods of the different predator populations may have contributed to differences in the olfactory response. The role of starvation in eliciting attraction to plants infested by their prey has been found in other phytoseiid species including *Typhlodromus pyri* Scheuten and *Metaseiulus occidentalis* (Nesbitt) (Sabelis and Van de Baan, 1983; Dicke, 1988b), and *Amblyseius andersoni* (= *A. potentillae*) (Garman) (Sabelis and van de Baan 1983, Dicke, 1988a).

Our study also investigated the sources of the volatiles that attract *T. manihoti* and *T. aripo*. When we gave the two predators a choice between *M. tanajoa* females isolated from cassava leaves and clean air, they were not able to detect the mites' presence, while they were clearly able to detect the presence of cassava leaves infested by the same number of *M. tanajoa* females. A comparison of these data with those reported by Sabelis and van de Baan (1983) and Sabelis *et al.* (1984) shows a similarity: isolated *T. urticae* adult females from infested lima bean plants were not attractive to *P. persimilis*. Koveos *et al.* (1995) also reported that possible odors released by *T. urticae* in the absence of plants play only minor role, if any, in the attraction of *A. andersoni*. Similar results were also found with *Spodoptera exigua* (Hübner) caterpillars and the parasitoid *Cotesia marginiventris* (Cresson) (Turlings *et al.*, 1991), *Pieris brassicae* (L.) and *P. rapae* (L.) caterpillars and the parasitoids *Cotesia glomerata* (L.) and *C. rubecula* (Marshall) respectively (Steinberg *et al.*, 1993; Geervliet *et al.*, 1994). Other herbivores can induce cassava volatiles as well and predators/parasitoids use odors emitted from herbivore-infested cassava plants for prey/host location. In olfactometer experiments, females of the parasitoid *Apoanagyrus lopezi* (formerly *Epidinocarsis lopezi*) preferred odors from mealybug-infested cassava plants to odors from non-infested cassava plants (Nadel and Alphen, 1987; Souissi, 1999; Souissi and Le Rü, 1999) or mealybug alone (Souissi, 1999; Souissi and Le Rü, 1999). Hammond (1988) found that adult *Diomus* sp. responded to volatile cues from mealybug-infested cassava leaves.

Our investigations do not reveal much difference between the two predator species regarding their attraction to infested cassava leaves in the Y-tube olfactometer. The most striking difference in their response is found when cassava leaves previously infested and then cleaned were offered. *Typhlodromalus aripo* has a stronger attraction to such leaves than *T. manihoti*. *Typhlodromalus manihoti* seems to have a somewhat higher threshold for perception of volatile cues from *M. tanajoa*-infested cassava leaves than *T. aripo*. Our data do not elucidate whether both phytoseiid species use the same chemicals as a source of information and whether cassava leaf biomass and/or area while keeping constant total mite density, affect the attraction of predators.

Acknowledgements

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3

Prey-related odor preference of the predatory mites *Typhlodromalus manihoti* and *Typhlodromalus aripo* (Acari: Phytoseiidae)

Désiré Gnanvossou, Rachid Hanna & Marcel Dicke

Abstract

Typhlodromalus manihoti and *T. aripo* are exotic predators of the cassava green mite *Mononychellus tanajoa* in Africa. In an earlier paper, we showed that the two predators were attracted to odors from *M. tanajoa*-infested cassava leaves. In addition to the key prey species, *M. tanajoa*, two alternative prey mite species, *Oligonychus gossypii* and *Tetranychus urticae* also occur in the cassava agroecosystem. Here, we used a Y-tube olfactometer to determine the attraction of the predators to odors from *O. gossypii*- or *T. urticae*-infested cassava leaves and their prey-related odor preference. When starved for 2 h, neither of the predator species was attracted to odors from *O. gossypii*- or *T. urticae*-infested leaves. Increasing predator starvation or doubling *O. gossypii* density yielded an attraction from *T. aripo* but not from *T. manihoti*. The presence of *O. gossypii* interacting either simultaneously on the same leaves or different sets of leaves with *M. tanajoa* does not alter the response of the two predators in detecting odors from *M. tanajoa*-infested leaves. In contrast, mixing odors from *T. urticae*-infested leaves with those from *M. tanajoa*-infested leaves impeded the attraction of *T. aripo* and *T. manihoti*, to the specific odors from *M. tanajoa*-infested leaves. Ecological advantages and disadvantages of the predators' behavior and possible implications for biological control against *M. tanajoa* are discussed.

Introduction

Chemicals of plant origin play an essential role during prey/host searching by carnivorous arthropods (e.g., Vet and Dicke, 1992; Tumlinson *et al.*, 1992). The production of herbivore-induced carnivore attractants can be specific to plant species/cultivars and to herbivore species that infest the plant (Takabayashi *et al.*, 1994; Dicke, 1999). This indicates that different herbivore species can induce different volatile blends (Takabayashi *et al.*, 1991; De Moraes *et al.*, 1998). Moreover, plants that are attacked by different herbivore species on different leaves are known to release the same compounds but with different ratios in the volatile blends (De Moraes *et al.*, 1998; Dicke, 1999). Herbivore-induced plant volatiles benefit predators/parasitoids by guiding the latter to their preferred host/prey on the foliage of the plants (Turlings *et al.*, 1991; Vet and Dicke, 1992). The discrimination by carnivorous arthropods among plant volatiles induced by different herbivore species has been reported for several predator and parasitoid species (e.g. Sabelis and van de Baan, 1983; Agelopoulos and Keller, 1994; Du *et al.*, 1998; De Moraes *et al.*, 1998). However, this has not been found in all studies (Geervliet *et al.*, 1996; Vet *et al.*, 1998; Vos *et al.*, 2001).

Three spider mite species are found infesting cassava in Africa. The most economically damaging and most widely spread species is the cassava green mite *Mononychellus tanajoa* (Bondar), which was accidentally introduced into Africa from its native range in South America (Nyiira, 1972). Large populations of this pest can inflict considerable damage to cassava growth (Yaninek *et al.*, 1989). A second, less economically important but widely distributed spider mite species in Africa, is the red spider mite *Oligonychus gossypii* (Zacher) (Yaninek and Onzo, 1988), but this species is not found on cassava in the Neotropics (G.J. De Moraes, personal communication). Both *M. tanajoa* and *O. gossypii*, however, co-occur on cassava foliage in Africa with overlapping distributions mostly in the middle stratum of the plants, as *M. tanajoa* largely infests the upper half of the cassava plant while *O. gossypii* infests the lower half of the cassava plant. A third spider mite species, the two-spotted spider mite *Tetranychus urticae* (Koch), is widely spread in South America (Bellotti *et al.*, 1987) but only occasionally infests cassava in Africa (Yaninek *et al.*, 1989).

The two predatory mite species *T. manihoti* and *T. aripo* are effective biological control agents of the target prey mite species and have been present in cassava fields in Africa since 1989 and 1993 respectively (Yaninek *et al.*, 1998; Yaninek *et al.*, 2001). Results from laboratory studies on the effects of food sources on development, survival and life table

characteristics of these predators, revealed that *T. aripo* can feed and develop to maturity on a wide range of diets, but requires tetranychid mite prey, primarily *M. tanajoa* and to a much lesser extent *O. gossypii* or *T. urticae* (chapter 4) or maize pollen (Yaninek *et al.*, 2001) for oviposition. On the other hand, *T. manihoti* can feed and develop on a narrower range of diets and requires *M. tanajoa* (Toko *et al.*, 1994; chapter 4) or to a lesser extent *O. gossypii* (chapter 4) for reproduction. These results have led to the hypothesis that odors from cassava plants infested either with *M. tanajoa*, *O. gossypii* or *T. urticae* may attract the predators *T. aripo* and *T. manihoti* but volatiles from leaves infested by *M. tanajoa* are expected to be more preferred.

In an earlier paper, we showed that *T. aripo* and *T. manihoti* were attracted by *M. tanajoa*-induced cassava plant volatiles (Gnanvossou *et al.*, 2001). So far, the ability of *T. aripo* and *T. manihoti* to discriminate between cassava volatiles induced by the two other tetranychid mite species found on cassava in Africa has not been investigated. Moreover, for plants attacked by more than one herbivore species on the same leaves, studies on behavioral response of carnivores are scarce (see Vos *et al.*, 2001; Shiojiri *et al.*, 2001 for two exceptions). Is a plant on which a target prey is feeding simultaneously with an alternative prey still attractive to predators? Phytoseiid mites do not feed without discrimination but prefer certain food types to others (Dicke *et al.*, 1989). Their prey preferences are expected to result in a maximization of reproductive success, in terms of contribution to future generations. Clearly, knowledge on prey preference in general and on prey-related odor preference in particular is needed to understand how predators/parasitoids or predatory mites in particular (in our case *T. manihoti* and *T. aripo*), respond to the target prey/host numbers in an environment where two or more prey/host species are present.

In this paper, we explore specifically the behavioral response of the two introduced phytoseiid species *T. manihoti* and *T. aripo* to odors from *M. tanajoa*-, *O. gossypii*- or *T. urticae*-infested leaves and to mixtures of odors from cassava leaves infested either with *M. tanajoa* and *O. gossypii* or with *M. tanajoa* and *T. urticae*. We address the following questions: (i) Do odors emitted from either *O. gossypii*-infested cassava leaves or from *T. urticae*-infested cassava leaves attract *T. manihoti* and *T. aripo*? (ii) Do *T. manihoti* and *T. aripo* prefer cassava volatiles induced by *M. tanajoa* over those induced by *O. gossypii* or *T. urticae*? This will be investigated by offering leaves infested by *M. tanajoa* versus leaves infested by either *O. gossypii* or *T. urticae* with different ratios of spider mite densities. (iii) Do the odors from *O. gossypii*-infested leaves or from *T. urticae*-infested leaves weaken the response of *T. manihoti*

and *T. aripo* to odors from *M. tanajoa* as a result of mixing of these odors sources? The answers to these questions should elucidate whether *T. manihoti* and *T. aripo* use olfaction to selectively locate certain prey species.

Materials and Methods

A. Cultures

Colonies of the two spider mite species *M. tanajoa* and *O. gossypii* were maintained indoors on potted cassava plants for one month at 26 ± 1 °C; 65-80% RH. Colonies of both species were started, and periodically restocked, from several hundred individuals collected from cassava fields in southern Benin. A colony of *T. urticae*, which originated from several hundred individuals collected from a colony maintained for several years on peanuts at the International Institute of Tropical Agriculture (IITA), Benin Station was also maintained on potted cassava for one month.

Colonies of *T. manihoti* and *T. aripo* were started from approximately a hundred adult females collected from cassava fields in southern Benin. They were maintained in the laboratory at 25 ± 1 °C and 80 ± 10% RH on detached cassava leaves infested by *M. tanajoa* as described by Mégevand *et al.* (1993). The whole rearing was kept on metallic shelves with feet isolated from the floor by a mixture of paraffin® liquid and water in plastic containers. The predatory mites had been maintained for 4 to 5 months under the aforementioned laboratory conditions prior to their use in the olfactometer experiments.

Plant materials. All cassava plants used in the experiments were collected from 4-5 week-old cassava plants (*Manihot esculenta* Crantz, variety "Agric") grown in plastic pots (volume: 2.5 l), filled with top soil collected from IITA experimental plots and maintained in a greenhouse (30 ± 5 °C; 70 ± 10% RH) at the IITA Benin Station.

B. Experimental procedures

Y-tube olfactometer tests. Attraction of predators to odor sources was monitored in two-choice tests using a closed system Y-tube olfactometer. The system, except for the compressor (SERBATOI® AUTOCLAVI; Type ELTO; Vol. 50) used to generate the air stream, was identical to that described by Takabayashi and Dicke, 1992. This olfactometer setup has been

extensively used for studying the olfactory responses of predatory mites (Sabelis and van de Baan, 1983; Takabayashi and Dicke, 1992), and the conclusions from these olfactometer experiments have been supported by greenhouse studies (Janssen, 1999).

Adult female *T. manihoti* and *T. aripo* were collected from the rearing units, where they had ample food available and were thus considered satiated at the time of removal from the cultures. They were individually enclosed in a plastic vial (10 mm diam. 40 mm long) and kept without food for either 2 or 10 h at 25-28 °C, 65-90% RH prior to the olfactometer bioassays. Female predators were placed individually at the base of an iron wire positioned in the middle of the Y-shaped glass tube, and parallel to the tube walls. The predators were observed until they reached the end of one of the arms, or for a maximum of five minutes, and were subsequently removed. Both predator species were tested alternately with the same odor sources. After a series of five mites, the odor sources were connected to the opposite arm of the olfactometer to correct for any unforeseen asymmetry in the experimental set-up. The number of females that chose either odor source or without choice was recorded for each predator species. Each test was repeated 3-6 times (on 3-6 consecutive days) with 20 predators per species and per day. New odor sources were used for each replicate.

Preparation of odor sources. Volatiles passing through the arms of the Y-tube olfactometer originated from different odor sources prepared as follows:

1. Predators' response to odors from either O. gossypii or T. urticae-infested leaves

We conducted a series of experiments to test the attraction of *T. manihoti* and *T. aripo* to odors emitted from either *O. gossypii*-infested leaves or *T. urticae*-infested leaves. Early in the morning, the petioles of eight newly collected young cassava leaves (3rd young leaf from the apex) from 4-5 week-old potted cassava plants were placed in water-filled vials sealed with parafilm. In the afternoon of the same day, four leaves were infested with 50, 100 or 200 adult female *O. gossypii* per leaf, or 100 adult female *T. urticae* per leaf. The same number of non-infested leaves was kept under the same conditions. All leaves were incubated for three days in plastic cages (length: 70 cm; width: 40 cm; height: 40 cm) at 26 ±1 °C, with continuous lighting provided by 2 fluorescent light sources. Mite-infested and non-infested leaves were incubated in separate cages. We used 2 h- and 10 h-starved predators to test the attraction to odors from *O. gossypii*-infested leaves and 2 h-starved predators only to test the attraction to odors from *T. urticae*-infested leaves.

2. Prey-related odor preference: choice between odors from M. tanajoa-infested leaves and either O. gossypii infested leaves or T. urticae-infested leaves

In the experiments where predators were offered a choice between odors from leaves infested with *M. tanajoa* versus leaves infested with *O. gossypii*, four leaves were infested with 50, 100 or 200 adult female *M. tanajoa* per leaf and four other leaves were each infested with 100 or 200 adult females *O. gossypii*. In the case of a choice between leaves infested with *M. tanajoa* versus leaves infested with *T. urticae* four leaves were infested with 25, 50 or 100 adult female *M. tanajoa* per leaf and four other leaves with either 25, 50 or 100 adult female *T. urticae* per leaf. The two categories of leaves (e.g. infested and non-infested) were incubated as described in the previous section. We used 2 h-starved predatory mites to conduct these experiments.

3. Effect of mixture of odor sources on predators' prey-related odor preference

To test whether prey-related odor preference by *T. aripo* and *T. manihoti* was affected by odors from cassava leaves infested by two prey mite species, a mixture of odor sources was established either by mixing leaves that were infested by either one of the two prey species or by preparing a mixed infestation of two prey species on the same leaves. For mixed infestations that consisted of combinations of leaves with single prey infestations, we used (a) *M. tanajoa* and *O. gossypii*, and (b) *M. tanajoa* and *T. urticae*, whereas for mixed infestations on the same leaves, we used (c) *M. tanajoa* and *O. gossypii*. Leaves with single species infestation and the control (non-infested) leaves were kept in four separate and isolated cages. Mixing of the odors induced by the two species incubated on different sets of leaves was done immediately prior to the beginning of each olfactometer experiment. In the case of a mixture of odors induced by two prey species on the same leaves, leaves with mixed prey infestation and those without prey were kept and isolated in separate cages. Densities of 100 or 25 adult female *M. tanajoa* and 100 adult female *O. gossypii* were used. For all experiments, we used 2h-starved predatory mites.

C. Statistical analyses

We used the one-tailed probability of the binomial test to compare the response of each predator species to odors from *M. tanajoa*, *O. gossypii* or *T. urticae*-infested leaves versus non-infested leaves, or odors from both *O. gossypii-* plus *M. tanajoa*-infested leaves versus non-infested leaves, and the two-tailed probability in the case of preference between two odor

sources consisting of infested leaves (Zar, 1984). Difference between predator species was tested by a 2 x 2 contingency table analysis based on a Chi-square, with Yates correction (Zar, 1984).

Results

1. Attraction to odors from cassava leaves infested with either O. gossypii or T. urticae

When 2 h-starved *T. aripo* and *T. manihoti* were given a choice between four leaves, each infested with 100 adult female *O. gossypii* and four non-infested leaves, neither species was attracted to the infested leaves. A similar set-up but with *T. urticae* instead of *O. gossypii* also did not yield a significant predator attraction to the infested leaves (Figure 1).
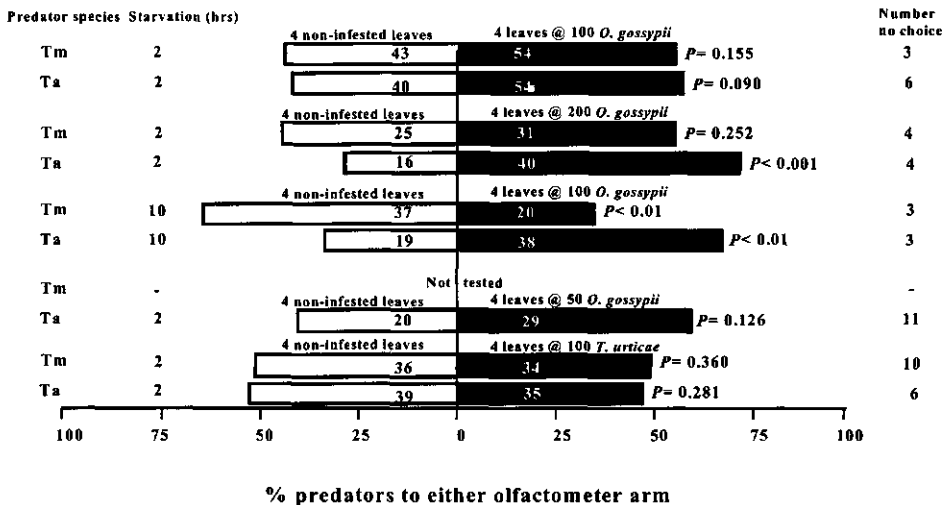

Figure 1. Effect of starvation and prey densities on the response of the predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta) when volatiles from either *Oligonychus gossypii*-infested leaves or *Tetranychus urticae*-infested leaves were offered in a Y-tube olfactometer versus non-infested leaves. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

However, when *O. gossypii* infestations were increased to 200 adult females per leaf, or when predator starvation period was increased from 2 to 10 h, 71% (*P*<0.001) and 67% (*P*<0.01) of the total number of *T. aripo* females tested respectively were attracted to odors from *O. gossypii*-infested leaves. In contrast, this increase in *O. gossypii* infestations and predator hunger period did not induce an attraction of *T. manihoti* to infested leaves (Figure 1).

2. Prey-related odor preference: choice between odors from M. tanajoa-infested leaves and O. gossypii-infested leaves

At equal densities (100 adult females per leaf) of *M. tanajoa* and *O. gossypii*, the majority of the *T. aripo* and *T. manihoti* females (59 and 76% respectively) chose the odors from *M. tanajoa*-infested leaves, but only *T. manihoti*'s response was significantly different from 50:50 (*P*=0.10 and *P*<0.001 for *T. aripo* and *T. manihoti* respectively; Figure 2). Increasing the *M. tanajoa* density to twice that of *O. gossypii* resulted in an attraction of 78% *T. aripo* (*P*<0.001) and 75% *T. manihoti* (*P*<0.001) to odors from *M. tanajoa*-infested leaves.
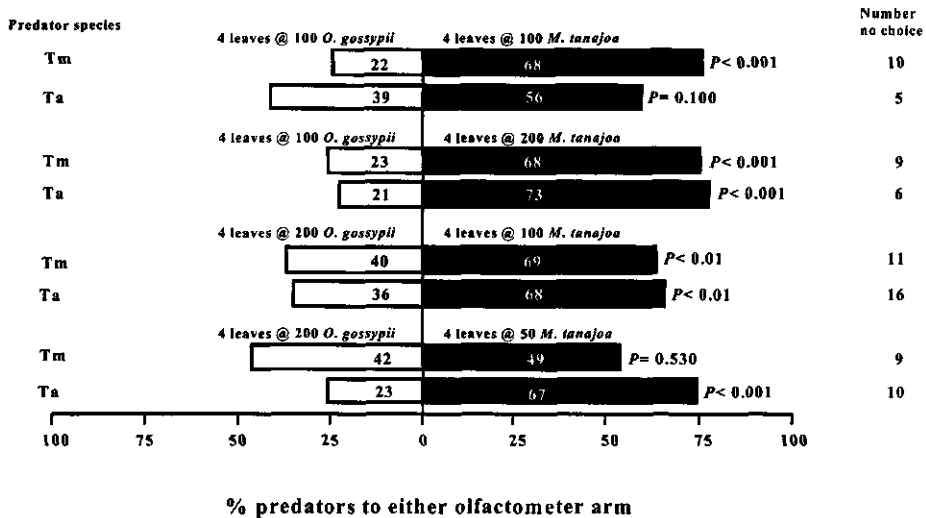

Figure 2. Response of 2 h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta), in a Y-tube olfactometer when offered simultaneously volatiles from *Mononychellus tanajoa*-infested cassava leaves versus *Oligonychus gossypii*-infested cassava leaves. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

The two predators were even attracted to *M. tanajoa*-infested leaves when the *M. tanajoa* density was half (100 per leaf) that of *O. gossypii* (200 per leaf) (*P*<0.01 for *T. aripo* and *P*<0.01 for *T. manihoti*) (Figure 2). At a 1:4 ratio of spider mite densities (50 *M. tanajoa* versus 200 *O. gossypii* per leaf), *T. aripo* significantly preferred the volatiles from *M. tanajoa*-infested leaves (74% of the total number of females tested chose this odor source) while *T. manihoti* did not show this preference (Figure 2).

3. Prey-related odor preference: choice between odors from M. tanajoa-infested leaves and T. urticae-infested leaves

At equal densities (100 adult females per leaf) of *M. tanajoa* and *T. urticae*, the majority of the *T. aripo* and *T. manihoti* females (56 and 60% respectively) preferred the odors from *M. tanajoa*-infested leaves but these responses were not statistically significant from 50:50 (Figure 3). When four leaves with 50 *M. tanajoa* per leaf were offered versus four leaves with 100 *T. urticae* per leaf, only the response of *T. manihoti* was significant in favor of the *M. tanajoa*-infested leaves (*P*<0.01; Figure 3).
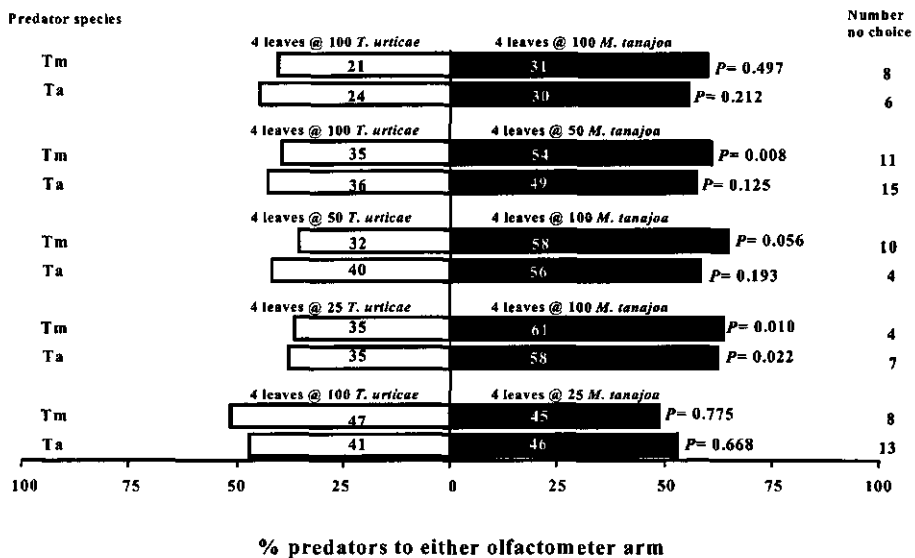

Figure 3. Response of 2 h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta) in a Y-tube olfactometer when offered simultaneously volatiles from *Mononychellus tanajoa*-infested cassava leaves versus *Tetranychus urticae*-infested cassava leaves. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

When 100 *M. tanajoa* and 50 *T. urticae* were used to infest two groups of four leaves, the majority of the two predators also chose the odors from *M. tanajoa*-infested leaves, but these responses were not statistically significant: 64% *T. manihoti* (P=0.056) and 58% *T. aripo* (P=0.193) (Figure 3). When four leaves, each infested with 100 adult female *M. tanajoa* versus four leaves each infested with 25 adult female *T. urticae* were offered, both *T. aripo* and *T. manihoti* were significantly attracted to *M. tanajoa*-infested leaves (P=0.022 and P=0.010 respectively). When the reverse situation was presented, neither *T. aripo* nor *T. manihoti* showed a preference to either odor source (Figure 3).

4. Effect of mixture of odor sources (either on the same leaf or on two different sets of leaves) on predators' prey preference

When four leaves each infested with 100 *M. tanajoa* plus four leaves each infested with 100 *O. gossypii* were offered together versus eight non-infested leaves, 64% *T. aripo* (P<0.05) and 70% *T. manihoti* (P<0.01) were attracted to the mixture of infested leaves (Figure 4).
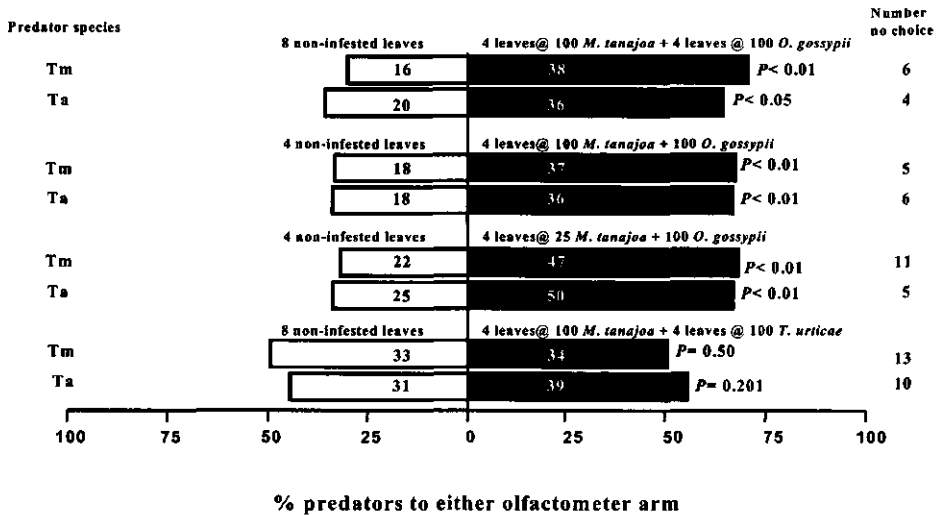

% predators to either olfactometer arm

Figure 4. Response of 2 h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta), in a Y-tube olfactometer when a choice was given between mixture of odors from *Mononychellus tanajoa*-*Oligonychus gossypii*-infested cassava leaves versus non-infested leaves or mixture of odors from *M. tanajoa*-*Tetranychus urticae*-infested cassava leaves versus non-infested leaves. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

The two predators were also significantly attracted when odors from four leaves each infested by 100 *M. tanajoa* plus 100 *O. gossypii* were offered versus four non-infested leaves; similar responses were also recorded when four leaves each infested with 25 adult female *M. tanajoa* plus 100 adult female *O. gossypii* were offered versus four non-infested leaves (Figure 4). No significant differences were found between predator species (*P*>0.05). In contrast, mixed odors from four *M. tanajoa*-infested leaves and four *T. urticae*-infested leaves that were offered together versus odors from eight non-infested leaves did not result in attraction of either of the two predator species (Figure 4).

Discussion

Our study shows that cassava plants infested with different herbivorous mite species emit information that appear to be specific to the herbivore species, as measured by the olfactory response of two phytoseiid predators that co-occur with the herbivores on cassava plants in Africa. We have previously shown that both *T. aripo* and *T. manihoti* were attracted to odors from cassava leaves infested by *M. tanajoa* (from 25 to 100 mites per leaf) (Gnanvossou *et al.*, 2001). That the two predators are sensitive to volatiles from *M. tanajoa*-infested cassava leaves is not surprising, as *M. tanajoa* is their primary prey on cassava, on which they reach their highest intrinsic rate of population increase (Yaninek *et al.*, 2001; chapter 4). Both predators are also able to regulate *M. tanajoa* densities in the field in their native range in South America (Braun *et al.*, 1989), and were introduced and established in Africa (Yaninek *et al.*, 2001).

In contrast to their response to *M. tanajoa*, *T. aripo* and *T. manihoti* are at best weakly attracted to *O. gossypii*, only with extended predator starvation and several hundred *O. gossypii* per leaf. This prey is not present on cassava in the predators' native range in South America (Jeppson *et al.*, 1975; Bellotti *et al.*, 1987). Only *T. manihoti* is able to multiply on *O. gossypii*, but this predator reaches a much lower intrinsic rate of population increase (r_m) than when *M. tanajoa* is offered as prey (chapter 4). Moreover, the attraction of *T. aripo* and *T. manihoti* to *O. gossypii*-infested leaves disappears when the predators are given a choice between leaves infested by *O. gossypii* and their preferred prey *M. tanajoa*, even at relatively low *M. tanajoa* infestation levels. What amount of volatiles and how many compounds in the volatile blends are emitted from *O. gossypii*-infested leaves compared with *M. tanajoa*-infested leaves, is still unknown. When offered a choice between *T. urticae*-infested and non-infested leaves, *T. aripo*

and *T. manihoti* were not attracted to the infested leaves. This lack of response is unlikely to be due to the lack of volatile emission from *T. urticae*-infested cassava leaves, as these leaves showed approximately similar damage levels as leaves infested by *M. tanajoa* to which both *T. aripo* and *T. manihoti* were strongly attracted. Moreover, other studies showed that phytoseiid predators such as *Phytoseiulus persimilis* Athias-Henriot, a specialist enemy of *T. urticae*, was attracted to volatiles from *T. urticae*-infested cassava leaves (M. Dicke, unpublished data). *Phytoseiulus persimilis* is also well known to respond to volatiles of a variety of plant species infested by *T. urticae* (Dicke and Sabelis, 1988).

What could be the mechanism that leads to odor preference of *T. aripo* and *T. manihoti*? We know from earlier studies on spider mite fresh weights, that an adult female *M. tanajoa* weighed $7.04 \pm 0.26\mu g$ (Yaninek and Gnanvossou, 1993), an adult female *O. gossypii* weighed $18.70 \pm 2.60\mu g$ (Gnanvossou, *et al.*, unpublished data), and that of *T. urticae* weighed $24.1 \pm 0.8\mu g$ (Sabelis, 1981). If we assume that the feeding rate of spider mites is positively correlated with their body weight, at equal spider mite numbers, one would expect the predators to respond to *O. gossypii* and *T. urticae*, because both of these spider mites are larger than *M. tanajoa*, and would inflict more damage to the cassava leaves. That the predators did not respond to either of the non-preferred prey and less common prey supports the hypothesis of herbivore species-specific cues (Sabelis and van de Baan, 1983; Takabayashi *et al.*, 1994; Dicke 1999), emitted by cassava. Furthermore, the two predators are not attracted to mechanically damage cassava leaves, which further supports that plant emits specific information to different types of damage. Spider mites are known to inject saliva into the cells of their host plant during the process of feeding (Tomczyk and Kropczynska, 1985) and the saliva injected may contain an elicitor that induces the production of volatiles in the plant (Turlings *et al.*, 1990; Mattiacci *et al.*, 1994). Thus, the elicitors of *M. tanajoa* compared to those of *O. gossypii* or *T. urticae* are likely to be different. As a consequence, the volatile blends induced by the herbivores, differently affect the behavioral response of the predatory mites *T. manihoti* and *T. aripo*.

Herbivore-induced specific volatiles in plants have been shown in other systems. The predatory mite *Phytoseiulus persimilis* Athias-Henriot discriminates between volatiles from apple foliage infested with the herbivorous mites *T. urticae* and *Panonychus ulmi* (Koch) (Sabelis and van de Baan, 1983; Sabelis and Dicke, 1985). *Amblyseius potentillae* (Garman) preferred volatiles from leaves infested with *P. ulmi* to those infested with *Aculus*

schlechtendali (Nalepa) or *T. urticae* (Dicke and Groeneveld, 1986). Similar data have been presented for the predatory mite *Typhlodromus pyri* Scheuten (Dicke, 1988). This predator preferred the volatiles induced by *P. ulmi* in apple foliage to those of *T. urticae* or *A. schlechtendali*, despite *P. ulmi* being outnumbered and outweighed by *T. urticae* or *A. schlechtendali*. A study by Venzon *et al.* (1999) showed that the predatory bug *Orius laevigatus* (Fieber) preferred volatiles from plants infested with spider mites to volatiles from plants with thrips in release-recapture experiments. Host related odor preference has also been demonstrated in the parasitoids *Aphydius ervi* Haliday (Du *et al.*, 1998) and *Cardiochiles nigriceps* (Vier.) (De Moraes *et al.*, 1998). However, *T. manihoti* and *T. aripo* do not seem to discriminate between odors from leaves infested with *M. tanajoa* and *T. urticae*. Studies on other carnivore-herbivore-plant systems have presented similar data (McCall *et al.*, 1993; Geervliet *et al.*, 1996; Vet *et al.*, 1998; Vos *et al.*, 2001).

Experience may influence the preference of predatory mites for herbivore-induced plant volatiles (Krips *et al.*, 1999; Drukker *et al.*, 2000). So far, no published data are present on the effect of experience on the discrimination by predators between odors from plants of one species that have been infested by different herbivore species. Since these have rather similar volatile blends (Dicke, 1999), learning may be relatively difficult (Vet *et al.*, 1998). The lack of attraction of the two predators to odors induced by the alternative prey mites might theoretically be attributed to the fact that females of *T. manihoti* or *T. aripo* used in our study had no previous experience either with odors induced by *O. gossypii* or *T. urticae* in cassava leaves. *Mononychellus tanajoa*, *O. gossypii* and *T. urticae* are recognized as a target prey and alternative prey mites respectively for *T. manihoti* and *T. aripo* and also for the experiments, the predators have been maintained on leaves infested with *M. tanajoa* only for more than 5 months. As a consequence we cannot exclude that the absence of previous experience of predators with the alternative prey may have affected their response. To exclude this, further experiments are necessary to investigate whether experience of the two predators either to odors emitted from *O. gossypii*-infested cassava leaves or *T. urticae*-infested cassava leaves before olfactometer tests, would affect their response to odors of the two prey species. Yet, the predatory mite *P. persimilis*, that is known to exhibit learning (Krips *et al.*, 1999; Drukker *et al.*, 2000) is attracted to bean leaves infested with a herbivore that cannot serve as prey, i.e. *Spodoptera* caterpillars, when the predators have been reared on bean plants infested with their prey *T. urticae* (Shimoda and Dicke, 2000). This suggests that in the phytoseiid mite *P. persimilis*, learning is not required to respond to volatiles related to an inferior prey species,

which is supported by data for other predatory mites (Dicke and Groeneveld, 1986; Dicke, 1988). On the other hand, it is likely that experience with inferior prey such as *O. gossypii* or *T. urticae* may have a negative effect on the attraction to this odor source when a better alternative is present. It has been shown that negative experiences (e.g., exposure to an inferior resource) can affect the response to odors in addition to positive experience (Vet *et al.*, 1998; Drukker *et al.*, 2000).

While most of our findings are consistent with previous findings on the response of predators and parasitoids to cues of plants infested by herbivorous mites and insects, we obtained some intriguing results concerning the response of *T. aripo* and *T. manihoti* to cues from leaves infested by both *M. tanajoa* and *T. urticae*. When a choice was given between leaves infested with *M. tanajoa* and leaves infested with *T. urticae*, the majority of *T. aripo* and *T. manihoti* preferred odors emitted from leaves with *M. tanajoa*, but this response apparently depended on the density of *T. urticae* relative to that of *M. tanajoa* – the predators' response was only significant when the density of *T. urticae* was only a quarter of that of *M. tanajoa*. However, when *T. urticae*-infested cassava leaves were mixed with *M. tanajoa*-infested leaves, this significantly reduced the attraction towards the odors from the *M. tanajoa*-infested leaves. From an ecological point of view, the presence of *T. urticae* may prevent *M. tanajoa* being attacked by its natural enemies *T. manihoti* and *T. aripo* and this would provide *M. tanajoa* with a form of enemy-free space.

Mononychellus tanajoa and *O. gossypii* can occur together on the same plant: *M. tanajoa* on foliage in the upper half of the plant and *O. gossypii* on foliage in the bottom half. However, towards the end of a prolonged dry season with the senescent bottom leaves completely absent from the plants, the two prey species have been found feeding on the same upper young leaves (A. Onzo, personal communication). In the case of mixing of odors from *O. gossypii*-infested cassava leaves with odors from *M. tanajoa*-infested cassava leaves, neither predator species was hampered in detecting the presence of the target prey, *M. tanajoa*. These results indicate that the presence of *M. tanajoa* could attract *T. manihoti* and *T. aripo* into patches occupied by both prey species which might enhance predation on *O. gossypii*, resulting possibly in a greater numerical response of the predators and hence a greater overall level of predation on *M. tanajoa*. This however remains a hypothesis that requires testing under semi-natural or natural conditions. Such indirect interactions between two non-competing prey species are common in

arthropod communities and have been reported in several studies with conclusive results (e.g., Huang and Sih, 1990; Holt and Lawton, 1994; Hanna *et al.*, 1997).

Acknowledgements

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4

Life history of the phytoseiid predators *Typhlodromalus manihoti* and *Typhlodromalus aripo* feeding on three species of spider mite

Désiré Gnanvossou, J. Steve Yaninek, Rachid Hanna & Marcel Dicke

Abstract

The performance of *Typhlodromalus manihoti* and *T. aripo*, two natural enemies of the cassava green mite *Mononychellus tanajoa*, was studied on three tetranychid prey mite species commonly found in the cassava agroecosystem in Africa. Populations of the two predator species were followed separately on each of the prey mite species, i.e. *M. tanajoa*, *Oligonychus gossypii* and *Tetranychus urticae*. Egg to adult development of *T. aripo* was shorter on *M. tanajoa* than on *T. urticae* as prey, while an intermediate value was recorded on *O. gossypii*. For *T. manihoti*, developmental time was shorter on *M. tanajoa* than on *O. gossypii* but no successful development occurred on *T. urticae*. The survival rates for both predators were similar on the three prey species. Both predatory mite species had a higher intrinsic rate of population increase (r_m) and net reproduction (R_0), shorter generation time and doubling time on *M. tanajoa* compared to *O. gossypii* and *T. urticae*. Furthermore, *T. manihoti* developed faster than *T. aripo* on either *M. tanajoa* or *O. gossypii*, while adult female *T. aripo* lived much longer than *T. manihoti* on the same prey mite species. Based on these life table results, a rank-order hierarchy of prey suitability for each of the two predator species was as follows: *M. tanajoa* > *T. urticae* > *O. gossypii* for *T. aripo* whereas it was *M. tanajoa* > *O. gossypii* > *T. urticae* for *T. manihoti*. Moreover, predator performance matches odor-related prey preference that was previously studied, if the key prey is compared to the two inferior prey species. The potential implications of our findings for the persistence of *T. aripo* and *T. manihoti* in the cassava agroecosystem in Africa are discussed.

Introduction

Plant-inhabiting mites of the family Phytoseiidae have received considerable interest over the last four decades as biological control agents of phytophagous mites. Many of these predatory mites can feed, develop and reproduce on a range of food sources including prey as well as non-prey substances such as pollen and honeydew (Abou-Awad *et al.*, 1989; James, 1989; Duso and Camporese, 1991; Bruce-Oliver *et al.*, 1996; McMurtry and Croft, 1997; Pratt *et al.*, 1999; Van Rijn and Tanigoshi, 1999). It has been reported that predatory mites preferentially select prey species on which they obtain maximum reproductive success (Sabelis, 1985b), suggesting that predatory mites display a hierarchy of preference among food sources. Thus, we expect predator foraging decisions with respect to different prey species to be related to subsequent payoff in terms of reproductive success, a relationship that has been shown for several predatory mites species in various systems e.g. including *Typhlodromus pyri* Scheuten (Dicke and de Jong, 1988), *Amblyseius andersoni* and *A. finlandicus* (Oudemans) (Dicke *et al.*, 1988). In the present study, we investigated the "preference-performance" relationship for two predatory mites *Typhlodromalus manihoti* Moraes and *T. aripo* DeLeon found on cassava (De Moraes *et al.*, 1994). The two phytoseiid species have been selected as potential natural enemies of the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) in Northern Brazil and were subsequently introduced into Africa by the International Institute of Tropical Agriculture (IITA) for the control of *M. tanajoa* (Yaninek *et al.*, 1993; Yaninek *et al.*, 1998). Initial studies that have been conducted in the laboratory showed that *T. manihoti* (Oduor, 1988; Klein, 1990; Bakker, 1993; Toko *et al.*, 1994) and *T. aripo* (Bakker, 1993) feed and develop on *M. tanajoa* and on a variety of plant-based foods including extrafloral nectar, pollen and fungal spores. Little was known, however, about comparative life history characteristics of the two predator species when feeding on different prey mite species.

Several species of phytophagous mites attack cassava *Manihot esculenta* Crantz (Euphorbiaceae) (Byrne *et al.*, 1983). *Mononychellus tanajoa* is by far the most common mite and widely distributed phytophagous mite on cassava both in the Neotropics (Bellotti *et al.*, 1987) and Africa (Nyiira, 1976). A second phytophagous spider mite *Oligonychus gossypii* (Zacher) is widely distributed on cassava in Africa (Yaninek and Onzo, 1988), but is not found on cassava in the Neotropics (G.J. De Moraes, personal communication). A third spider mite species *Tetranychus urticae* (Koch) is also widely distributed in the Neotropics

and in Africa, but it occurs much less on cassava than on other vegetation associated with the cassava agroecosystems (Jeppson *et al.*, 1975; Bellotti *et al.*, 1987).

Predators are expected to select prey preferentially by means of induced plant volatiles on which they can obtain maximum reproductive success (Sabelis, 1985b; Dicke *et al.*, 1988). This exciting behavior of predators has been investigated during the past decades (Dicke, 1988; Dicke *et al.*, 1998; Sabelis *et al.*, 1999). By means of an olfactometer, *T. manihoti* and *T. aripo* are known to display a preference for volatiles emitted from cassava leaves infested by *M. tanajoa* over those emitted from cassava leaves infested by *O. gossypii* (chapter 3). However, when given a choice between volatiles emitted from cassava leaves infested by *M. tanajoa* and volatiles emitted from cassava leaves infested by *T. urticae*, neither predator showed a preference for either volatile. We would therefore expect *T. aripo* and *T. manihoti* to perform better on *M. tanajoa* than on *O. gossypii* and *T. urticae*.

The two predator species were especially selected for this study because of their long persistence on cassava in the near absence of *M. tanajoa* (Yaninek *et al.*, 1998). This adaptation suggests that the two predators probably switch to alternative prey either on cassava or on alternative host plants. In Africa, cassava is often infested with mixed populations of the exotic pest, *M. tanajoa* and the indigenous spider mite, *O. gossypii*. *Tetranychus urticae* is rarely found on cassava but can be common on host plants associated with cassava production. Presently, we do not know whether the behavior of the predatory mites *T. aripo* and *T. manihoti* to herbivore-induced cassava plant volatiles relates to herbivore suitability. It is expected that differences in odor-related prey preference of the two predators, as previously measured (chapter 3) is reflected in predator performance. The present work was undertaken to give answer this question. The study reported here investigates (i) the suitability of *M. tanajoa*, *O. gossypii* and *T. urticae* as food for the development of juvenile stages and on adult female's reproduction of both *T. aripo* and *T. manihoti*, and (ii) whether relative performance matches prey-related odor preference.

Materials and Methods

Predator and prey cultures. Cohorts of *T. manihoti* and *T. aripo* used in this study were obtained from cultures of the two predators initiated from individuals collected from cassava fields in Mankesim, Ghana in June 1994 and Djèrègbé, Benin in January 1996. Predator colonies were maintained in the laboratory at 25 ± 1 °C and $80 \pm 10\%$ RH on detached

cassava leaves infested by *M. tanajoa* as described by Mégevand *et al.* (1993). The petioles of these leaves were placed in water-filled vials sealed with parafilm to keep them hydrated. The vials with leaves were placed in a plastic tray (35 x 35 x 6 cm) and covered with a similar tray. The whole setup was kept on metallic shelves with feet isolated from the floor by a mixture of paraffin® liquid and water in plastic containers. New prey infested leaves were added daily to the rearing units. The predatory mite populations had been maintained over 6 months under the aforementioned laboratory conditions prior to their use.

Three prey mite species, i.e. *M. tanajoa*, *O. gossypii* and *T. urticae*, commonly found in the cassava agroecosystem, were offered in no choice situations to *T. aripo* and *T. manihoti*. The three prey mite species cultures were established indoors on potted cassava plants for one month at 26 ± 1 °C; 65-85% RH. All stages of the three prey species were used as food for the predators. The population of *M. tanajoa* was started from individuals obtained from a colony reared on cassava "trees" in a greenhouse (Haug *et al.*, 1987). *Tetranychus urticae* was obtained from a colony maintained on peanut plants in a greenhouse at IITA whereas a culture of *O. gossypii* was established from approximately 100 adult females collected from cassava in southern Benin.

Predator feeding experiments. The feeding experiments were conducted in arenas consisting of a cassava leaf disk (2.5 cm in diameter) placed in a mini Petri dish (diam.: 26 mm; height: 9 mm) lined at the bottom (abaxial surface up) with a 1 mm layer of agar (Bacto-Agar Difco). The Agar provided moisture to the leaf disk when the dish was closed with a lid. Holes of 3 mm in diameter, were cut into opposite sides of each arena and covered with 0.1 mm mesh for air circulation inside the arena.

A cohort of same-age individuals was started using eggs deposited over a 6 to 24-h period by female predators isolated from laboratory cultures. Eggs were isolated and placed on each leaf disk, in the mini Petri dish. The experiment was replicated 30 to 100 times for each treatment. Prey was provided daily by brushing all stages of the respective prey species, while assuring that a minimum of 20 actives (adults and immature stages) and 40 eggs were provided to each leaf disk. Leaf disks were changed every two to three days to maintain good leaf quality and minimize the growth of saprophytic fungi.

All trials were maintained in controlled climate chambers at a temperature of 25 ± 1 °C and 70-85% RH. Observations on development, survivorship and oviposition were made every 12-24h intervals. Prior to molting of female deutonymphs to adults, two adult males of

the respective species, obtained from the laboratory cultures, were added to each arena to encourage mating soon after the protonymphal moult. The added males were removed immediately after the initiation of oviposition. Developmental period was determined only for individuals that reached the adult stage. Individuals whose larval stage was not recorded (i.e. the larval development was so fast that it was not possible to record it before the individuals had molted to protonymphal stage) were not included in the determination of their larval periods. The sex ratio was determined based on the proportion of females in the progeny.

Statistical analysis. We used one-factor ANOVA (with prey species as the independent variable) to test for significant differences caused by diet (e.g. prey species) for the following developmental parameters of *T. aripo* and *T. manihoti*: duration of each life stage, total duration of development from egg to adult, duration of preoviposition, oviposition and post-oviposition periods, and adult longevity. We compared parameter means using the Student-Newman-Keuls (SNK) only when the ANOVA F-values were significant at $P<0.05$. We used the LIFETEST Procedure with Wilcoxon statistics for determination of Chi-Square probabilities to compare survivorship curves of the two predator species on the three spider mite species. All the above statistical analyses were conducted with SAS (SAS Institute Inc., 2000). Finally, we calculated the life table statistics using the Jacknife Procedure described by Hulting *et al.* (1990) and tested differences between the intrinsic rate of increase (r_m) and the net reproduction (R_o) among populations using Student-Newman-Keuls test based on Jacknife associated standard errors (Hulting *et al.*, 1990; Zar, 1984).

Results

Effect of diet on immature and adult female development

When either predator species was offered a single prey species, consisting of *M. tanajoa, O. gossypii* or *T. urticae* only *T. aripo* successfully fed on all three diets (Table 1a). *Typhlodromalus manihoti* did not successfully develop on *T. urticae* (Table 1b). There were significant effects of prey species on immature development of both predators species. For *T. aripo*, egg to adult development was significantly faster on a diet of *M. tanajoa* (5.5 days) than on a diet of *O. gossypii* (8.1 days), while development on a diet of *T. urticae* resulting in an intermediate value (7.2 days). Furthermore, there was a significant difference between the longevities of females reared on *M. tanajoa, O. gossypii* or *T. urticae* ($P<0.05$). Pre-

oviposition and post-oviposition periods for *T. aripo* were shorter on *M. tanajoa* (2.6 days and 1.9 days respectively) and *T. urticae* (2.5 days and 2.9 days respectively) than on *O. gossypii* (6.3 days and 8.3 days respectively) (Table 1a).

Table 1. Effect of diet on the duration of instars (days; mean ± SD) of the predatory mites *Typhlodromalus aripo* **(a)** and *T. manihoti* **(b)** at 25 °C

Parameter	Diet		
	M. tanajoa	*O. gossypii*	*T. urticae*
(a)			
Egg	2.1 ± 0.5b (N* = 60)	2.1 ± 0.5b (N = 29)	2.3 ± 0.4a (N = 55)
Larva	1.0 ± 0.0b (N = 58)	1.4 ± 0.5a (N = 27)	1.4 ± 0.5a (N = 55)
Protonymph	1.2 ± 0.5c (N = 60)	2.7 ± 1.0a (N = 29)	1.7 ± 0.8b (N = 55)
Deutonymph	1.2 ± 0.5b (N = 60)	1.9 ± 1.7a (N = 29)	1.8 ± 0.9a (N = 55)
Egg-adult	5.5 ± 0.8c (N = 60)	8.1 ± 1.9a (N = 29)	7.2 ± 1.0b (N = 55)
Pre-oviposition	2.6 ± 0.8b (N = 35)	6.3 ± 0.8a (N = 5)	2.5 ± 0.7b (N = 35)
Oviposition	9.5 ± 2.9a (N = 35)	3.0 ± 2.4c (N = 5)	7.2 ± 2.7b (N = 35)
Post-oviposition	1.9 ± 1.1b (N = 35)	8.3 ± 4.3a (N = 5)	2.9 ± 2.4b (N = 35)
Female longevity	19.6 ± 3.3b (N = 35)	25.1 ± 10.2a (N = 5)	19.8 ± 3.5b (N = 35)
(b)			
Egg	2.1 ± 0.5a (N = 61)	1.8 ± 0.4b (N = 52)	1.5 ± 0.0 (N = 1)
Larva	1.1 ± 0.3a (N = 61)	1.1 ± 0.5a (N = 41)	1.0 ± 0.0 (N = 1)
Protonymph	1.1 ± 0.3b (N = 61)	1.8 ± 0.8a (N = 52)	4.0 ± 2.1 (N = 1)
Deutonymph	0.9 ± 0.3b (N = 61)	1.3 ± 0.6a (N = 52)	2.0 ± 0.0 (N = 1)
Egg-adult	5.2 ± 0.4b (N = 61)	5.8 ± 1.0a (N = 52)	8.5 ± 0.0 (N = 1)
Pre-oviposition	1.2 ± 0.4b (N = 36)	2.1 ± 0.5a (N = 30)	0.0 ± 0.0 (N = 1)
Oviposition	7.2 ± 3.4a (N = 36)	7.8 ± 4.4a (N = 30)	0.0 ± 0.0 (N = 1)
Post-oviposition	1.8 ± 1.4a (N = 36)	1.7 ± 1.4a (N = 30)	0.0 ± 0.0 (N = 1)
Female longevity	14.9 ± 4.7a (N = 36)	17.2 ± 4.7a (N = 30)	11.0 ± 0.0 (N = 1)

Means ± S.D. within a row followed by the same letter are not significantly different at $P = 0.05$ (Student-Newman-Keuls multiple range test); *N = number of females in the cohort

Regarding *T. manihoti*, only one female had completed its life cycle when *T. urticae* was offered as food. When this predator species was fed either *M. tanajoa* or *O. gossypii*, there

was a significant difference in the egg to adult developmental period and the pre-oviposition period ($P<0.05$). However, there was no significant difference in the oviposition, post-oviposition or longevity periods of females ($P>0.05$) (Table 1b).

Effect of diet on adult female reproduction and survivorship

The age-specific fecundity of females of *T. aripo* was delayed compared to that of *T. manihoti* regardless of the diet (Figures 1 & 2). Peak fecundity for both predators was recorded between the first 10 and 15 days (e.g. 3-10 days after the first eggs were laid) of the females' life span (Figures 1 & 2).
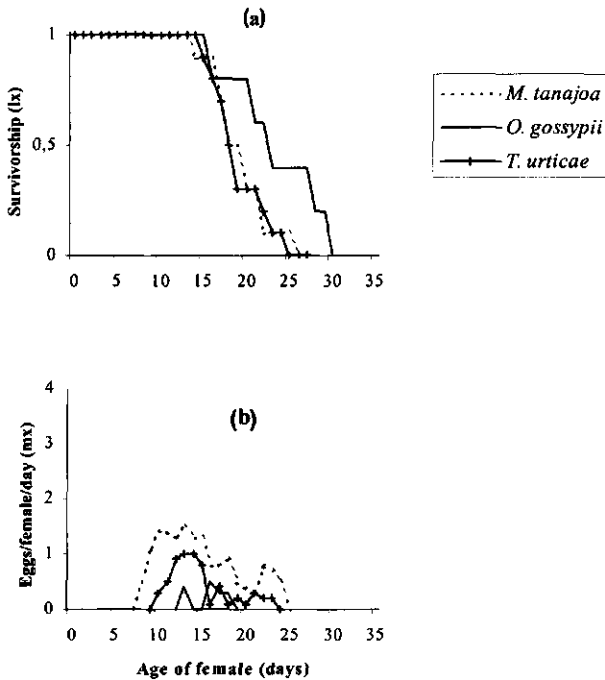

Figure 1. Age-specific survival (a) and fecundity (b) of *T. aripo* on three prey mite species. Day 0 is the day on which the mother was born.
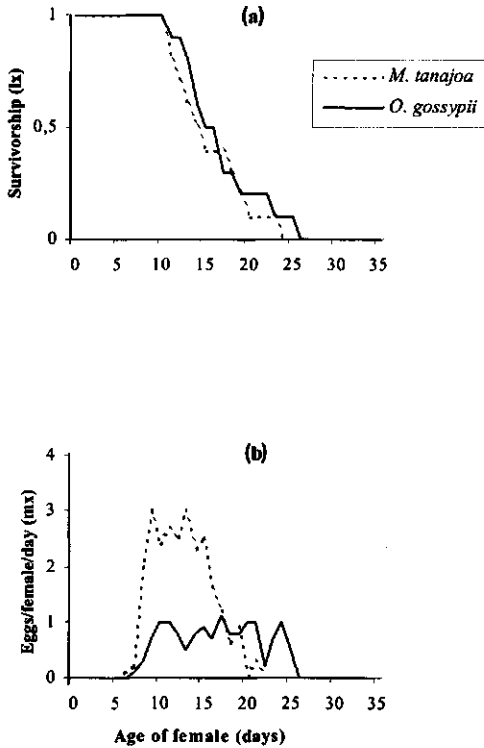
Figure 2. Age-specific survival (**a**) and fecundity (**b**) of *T. manihoti* on two prey mite species. Day 0 is the day on which the mother was born.

The highest peak of age-specific fecundity (Figures 1 & 2) and the highest female fecundity (Table 2) were obtained when populations of the two predators were fed *M. tanajoa*. *Typhlodromalus aripo* females survived longer on *O. gossypii* than on *M. tanajoa* or *T. urticae* as food (Figure 1a) whereas *T. manihoti* populations survived equally on both *M. tanajoa* and *O. gossypii* (Figure 2a). Wilcoxon statistics did not reveal any significant difference among survival rates of different populations either reared on *M. tanajoa*, *O. gossypii* or *T. urticae* for *T. aripo* (*P*=0.22) or for *T. manihoti* reared on *M. tanajoa* or *O. gossypii* (*P*=0.28).

Effect of diet on life table characteristics

Prey species had substantial and significant effect on all the life table parameters of both predators, including intrinsic rate of increase (r_m), net reproductive rate (R_o), mean generation time (GT), doubling time (DT), fecundity (eggs/female), and sex ratio (proportion female progeny). For *T. aripo*, r_m, R_o and fecundity were respectively 2.1, 2.3 and 2.4 fold higher on a diet of *M. tanajoa* compared with a diet of *T. urticae* (Table 2a; $P<0.05$).

Table 2. Effect of diet on life table characteristics of the predatory mites *Typhlodromalus aripo* (**a**) and *T. manihoti* (**b**) at 25 °C

Parameter	Diet		
	M. tanajoa	*O. gossypii*	*T. urticae*
(a)			
Intrinsic rate of increase, r_m (day^{-1})	$0.153 \pm 0.006a$	$0.000 \pm 0.000c$	$0.073 \pm 0.007b$
Net reproduction, R_o (females per female	$6.20 \pm 0.49a$	$0.29 \pm 0.18c$	$2.66 \pm 0.26b$
Mean generation time (days)	11.96	-	13.46
Doubling time (days)	4.54	-	9.53
Fecundity (eggs per female)	$12.0 \pm 4.8a$	$1.2 \pm 0.4c$	$5.1 \pm 2.9b$
Female progeny	55	33	64
(b)			
Intrinsic rate of increase, r_m (day^{-1})	$0.234 \pm 0.009a$	$0.103 \pm 0.012b$	-
Net reproduction, R_o (females per female	$11.54 \pm 1.49a$	$3.42 \pm 0.49b$	-
Mean generation time (days)	10.48	11.73	-
Doubling time (days)	2.97	6.61	-
Fecundity (eggs per female)	$17.3 \pm 12.9a$	$6.8 \pm 6.0b$	0.0 ± 0.0
Female progeny	72	67	-

Means $\pm$ S.D. within a row followed by the same letter are not significantly different at $P = 0.05$ (Student-Newman-Keuls multiple range test).

In addition, both GT and DT were shorter (0.89x and 0.48x respectively) on a diet of *M. tanajoa* than on a diet of *T. urticae*, while the sex ratio was higher on *T. urticae* (64%) than on *M. tanajoa* (55%) diet (Table 2a). Similar to *T. aripo*, life table parameters for *T. manihoti* were significantly different on *M. tanajoa* from those on the alternative prey

O. gossypii. This predator's life table parameters could not be calculated on a diet of *T. urticae*, as female reproduction and survivoral on this prey were nil (only on female of an original cohort of 60 eggs survived long enough to reproduce). For *T. manihoti*, r_m, R_o and female fecundity were respectively 2.3, 3.4 and 2.5 fold higher on a diet of *M. tanajoa* compared with a diet of *O. gossypii* (Table 2b; $P<0.05$). In addition, both GT and DT were shorter (0.89x and 0.45x respectively) on a diet of *M. tanajoa* than on a diet of *T. urticae,* but unlike *T. aripo*, sex ratio was higher on *M. tanajoa* (72%) than on *O. gossypii* (67%) diet (Table 2b).

Discussion

Our study showed that *T. aripo* fed, survived and developed on different spider mite species such as *M. tanajoa, O. gossypii* and *T. urticae* whereas *T. manihoti* developed and survived on *M. tanajoa* and *O. gossypii* only. The type of food greatly affected the female's development and fecundity. Feeding on suitable food resulted in a faster development of pre-imaginal stages, a reduction in adult female pre-ovipostion, oviposition and post-ovipositon periods. A reduced female longevity was also recorded on the suitable diet due probably to a trade-off in energy spent on prey consumption versus oviposition. Female longevity and fecundity seem to be inversely correlated. The same trend was observed in previous studies on *Iphiseius degenerans* (Berlese) fed broad bean pollen, castor pollen and spider mites (Van Rijn and Tanigoshi, 1999) and on *Rhynocoris fuscipes* (Fabricius) fed on *Spodoptera litura* Fab., *Earias vittella* Fab., and *Corcyra cephalonica* Stainton (Edward George, 2000). These authors reported that the life span of female predators was reduced and the intrinsic rate of increase (r_m) was higher on the suitable diet (broad bean pollen and *S. litura* for the two predators respectively) than on the other diets. A rank-order hierarchy of prey suitability shows that *M. tanajoa* and *T. urticae* were more suitable prey than *O. gossypii* for *T. aripo* while *M. tanajoa and O. gossypii* were more suitable than *T. urticae* for *T. manihoti*. However, for both predator species, *M. tanajoa* was the most suitable food source compared to the prey mite species *O. gossypii* and *T. urticae*. These results might be explained by a difference in nutritional value or quality between prey species. The consumption of high quality prey may favour a high conversion of food into egg biomass, and therefore a high intrinsic rate of increase of the predator (Sabelis, 1985a; Bruce-Oliver and Hoy, 1990).

Mean generation time and doubling time for both predator species were shorter on *M. tanajoa* food than on *O. gossypii* or *T. urticae*. A short doubling time will lead to fast build-up of the predator population and consequently a suppression of the target prey *M. tanajoa* in a relatively short time. Because *T. manihoti* has a shorter doubling time compared to *T. aripo* when fed *M. tanajoa*, under optimal food and environmental conditions, *T. manihoti* populations might increase more rapidly than a *T. aripo* population. However, optimal values for life history traits are expected to depend upon environmental conditions (Johansson *et al.*, 2001). Factors such as saturation deficit (Bakker *et al.*, 1993; Mégevand, 1997) could limit egg hatchability of *T. manihoti* and hamper its survival, to the extent it affects its population dynamics and persistence in cassava fields. The population increase of *T. manihoti* can have ecological consequences for *T. aripo* as intraguild interactions may occur between the two predators during subsequent low prey availability. However, *T. aripo*'s foraging strategy (Onzo *et al.*, in prep.) and habitat preference limit the magnitude of the interspecific interactions, leading to coexistence with its intraguild predator *T. manihoti* in the same habitat (chapter 5).

Typhlodromalus manihoti performs better on *M. tanajoa* and *O. gossypii* compared to *T. aripo*. The low reproductive rate of *T. aripo* on *M. tanajoa* may not be the result of a combination of low feeding rate and low efficiency of converting prey into offspring compared to *T. manihoti* (Yaninek *et al.*, 2001). Nonetheless, *T. aripo* did better egg production on *T. urticae* than did *T. manihoti*. It is well known that *T. urticae* has the ability to construct a complex web on the leaf surface of its host plant (Sabelis, 1981; Saîto, 1985). Behavioral investigations revealed that the ability of Phytoseiidae to penetrate the web is related to chaetotaxy (Sabelis and Bakker, 1992). The two-spotted spider mite may be less favorable to *T. manihoti* because of the structure of its margino-dorsal setae (De Moraes *et al.*, 1994). However, in our study, *T. urticae* females were renewed every 2-3 days so no dense web was found in the experimental units. Future research should put emphasis on whether the web of *T. urticae* impedes foraging behavior, predation efficiency and fecundity of both *T. aripo* and *T. manihoti*.

Our results on life table characteristics of *T. manihoti* can explain previous data on the prey-related odor preference (chapter 3). *Typhlodromalus manihoti* did not prefer odors emitted from cassava leaves infested with *T. urticae* over those from non-infested leaves and also did not perform well on this prey. However, *T. aripo* was not attracted to odors from *T. urticae*-infested cassava leaves (chapter 3) and still *T. aripo* can feed and reproduce on *T.*

urticae although its reproductive success is lower than on *M. tanajoa* as food. *Typhlodromalus aripo* was attracted to *M. tanajoa*-infested cassava leaves (Gnanvossou *et al.*, 2001). Regarding *O. gossypii*, the life table results probably explain why both predatory mites prefer *M. tanajoa*-induced odors over *O. gossypii*-induced odors. Overall, the results showed that *T. aripo* and *T. manihoti* fed *O. gossypii* and *T. urticae* have a lower reproduction rate compared to conspecifics fed *M. tanajoa*. When reared on *M. tanajoa* the two predator species developed faster than on the other two prey mite species. Cassava leaves infested by *O. gossypii* and *T. urticae* are less attractive than *M. tanajoa*. Phytoseiids are expected to search for the most profitable prey species and use herbivore-induced volatiles for that purpose. Therefore, when the two alternative prey mites as a whole are compared with the key prey mite, prey-related odor preference and predator performance are nicely correlated.

Acknowledgements

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5

Role of plant volatiles in niche segregation and interspecific competition between two predatory mites

Désiré Gnanvossou, Rachid Hanna & Marcel Dicke

Abstract

Niche use by carnivorous arthropods is a vital component in their evolutionary ecology. In Africa, *Typhlodromalus manihoti* and *T. aripo*, two predators of the cassava green mite *Mononychellus tanajoa* occupy different parts of cassava foliage. In the present study, niche segregation by these two predators, as mediated by prey-induced infochemicals was investigated. In response to prey feeding damage, cassava plant tissues emit volatile blends, which act as attractants for predators. When given a choice between old cassava leaves infested with *M. tanajoa* and either apices or young cassava leaves infested with *M. tanajoa*, *T. aripo* displayed a marked preference for odors emitted from either infested apices or infested young leaves over infested old leaves but showed no preference for odors from apices versus young leaves both infested with *M. tanajoa*. *Typhlodromalus manihoti* did not discriminate between volatiles from the three infested cassava plant parts. The niches of the two predator species were partially segregated. The distribution of prey species, intraguild predation and competition are likely to play a role in this. Our data show that the predator *T. aripo* uses differences in volatile blends released by infested cassava plant parts and restricted its fundamental niche to a realized niche, which enables coexistence with its competitor *T. manihoti*.

Introduction

According to the theory of the ideal free distribution, competitors are equal in all respects and 'free' to settle in any patch (Kacelnik *et al.*, 1992). Behavioral properties of individuals in communities may affect the distribution of competitors, hence leading to competition or niche segregation (Kacelnik *et al.*, 1992). The analysis of availability of food resources and habitat use is an important first step in the study of insect community organization. Understanding the mechanisms of ecological coexistence or segregation of species is an important issue in community ecology (Rosenzweig, 1981; Aldridge, 1986; Brown, 1989, Ziv *et al.*, 1993; Arlettaz *et al.*, 1997; Chesson, 2000; Barbosa *et al.*, 2001; Nakashizuka, 2001). Avoidance of competition can lead to non-overlapping patterns of resource utilization (Janssen *et al.*, 1995, 1997). Although niche overlap is a prerequisite for exploitative competition, it only leads to competition when resources are in short supply (Pianka, 1976). In carnivorous arthropod communities, host or prey habitat location is a first and major step in the foraging process of carnivores and this is enhanced by infochemicals released by herbivore-damaged plants that are indirectly associated with the presence of herbivores (Dicke and Vet, 1999; Sabelis *et al.*, 1999). These cues can mediate carnivore distributions and interactions such as competition and niche segregation (Janssen *et al.*, 1998; Geervliet *et al.*, 2000).

The composition of volatile blends emitted from herbivore-infested plants is affected by biotic and abiotic factors (reviewed in Takabayashi *et al.*, 1994b and Dicke, 1999) including herbivore species and stage (Sabelis and van de Baan, 1983; Sabelis *et al.*, 1984; Dicke, 1988; Takabayashi *et al.*, 1991; De Moraes *et al.*, 1998), plant species (Dicke and Sabelis, 1988; Takabayashi *et al.*, 1991; Turlings *et al.*, 1993; Takabayashi and Dicke, 1996) and plant tissues (Turlings *et al.*, 1993; Takabayashi *et al.*, 1994a). Many studies have demonstrated the effects of the host plant on within-plant distribution of herbivores (Gianoli, 1999) and parasitoid foraging (De Moraes and Lewis, 1999; Geervliet *et al.*, 2000). In contrast, much less well documented are the effects of volatiles from different parts of the same plant on the foraging decisions of predator species and consequently on predator distributions over a plant. In this paper, we investigated whether the two predatory mites of the cassava green mite, *T. manihoti* and *T. aripo*, show differential preferences to volatiles from different cassava plant tissues.

Our experimental system for this study consisted of cassava plants (*Manihot esculenta*) (Euphorbiaceae), the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) and the two predatory mites, *T. aripo* and *T. manihoti*. In the African cassava agroecosystem, *M. tanajoa* is the most abundant tetranychid mite species and generally occurs on the upper half of the plant (Yaninek *et al.*, 1989, 1991). The two phytoseiid species *T. manihoti* and *T. aripo* use *M. tanajoa* as the main prey but their spatial distribution within the cassava plant differs. On cassava in Africa, where *T. manihoti* and *T. aripo* have been present since 1989 and 1993 respectively, *T. aripo* displays a diurnal foraging pattern – it inhabits the terminal growing point (or apex) of the cassava branches during the daylight hours and undertakes foraging bouts on young cassava leaves below the terminal points during much of the night hours (Onzo *et al.*, in prep.). Moreover, all of *T. aripo*'s reproduction takes place in the apex, whereas *T. manihoti* forages and reproduces on both young and old leaves (more on young than on old) but not in the apices of cassava plants. The mechanisms underlying the differential distributions of the two predator species are not well known. In this paper, we investigate if distributions of the predators on the cassava plant are related to infochemicals released by different plant tissues when infested by *M. tanajoa*.

Materials and Methods

Cassava plant tissues. Plant tissues (e.g. apex, 3rd leaf from top and 3rd leaf from bottom were collected from 4-5 week old cassava 'trees' that had ca 9 leaves per branch. A cassava tree is a vertical production system of cassava leaves used for mite rearing (Haug and Mégevand, 1989). It consists of a plastic sleeve 1.8 m long and 12 cm diameter filled with ground coconut husks and suspended in a metal frame. Sixty-eight cassava cuttings are planted along the sleeve into the ground coconut husks. The cassava trees were grown in a greenhouse (30 ± 5 °C; 70 ± 10% RH) at the International Institute of Tropical Agriculture in Benin (IITA-Benin). Cassava trees were watered as needed with water containing a fertilizer (LUWASA salt) with the following composition: 15% N, 7% P$_2$O$_5$, 6% MgO, 0.12% Fe and traces of B, Cu, Mn, Zn, Mo, and Co.

Tissue weight determination. The fresh weights of plant tissues (e.g. apex, 3rd leaf from top and 3rd leaf from bottom) were determined with an electronic balance. A single leaf was

placed into a pre-weighed paper envelope (16.0 cm long and 11.3 cm wide, and weighing an average of 3.45 ± 0.31 g when empty). Since the apex of a cassava shoot is very small compared to the leaves, an average weight was determined based on group measurements (2 apices in each envelope). Thirty replicate envelopes per tissue age were evaluated in five temporally distinct trials. The height of the stems on which the apex and leaves were collected was measured and the total number of leaves was systematically counted.

Spider mite and predator cultures. Colony of the *Mononychellus tanajoa* was maintained indoors on potted cassava plants for one month at 26 ± 1 °C; 65-80% RH, and was periodically restocked from several hundred individuals collected from cassava fields in southern Benin. Colonies of *T. manihoti* and *T. aripo* were started from approximately a hundred adult females collected from cassava fields in southern Benin. They were maintained in the laboratory at 25 ± 1 °C and 80 ± 10% RH on detached cassava leaves infested by *M. tanajoa* as described by Mégevand *et al.* (1993). Both young and old infested cassava leaves were used for rearing the two predatory mite species. The petioles of these leaves were placed in water-filled vials sealed with parafilm to keep them hydrated. The vials with leaves were placed in a plastic tray (35 x 35 x 6 cm) and covered with a similar tray. The whole setup was kept on metallic shelves with feet isolated from the floor by a mixture of paraffin® liquid and water in plastic containers. New prey infested leaves were added daily to the rearing units. The predatory mites had been maintained for 5 to 6 months under the aforementioned laboratory conditions prior to their use in the olfactometer experiments.

Olfactometer experiments: Attraction of predators to odor sources was determined in two-choice tests by using a closed system Y-tube olfactometer for the following comparisons: (i) infested or non-infested plant tissues versus clean air, (ii) infested versus non-infested plant tissues, and (iii) infested tissues of different plant parts (apex, 3rd leaf from top and 3rd leaf from bottom) in pairwise tests. The olfactometer system, except for the compressor (SERBATOI® AUTOCLAVI; Type ELTO; Vol. 50) used to generate an air stream, was identical to that described by Takabayashi and Dicke, 1992. This olfactometer setup has been extensively used for studying the olfactory responses of predatory mites (Sabelis and van de Baan, 1983; Takabayashi and Dicke, 1992) and the conclusions from these olfactometer experiments have been supported by greenhouse studies (Janssen, 1999).

Adult female *T. manihoti* and *T. aripo* were collected from the rearing units where they had ample food available and were thus considered satiated at the time of removal from the cultures. They were individually enclosed in a plastic vial (10 mm in diameter and 40 mm long) and kept without food for 2 h at 25-28 °C, 65-90% RH prior to the olfactometer bioassays. Female predators were placed individually at the base of an iron wire that was positioned in the middle of the Y-shaped glass tube, and parallel to the tube walls. The predators were observed until they reached the end of one of the arms, or for a maximum of five minutes, and were subsequently removed. Both predator species were tested alternately with the same odor sources. After a series of five mites, the odor sources were connected to the opposite arm of the olfactometer to correct for any unforeseen asymmetry in the experimental set-up. The number of females that chose either odor source or did not choose was recorded for each predator species. Each test was repeated 3-5 times (on 3-5 consecutive days) with 20 predators per species per repetition. New odor sources were used for each repetition.

Preparation of odor sources. Leaves and apices used as odor sources in olfactometer bioassays were detached from cassava plants in the morning. Immediately upon detachment, leaves were placed with the petiole in water-filled glass vials (0.8 cm in diameter and 4 cm deep) and apices with the stem end (10 cm below the apex) in water-filled plastic vials (2.7 cm in diameter and 6.5 cm deep) that were sealed with parafilm to keep the plant tissues fresh and hydrated during the course of the experiment. Each glass vial contained one leaf and each plastic vial contained eight apices. The leaf tissue treatment consisted of two types of leaves of different age classes selected from 5-week old cassava trees with the 3rd leaf from the apex representing the young leaves and the 3rd leaf from the bottom representing the old class leaves. In the afternoon of the same day, all or only half of the total number of leaves or apices collected were infested with spider mites. We kept constant both tissue weight and number of mites used for initial infestations. The number of leaves or apices used in the experiment was based on their predetermined fresh weight. Based on tissue biomass, 72 apices were equivalent to five old leaves or four young leaves. We used a similar number of spider mites, 400 adult female *M. tanajoa*, to infest each amount of plant tissue. Plant tissues were then incubated for three days in plastic cages (length: 70 cm; width: 40 cm; height: 40 cm) at 26 ± 1 °C and under continuous light provided by two fluorescent light sources above each cage. *Mononychellus tanajoa*-infested and non-infested tissues were incubated in separate cages kept in the same room.

Statistics. We used the one-tailed probability of the binomial test to compare the response of each predator species to odors from the *M. tanajoa*-infested apices and young or old leaves versus either clean air or non-infested tissues, and the two-tailed probability in pairwise comparison of *M. tanajoa*-infested plant tissues of different age (i.e., tissue-related odor preference) (Zar, 1984). Females that did not walk to the far end of one of the arms and those that did not make a choice were not included in the statistical analysis. Finally, we used 2 x 2 contingency table analysis based on Chi-square, with Yates correction to test between-predator differences in their response to all the treatments (Zar, 1984).

Results

1. Biomass data of cassava plant tissues

The average fresh weights of the apex, 3rd leaf from the top and 3rd leaf from the bottom were 0.034 ± 0.010, 0.62 ± 0.10 and 0.48 ± 0.05g (mean $\pm$ SD) respectively (Table 1).

Table 1. Fresh weight (g) of cassava plant tissues (4-5 week old) collected from cassava trees grown in a greenhouse. Means $\pm$ SD are presented

Trial	Rep./ trial	Number of tissue per replicate Apex	Leaf	Stem height (cm)	Number of leaves per stem	Fresh weight (g) Apex	3rd YL*	3rd OL*
1	30	2	1	58.2±8.9	8.8±0.6	0.037±0.007	0.56±0.14	0.49±0.20
2	30	2	1	58.3±10.0	9.3±0.8	0.050±0.078	0.68±0.14	0.55±0.16
3	30	2	1	61.5±7.0	9.3±0.5	0.028±0.006	0.76±0.15	0.52±0.13
4	30	2	1	56.8±9.6	9.2±0.6	0.022±0.004	0.55±0.15	0.44±0.12
5	30	2	1	57.1±10.3	9.3±0.6	0.031±0.005	0.54±0.18	0.42±0.19
Pooled mean				58.4±1.9	9.2±0.2	0.034±0.010	0.62±0.10	0.48±0.05

*YL = young leaf; OL = old leaf

The biomass of a 3rd young leaf was 18.3 fold greater than that of an apex, and 1.3 fold greater than that of a 3rd old leaf, and the biomass of the 3rd old leaf was 14.3 fold greater than that of an apex. The number of units of plant tissue taken during each olfactometer experiment (experiment 3) was a function of tissue biomass. At the moment the samples were taken, the mean ($\pm$ SD) of the stem height and of the number of leaves per stem was 58.4 $\pm$ 1.9 cm and 9.2 $\pm$ 0.2 leaves respectively.

2. Olfactometer experiments:

Experiment 1: Predator responses to volatiles from either infested or non-infested cassava tissues versus clean air

Typhlodromalus aripo and *T. manihoti* were significantly attracted to odors emitted from 72 *M. tanajoa*-infested cassava apices over clean air (*P*<0.01) (Figure 1).
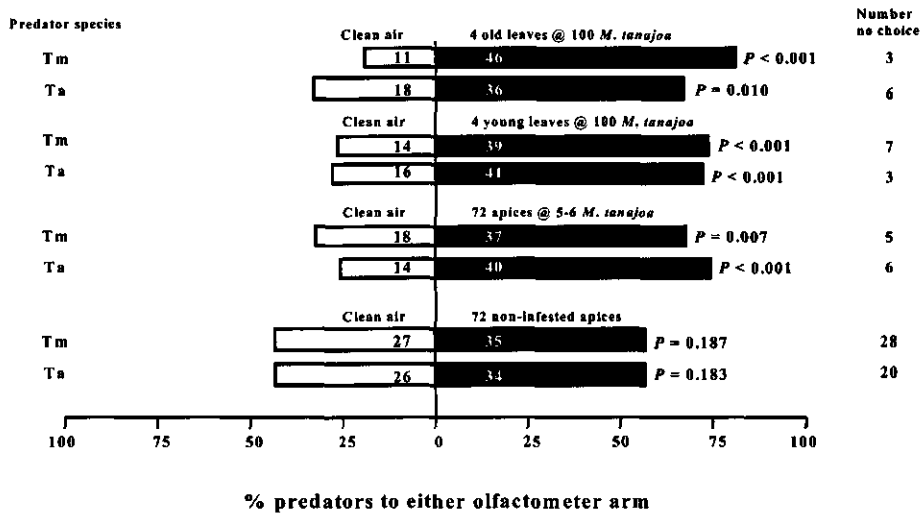

% predators to either olfactometer arm

Figure 1. Response of 2h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta), in a Y-tube olfactometer when a choice wa given between volatiles from *M.tanajoa*-infested leaves/apices or non-infested apices versus clean air. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

Typhlodromalus aripo showed a greater response (74.1%) to infested apices than *T. manihoti* (67.3%) but no significant difference was found between the two predators (*P*=0.352). In contrast, neither predator species discriminated between 72 non-infested apices and clean air. When given a choice between four *M. tanajoa*-infested young leaves and clean air, the two predators were significantly (P<0.01) and similarly (*P*=0.873) attracted to infested young leaves. Both predator species were more attracted to four *M. tanajoa*-infested old leaves than to clean air. However, *T. manihoti* showed a significantly greater response (80.7%) to infested old leaves than *T. aripo* (66.7%) (*P*=0.036; Figure 1).

Experiment 2: Predator responses to volatiles from infested versus non-infested cassava tissues

Seventy-two *M. tanajoa*-infested apices significantly attracted *T. aripo* and *T. manihoti*
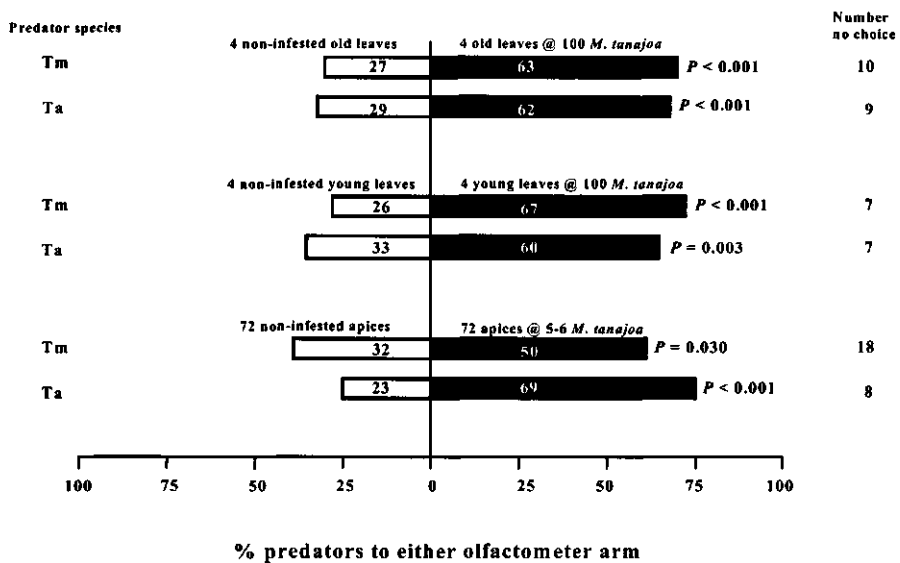

% predators to either olfactometer arm

Figure 2. Response of 2h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta), in a Y-tube olfactometer when volatiles from either the *M.tanajoa*-infested leaves/apices or non-infested leaves/apices were offered. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

when 72 non-infested apices were offered as alternative, but *T. aripo* showed a significantly greater attraction (75.0%), than *T. manihoti* (61.0%) (*P*=0.049; Figure 2). When given a choice between four *M. tanajoa*-infested young leaves and four non-infested young leaves or between four *M. tanajoa*-infested old leaves and four old non-infested leaves, the two predators were significantly and similarly attracted to infested leaves regardless of leaf age (Figure 2). The responses of the two predator species were not significantly different (young leaves, *P*=0.361; old leaves, *P*=0.878).

Experiment 3: Predator responses to infested tissues of different age in two-choice situations

Typhlodromalus aripo and *T. manihoti* showed no clear preference when 72 *M. tanajoa*-infested cassava apices were offered versus four *M. tanajoa*-infested young leaves (Figure 3).
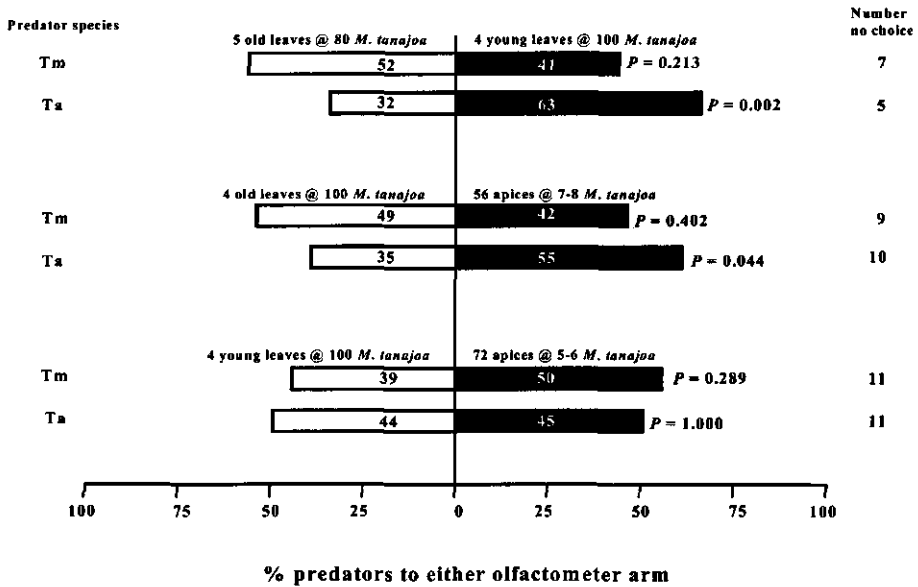

Figure 3. Response of 2h-starved predatory mites *Typhlodromalus manihoti* (Tm) and *T. aripo* (Ta), in a Y-tube olfactometer when given simultaneously volatiles from *M. tanajoa*-infested tissues of different ages. Numbers in the bar segments represent the total number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

However, when given a choice between four *M. tanajoa*-infested old leaves and 56 *M. tanajoa*-infested apices, *T. aripo* was significantly attracted to infested apices (61.1%) than to

old infested leaves (38.9%), while *Typhlodromalus manihoti* did not discriminate between these odor sources. A similar differential response by the two predator species to the infested young leaves was recorded when four *M. tanajoa*-infested young leaves were offered versus five *M. tanajoa*-infested old leaves. *Typhlodromalus aripo* showed a significantly greater attraction to *M. tanajoa*-infested young leaves (66.3%) than *M. tanajoa*-infested old leaves (33.7%). *Typhlodromalus manihoti* did not discriminate between these odor sources (Figure 3).

Discussion

Our data show that the attraction of *T. aripo* to volatiles emitted from *M. tanajoa*-infested cassava plant tissues is affected by the age of the infested tissues: infested apices or infested young leaves were preferred over infested old leaves when these were offered simultaneously. However, *T. manihoti* does not discriminate between volatiles from infested young tissues (i.e. apex and young leaves) and volatiles from infested old leaves. Thus, *M. tanajoa*-induced cassava plant volatiles from tissues of different ages evoke different behavioral responses in the two predatory mite species. These results fit the observations on the spatial distribution patterns of the two predatory mite species on cassava plant foliage: *T. manihoti* inhabits young and old leaves but preferentially forages on young cassava leaves whereas *T. aripo* inhabits the cassava apices and forages on young leaves (Bakker, 1993; Onzo *et al.*, in prep.). In our study, we used similar numbers of spider mites to infest similar biomass of different plant tissues and still we have differences in the attraction to infested cassava plant tissues by the two predatory mites. Why would *T. aripo* and *T. manihoti* differ in this way? Discrimination by predators and parasitoids, between tissues of different ages exists and quantitative differences in volatile blends have been recorded between tissues of different ages of cucumber plants (Takabayashi *et al.*, 1994a) and of cotton plants (Turlings *et al.*, 1993). The predatory mite *Phytoseiulus perimilis* Athias-Henriot was attracted to spider-mite infested young cucumber leaves but not to spider-mite infested old cucumber leaves and some chemical differences between the blends of infested young and old cucumber leaves were reported (Takabayashi *et al.*, 1994a)

Irrespective of the mechanism, each of the two predatory mite species *T. aripo* and *T. manihoti* make different decisions when presented with exactly the same information. Two

herbivore mites occur on cassava plants: *M. tanajoa* that occurs on the younger foliage and *Oligonychus gossypii* that occurs on the older leaves of the plants (Yaninek and Onzo, 1988; Yaninek *et al.*, 1989). For both predators, *M. tanajoa* is a key prey. However, while *O. gossypii* can serve as prey to *T. manihoti*, *O. gossypii* is not a suitable prey for *T. aripo* (chapter 4). Therefore, *T. aripo* would be expected to prefer to forage on young leaves where high populations of the key prey *M. tanajoa* are found, whereas *T. manihoti* would be expected to forage throughout the cassava plant. This is exactly what we found during our investigation with a Y-tube olfactometer.

When two species, that overlap in resource use, meet, they may coexist through resource partitioning or one may outcompete the other (Tokeshi, 1999). The degree of preference between young and old cassava plant tissues exhibited by *T. aripo* may be explained in part by intraguild interactions with *T. manihoti*, such as resource competition and intraguild predation. Differences in searching behavior can reduce overlap in prey use, which may facilitate coexistence of predators searching for the same prey species (Johnson and Hubbell, 1975; van Alphen *et al.*, 1991; Arlettaz *et al.*, 1997; Arlettaz, 1999). A recent study has demonstrated that *T. aripo* showed temporal partitioning of resources on young leaves: they are mostly active during the first part of the night in foraging on the upper young leaves of cassava foliage and move to the apex where the predators aggregate as soon as the gut is full (Onzo *et al.*, in prep.). Thus, the cassava apex is considered a refuge for this predator species, for protection against competitors and hard conditions. Moreover, in addition to restriction of its niche, *T. aripo* restricts time to foraging on that part of the foliage where it shares resources with its competitor *T. manihoti* possibly to escape from intraguild predation. Many animals modify their microhabitat or refuge use according to levels of predation risks and infochemicals can mediate this (reviewed in Dicke and Grostal, 2001). Apart from differential responses to herbivore-induced plant volatiles, predatory mites may also exploit volatiles related to predator invasion in prey patch (Janssen *et al.*, 1997) and this has also been shown for the predatory mites *T. manihoti* and *T. aripo* (chapter 6). The two predators prefer volatiles from a prey patch that is not invaded by predators over a prey patch invaded by conspecific or heterospecific predators. When given a choice between volatiles from a prey patch invaded by conspecific and heterospecific predators, most predators go to the volatiles from the patch invaded by heterospecifics, but this is only statistically significant for the choice of *T. manihoti* (chapter 6). In conclusion, the predatory mites *T. manihoti* and *T. aripo* have differential distributions over cassava plants and differential responses to

infochemicals are likely to play a role in this. Arthropods are known to exploit many types of infochemicals during foraging for resources and avoiding enemies or competitors so as to maximize fitness (Bell and Carde, 1984; Dicke and Sabelis, 1992; Janssen *et al.*, 1998; Sabelis *et al.*, 1999; Dicke and Vet, 1999) and the data presented here add an intriguing example to the literature.

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6

Odor-mediated intraguild interactions among three predatory mites

Désiré Gnanvossou, Rachid Hanna & Marcel Dicke

Abstract

Carnivorous arthropods exhibit complex intraspecific and interspecific behavior among themselves when they share the same habitat and food resources. They should simultaneously search for adequate food and habitat for themselves and their offspring and in the meantime avoid becoming food for other organisms. This behavior is of great ecological interest in conditions of low prey availability. We examined by means of an olfactometer, how volatile chemicals from prey patches with presence of competitors contribute in shaping ecological communities. We used the exotic predatory mites *Typhlodromalus manihoti* and *T. aripo* and the native predator species *Euseius fustis*. *Mononychellus tanajoa* is a common prey species for the three predators. As odor sources we used *M. tanajoa*-infested cassava leaves or apices with or without presence of predators. *Typhlodromalus manihoti* avoided patches inhabited by the heterospecifics *T. aripo* and *E. fustis* or by conspecifics when tested against a patch without predators. Similarly, both *T. aripo* and *E. fustis* females avoided patches with con- or heterospecifics when tested against a patch without predators. When one patch contained *T. aripo* and the other *T. manihoti*, females of the latter preferred the patch with *T. aripo*. Thus, *T. manihoti* is able to discriminate between odors from patches with con- and heterospecifics. Our results show that the three predatory species are able to assess prey patch profitability using volatiles. Under natural conditions and when food becomes scarce, the three predatory mites might be involved in interspecific and/or intraspecific interactions that can substantially affect population dynamics of the predators and the prey.

Introduction

Interactions between predators that use similar resources are receiving intensified attention from ecologists (Polis *et al.*, 1989; Polis and Holt, 1992; Rosenheim *et al.*, 1993, 1995; Janssen *et al.*, 1998; Lucas *et al.*, 1998). In many natural communities and at the same trophic level, one or more species may act not only as competitors but also as predators of other species. This phenomenon is termed intraguild predation (Polis *et al.*, 1989; Rosenheim *et al.*, 1995; Holt and Polis, 1997) whereas predation on conspecifics is termed cannibalism (Polis, 1981; Schmidt *et al.*, 1998). Intraguild predation can be seen as an extreme form of competition and may affect the distribution and abundance of species over patches (Polis and McCormick, 1987; Lucas *et al.*, 1998). Predation between and within species is promoted when the risks associated with capturing the victim and feeding on it are relatively low (Schmidt *et al.*, 1998). Many studies have been devoted to intraguild predation (Polis and McCormick, 1987; Fletcher and Underwood, 1987; Wissinger and McGrady, 1993; Polis and Holt, 1992; Rosenheim *et al.*, 1993; Lucas *et al.*, 1998; Roda *et al.*, 2000) and cannibalism/intraguild predation (Croft and Croft, 1993; Croft *et al.*, 1996; MacRae and Croft, 1997; Schausberger, 1997, 1999) in various systems.

Factors internal to predators (Lucas *et al.*, 1998) and external factors (Lucas *et al.*, 1998; Roda *et al.*, 2000) are known to affect the magnitude of intraguild interactions. Among the external factors, volatiles might play an important role by mediating avoidance of cannibalism or intraguild predation (Janssen *et al.*, 1997). Plants that are infested by herbivores can indirectly protect themselves against these herbivores by releasing volatiles that attract natural enemies of the herbivores (Sabelis and van de Baan, 1983; Sabelis and Dicke, 1985; Tumlinson *et al.*, 1993; Dicke, 1999). Chemical cues can also mediate avoidance within and between herbivores and predators/parasitoids. Between the third and the second trophic levels, chemical stimuli from predators can influence the searching behavior of prey, resulting in the avoidance of predators and therefore in a reduced predation risk (Kriesch and Dicke, 1997; Grostal and Dicke, 1999, 2000; Pallini *et al.*, 1999; reviewed in Lima and Dill, 1990; Dicke and Grostal, 2001). Competitive interactions within the second trophic level have also been investigated for spider mites and bark beetles. In the case of spider mites, densities of *Tetranychus urticae* were higher on control plants than on plants previously infested with conspecifics or the heterospecific *Eotetranychus carpini borealis*. This negative effect was mediated by the plant, which may produce chemicals that signal the

presence of the first colonizer (Bounfour and Tanigoshi, 2001). In the case of spruce bark beetles, *Ips typographus* and *Pityogenes chalcographus* (Coleoptera: Scolytidae) preferred to visit non-infested spruce plants to spruce plants occupied by conspecifics or heterospecifics (Byers, 1993). Within the third trophic level, recent studies have shown that volatile cues mediated interactions between two parasitoid species. *Leptopilina heterotoma* Thompson (Hymenoptera: Eucoilidae) (Janssen *et al.*, 1991; Janssen *et al.*, 1995a,b) an endoparasitoid attacking *Drosophila* larvae, avoided odors from stinkhorn patches when another, closely related parasitoid species, *Leptopilina clavipes* (Hartig) (Hymenoptera: Eucoilidae) was present on these patches (Janssen *et al.*, 1995a,b). Similarly, the generalist parasitoid *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) avoided odors from *Pieris rapae*-infested Brussels sprouts leaves on which the specialist *C. rubecula* (Marshall) (Hymenoptera: Braconidae), a superior competitor, was parasitizing *P. rapae* (Geervliet *et al.*, 1998).

Not much is known about odor-mediated interactions between conspecific or between con- and heterospecific predatory mites. When females of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) were offered a choice between the odors of bean leaves infested by their prey *Tetranychus urticae* and bean leaves infested by both *T. urticae* plus conspecific predatory mites, they preferred the odors emanating from prey patches without conspecifics (Janssen *et al.*, 1997). It was suggested that the cues responsible for this discrimination are produced by adult spider mites in predator-infested spider mite colonies. The cues are produced even when the spider mites are exposed to volatiles from their predators (i.e. predators positioned upwind from the prey patch), so physical contact is not needed before obtaining a response from the spider mites (Janssen *et al.*, 1997). *Phytoseiulus persimilis* did not show avoidance of prey patches invaded by any of three heterospecific predators, i.e. a predatory mite, a predatory bug and an omnivorous thrips (Janssen *et al.*, 1999).

We use as system two phytoseiid species exotic to Africa, *Typhlodromalus manihoti* Moraes and *T. aripo* DeLeon, and the most common native phytoseiid species on cassava in Africa, i.e. *Euseius* (= *Amblyseius*) *fustis* (Pritchard and Baker). In 1989 and 1993, *T. manihoti* and *T. aripo* respectively, were identified by the International Institute of Tropical Agriculture (IITA) as potential biological control agents of *Mononychellus tanajoa* (Bondar) and were introduced to Africa for releases (Yaninek *et al.*, 1993; Yaninek *et al.*, 1998). *Typhlodromalus aripo* in particular has been reported to show diurnal behavioural patterns. It

lives in the cassava apices and moves down to feed on *M. tanajoa* on young leaves during the night hours (Onzo *et al.*, in prep). *Typhlodromalus manihoti* is known to reside on young and old leaves, but prefers to forage on young leaves (Yaninek *et al.*, 2001). The native predatory mite *E. fustis*, like *T. manihoti*, resides on young and old leaves, but prefers old to young leaves (R. Hanna unpubl. data). In contrast to *T. aripo* and *T. manihoti*, which are effective predators of *M. tanajoa* and indeed prefer this prey to others (chapter 3), *E. fustis* is highly generalist and does not prefer to feed on *M. tanajoa* (Bruce-Oliver *et al.*, 1996; McMurtry and Croft, 1997). Both *T. aripo* and *T. manihoti*, and to certain extent *E. fustis* have overlapping niches as they forage in the same prey patches and share the same extraguild prey *M. tanajoa*. The purpose of the present study is to explore intra- and interspecific interactions among members of the third trophic level with emphasis on the role of volatile chemicals in the avoidance of intraguild predators and their possible ecological consequences. The main questions addressed are: (i) Do *T. aripo*, *T. manihoti* or *E. fustis* detect the presence of heterospecifics in prey patches and avoid visiting these patches? (ii) Do conspecific predatory mites avoid each other? (iii) Do *T. aripo* and *T. manihoti* distinguish between con-and heterospecifics? (iv) What could be the ecological consequences in the case of strong interactions between *T. aripo* and *T. manihoti*?

Materials and Methods

Spider mite and predator cultures. The *Mononychellus tanajoa* population was collected from cassava fields in southern Benin. The culture was maintained indoors on 2-4 week-old potted cassava plants grown in ground coconut husks medium at 26 ± 1 °C; 65-80% RH and was renewed periodically with mites collected from the same area in southern Benin. The predatory mites *T. manihoti* and *T. aripo* were collected from southern Benin where they have been present since 1989 and 1993 respectively. The third predatory mite *E. fustis*, a native species, was collected in the same period and in the same region as *T. manihoti* and *T. aripo*. The three predatory mite species were maintained in the laboratory at 25 ± 1 °C and 80 ± 10% RH on detached cassava leaves infested with *M. tanajoa* as described by Mégevand *et al.*, 1993. The whole setup was kept on metallic shelves with feet isolated from the floor by a mixture of paraffin® liquid and water in plastic containers. New infested leaves were added daily to the rearing units. The predatory mites had been maintained 4 to 5 months

under the aforementioned laboratory conditions prior to their use in the olfactometer experiments.

Y-tube olfactometer tests. The behavioral response of predators to odor sources was monitored in two-choice tests using a closed system Y-tube olfactometer. The system, except for the compressor (SERBATOI® AUTOCLAVI; Type ELTO; Vol. 50) used to generate an air stream, was identical to that described by Takabayashi and Dicke (1992). This olfactometer setup has been extensively used for studying the olfactory responses of predatory mites (Sabelis and van de Baan, 1983; Takabayashi and Dicke, 1992) and the conclusions from these olfactometer studies are supported by greenhouse studies (Janssen, 1999).

Adult female *T. manihoti* and *T. aripo* were collected from the rearing units, where they had ample food available and were thus considered satiated at the time of removal from the cultures. They were individually enclosed in plastic vials (10 mm diam. 40 mm long) and kept without food for 2h at 25-28 °C; 65-90% RH prior to the olfactometer bioassays. Female predators were placed individually at the base of an iron wire that was positioned in the middle of the Y-shaped glass tube, and parallel to the tube walls. The predators were observed until they reached the end of one of the arms, or for a maximum of five minutes and were subsequently removed. After a series of five mites, the odor sources were connected to the opposite arm of the olfactometer to correct for any unforeseen asymmetry in the experimental set-up. The number of females that chose either odor source or without choice was recorded for each predator species. Each test was repeated 4-5 times (4-5 consecutive days) with 20 predators per species per day. New odor sources were used for each replicate.

Preparation of odor sources. To investigate the avoidance of the three predator species, air passing through the arms of the Y-tube olfactometer originated from different odor sources that were prepared as follows:

Step 1. Two groups of four young cassava leaves each with 100 adult *M. tanajoa* females or two groups of 72 cassava apices (equivalent to four young leaves, see chapter 5) each with 5-6 adult *M. tanajoa* females were incubated for two days in plastic cages (length: 70 cm; width: 40 cm; height: 40 cm), with two fluorescent light sources above each cage at 26 ± 1°C. The control group consisting of 72 apices was kept clean of *M. tanajoa*.

Step 2. Two days after infestation with *M. tanajoa,* each group of four leaves was put in separate jars and was then connected simultaneously to the arms of the Y-tube, and 20 starved adult female predators (2 h-starved) were offered a choice between the two odor sources (i.e. infested cassava leaves versus non-infested cassava leaves) to test for differential attraction of the predators. We did not find a significant attraction to one of the two odor sources in any of these tests (data not shown). In the case of cassava apices, this step was not carried out to avoid loosing some mites from the initial number deposited.

Step 3. The same day after step 2, the cassava leaves with prey and their eggs were laid upside down on water-saturated cotton wool at the bottom of a plastic box. Leaves in contact with water-soaked cotton wool stayed fresh and the spider mites could not escape. For the cassava apices, the portion of the stem at the bottom part was placed in water-filled vials sealed with parafilm to keep the apices hydrated. Then, 20 adult female predators (5 adult females per leaf, regardless of the species) were added to the group of leaves that had attracted most predators in step 2. When the two groups of infested leaves attracted equal number of predators, the container that received the adult female predators was randomly selected. For apices (infested or non-infested), the container that received the predators was randomly selected since step 2 was omitted. The two odor sources consisting of leaves/apices with or without predators were kept overnight in two different cages as described in step 1.

Step 4. The next day (i.e. 3 days after infestation of leaves with prey and 1 day after adding predators to prey patches), the relative attractiveness of the two odor sources consisting of leaves/apices with adult female prey and prey eggs and leaves/apices with adult female prey, prey eggs, adult female predator and predator eggs was tested. Two hour-starved predators were used for the tests. The data discussed in this paper were obtained from this last step.

Statistics. We used the one-tailed probability of the binomial test to test the response of predator species to odors from prey patches with or without predators and the two-tailed probability in the case of preference between odors from both prey patches invaded by predators. We tested the difference between predator species with Chi-square analysis with Yates correction; data were arranged in a 2 x 2 contingency table (Zar, 1984). Numbers of adult female *M. tanajoa* and eggs in the prey patches used as odor sources were compared with a t-test.

Results

1. Response of predators to odors from leaves or apices with M. tanajoa plus T. aripo

When offered a choice between odors from leaves infested with *M. tanajoa* only and odors from leaves with *M. tanajoa* plus *T. aripo*, the majority of *T. manihoti*, *T. aripo* and *E. fustis* chose the leaves without *T. aripo* (Figure 1).
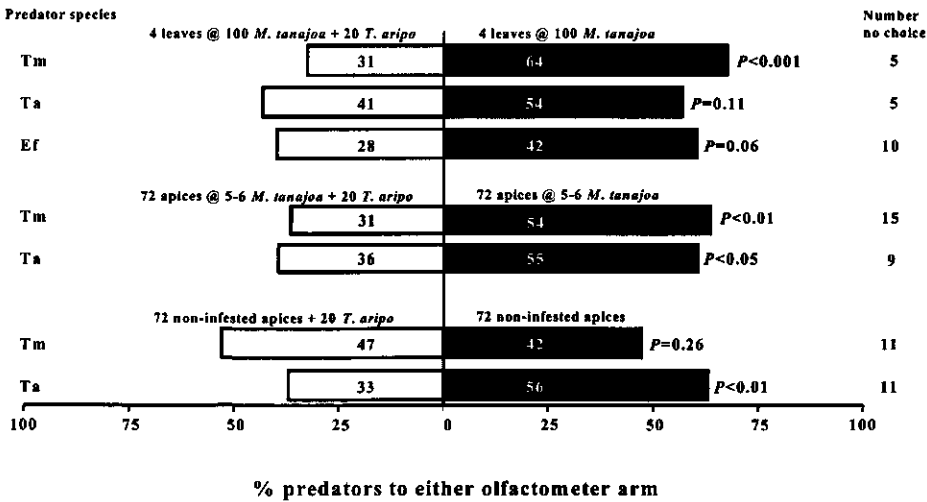

Figure 1. Response of 2h-starved predatory mites *T. manihoti* (Tm), *T. aripo* (Ta) and *E. fustis* (Ef) in an olfactometer when offered the choice between odors from leaves with *M. tanajoa* or apices either with or without *M. tanajoa* (right side of the bars) and leaves or apices with *M. tanajoa* and predatory mite *T. aripo* (left side of the bars). Numbers in the bar segments refer to overall number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

However, this was only statistically significant for *T. manihoti* ($P<0.001$). The numbers of adult female *M. tanajoa* and eggs on the leaves with *T. aripo* were not statistically different from those on leaves without *T. aripo* ($P>0.05$). In the case where infested apices were used, *T.aripo* and *T. manihoti* (*E. fustis* was not tested) significantly avoided the apices with *T. aripo* ($P<0.05$). The numbers of adult female *M. tanajoa* and eggs on the apices with *T. aripo* were not statistically different from those on apices without *T. aripo* ($P>0.05$). *Typhlodromalus aripo* also avoided non-infested apices with *T. aripo* when the alternative

was non-infested apices (*P*<0.01), whereas *T. manihoti* did not show a clear preference to non-infested apices with or without *T. aripo* (Figure 1).

2. Response of predators to odors from leaves with M. tanajoa plus T. manihoti

All three predator species, *T. aripo*, *T. manihoti* and *E. fustis*, avoided *M. tanajoa*-infested cassava leaves harboring *T. manihoti* when with the alternative was *M. tanajoa*-infested cassava leaves only (*P*<0.05) (Figure 2). The numbers of adult female *M. tanajoa* and eggs on the leaves with *T. manihoti* were not statistically different from those on leaves without *T. manihoti* (*P*>0.05).
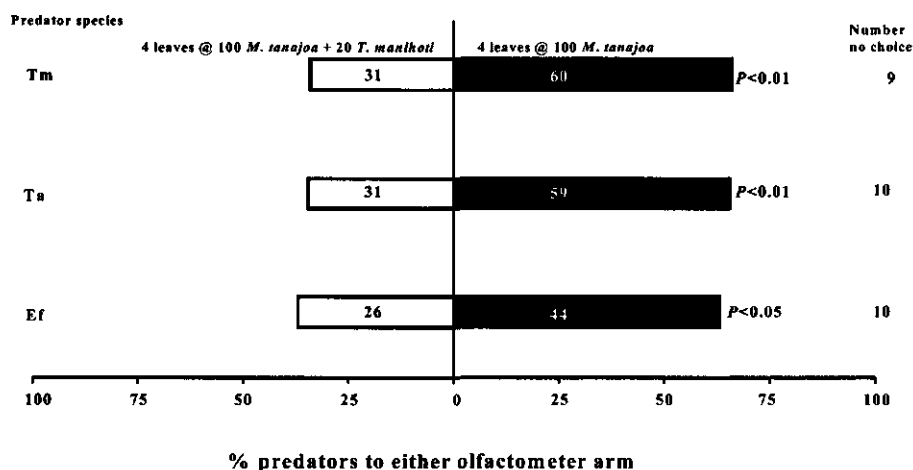

Figure 2. Response of 2h-starved predatory mites *T. manihoti* (Tm), *T. aripo* (Ta) and *E. fustis* (Ef) in an olfactometer when offered the choice between odors from leaves with *M. tanajoa* (right side of the bars) and leaves with *M. tanajoa* and predatory mite *T. manihoti* (left side of the bars). Numbers in the bar segments refer to overall number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

3. Response of predators to odors from leaves with either M. tanajoa plus T. aripo or M. tanajoa plus T. manihoti

When odors from two prey patches infested with *M. tanajoa* and occupied by *T. aripo* at one side and *T. manihoti* at the other, significantly more *T. manihoti* chose the patch with the heterospecific predator *T. aripo* (*P*<0.05); in contrast *T. aripo* distributed evenly over the two odor sources (Figure 3). The numbers of adult female *M. tanajoa* and eggs on the leaves with *T. manihoti* were not statistically different from those on leaves with *T. aripo* (*P*>0.05).
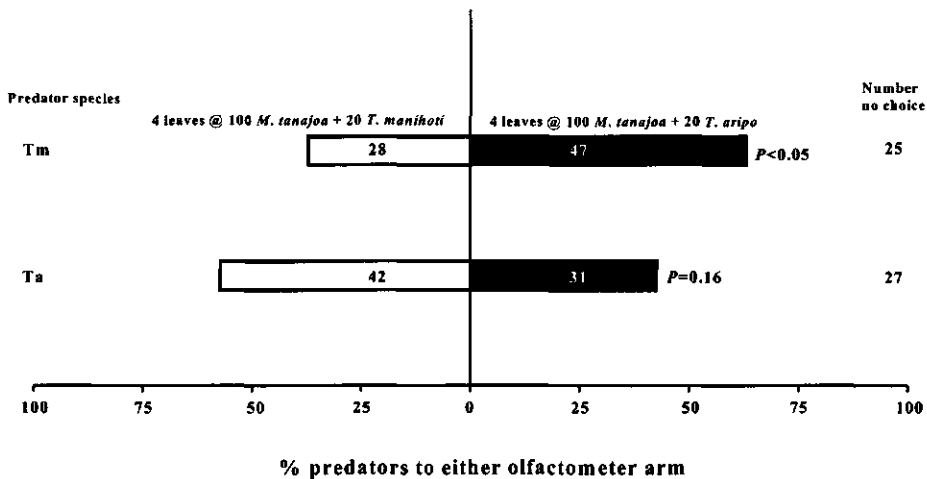

Figure 3. Response of 2h-starved predatory mites *T. manihoti* (Tm) and *T. aripo* (Ta) in an olfactometer when offered simultaneously the choice between odors from leaves with *M. tanajoa* and predatory mite *T. aripo* (right side of the bars) and leaves with *M. tanajoa* and predatory mite *T. manihoti* (left side of the bars). Numbers in the bar segments refer to overall number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

4. Response of predators to odors from leaves with M. tanajoa plus E. fustis

When the prey patch was colonized by the native predator *E. fustis*, all three predators tested, *T. manihoti*, *T. aripo* and *E. fustis*, significantly avoided the patch with *E. fustis* and preferred the odors from the control patch that was infested by *M. tanajoa* only (*P*<0.05) (Figure 4). The numbers of adult female *M. tanajoa* and eggs on the leaves with *E. fustis* were not statistically different from those on leaves without *E. fustis* (*P*>0.05).
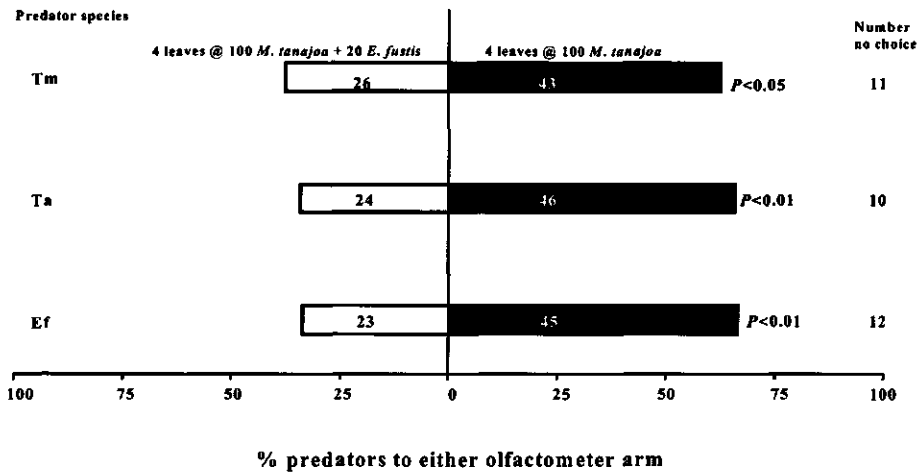
Figure 4. Response of 2h-starved predatory mites *T. manihoti* (Tm), *T. aripo* (Ta) and *E. fustis* (Ef) in an olfactometer when offered the choice between odors from leaves with *M. tanajoa* (right side of the bars) and leaves with *M. tanajoa* and predatory mite *E. fustis* (left side of the bars). Numbers in the bar segments refer to overall number of predators that chose either olfactometer arm. Probabilities on the right side of bars are for binomial test.

Discussion

Interspecific interactions

In pairwise interactions, *T. aripo* avoided patches colonized by the heterospecific predators *T. manihoti* and *E. fustis*. Similarly, *T. manihoti* avoided patches colonized by the heterospecific predators *T. aripo* and *E. fustis* whereas both odor sources had been equally attractive when only the prey was present (before addition of predators to one of them). However, *T. manihoti* preferred odors from infested leaves plus *T. aripo* to infested leaves plus conspecific predators. This behavior of *T. manihoti* indicates that it might rather prey on or compete with heterospecifics than conspecifics. The presence of a predator species in a prey patch greatly influences the attraction of another predator species to that prey patch. The latter frequently avoids encounters with the former (Schmidt *et al.*, 1998), although this is not always the case (Janssen *et al.*, 1999). The preference of predators for odors from prey patches without competitors over odors from prey patches with predators may be mediated by

volatiles from the predators, from the prey or from the host plant. Which mechanism mediates the observed responses in this case remains to be investigated thoroughly. Yet, *T. aripo* avoids odors from conspecifics on non-infested apices, which suggests that the cues are produced by the predators. However, in the same experiment *T. manihoti* did not discriminate between odors from apices with and without *T. aripo*. In all tests, the number of prey mites and prey eggs were similar for predator-invaded and predator-free prey patches. Thus, the olfactometer data could not have been influenced by significant differences in prey numbers and thus by differences in prey-induced plant odors. In another predator-prey system, there are strong indications that prey mites exposed to predators or their odors emit the volatiles that are avoided by conspecific predators (Janssen *et al.*, 1997). Whether this is the case here as well, still needs to be investigated.

Intraspecific interactions

Typhlodromalus aripo avoids conspecific predators on apices. In the case of conspecific predators on leaves, the majority of *T. aripo* went to leaves without *T. aripo* but the distribution was not significantly different from 50:50. Given that *T. aripo* aggregates in apices (Onzo *et al.*, in prep.), we had not expected to find that the predators avoid volatiles related to conspecifics on spider mite-infested cassava apices and presently it is hard to say why *T. aripo* shows such behavior. Under natural conditions, cassava apices with *T. aripo* are not infested with spider mites and it may be that the predators respond differently to odors from infested apices with conspecifics than to non-infested apices with conspecifics. Similarly, the predator *P. persimilis* is repelled by odors from a prey patch with conspecific predators while so far there is no evidence that cannibalism occurs in *T. aripo*. The study by Janssen *et al.*, 1997 shows a similar situation: *P. persimilis* moved away from conspecifics though it aggregated in prey patches to some extent. These authors reported that volatiles that result in avoidance by *P. persimilis* of leaves with conspecifics are not produced by the predators themselves but are probably produced by prey in response to presence of predators or in response to odors of predators. In our context, the presence of *M. tanajoa* is not essential for the avoidance response as it is also recorded when *T. aripo* is offered as odor source on non-infested cassava apices. Further research should investigate this surprising result in more details. *Typhlodromalus manihoti* and *E. fustis* do also avoid conspecific predators. Apparently, the phenomenon of conspecific avoidance is not an exception among predatory mites species and among parasitoids (Janssen *et al.*, 1995a,b; Geervliet et al. 1998).

Ecological consequenses

The behavioral patterns of the three predatory mite species cannot be evaluated without information on ecological consequences. When biological control agents are used to suppress a pest population, the introduction of more than two species often is considered to be favorable (Croft and MacRae, 1992a,b; Spiller and Schoener, 1994). If *T. aripo* and *T. manihoti* inhabit the same cassava plant and feed on a common prey species *M. tanajoa*, interspecific interactions such as those reported in the present study, could lead to competitive exclusion, suggesting that a potentially more effective predator species (i.e. *T. aripo*), is outcompeted by a better competitor (i.e. *T. manihoti*). As a consequence, competition for resources could affect the reproductive potential and survivorship of *T. aripo* and ultimately influence the population dynamics. Though mixed releases of *T. aripo* and *T. manihoti* result in competition and intraguild predation, niche segregation and differences in searching behavior on cassava plants (chapter 5) reduce overlap in resources use, which facilitates coexistence. Under natural conditions, mixed *T. aripo* and *T. manihoti* releases did not actually represent constraints on the efficacy of the control of *M. tanajoa* as reported by Onzo *et al.* (in prep.). These results indicate that the interactions between the two predatory mites species do not lead to less effective suppression of *M. tanajoa*.

Euseius fustis is the most common phytoseiid species found on cassava in Africa, and does better on pollen than on *M. tanajoa* (Bruce-Oliver *et al.*, 1996). It usually reaches highest densities when *M. tanajoa* is scarce or absent and when pollen appears to be more abundant (Bruce-Oliver *et al.*, 1996) and therefore, seems to be a good species to integrate in intraguild interactions with the two exotic predatory mites on cassava. This species is most likely to a show similar interaction pattern with each of the two exotic predator species. It avoided the two exotic predatory mites suggesting a reduction of competition leading to a better establishment and expansion of the exotic predators for an effective regulation of the key pest population.

Further research should put emphasis on how to use all three predator species together in the intraguild interactions instead of pairwise interactions as reported in the present study. We also suggest to vary the densities of predators and/or prey in prey patches and/or the starvation level of predators in future studies, to investigate to what extent density-dependence affects the intraguild interactions and competition among *E. fustis, T. aripo* and *T. manihoti*.

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7

General discussion

Arthropods are well known to exploit chemical information (Bell and Carde, 1984; Dicke and Sabelis, 1992). They may use chemical information from resources, from competitors and from enemies. In fact, there is an infochemical web superimposed on a food web (Dicke and Vet 1999; Sabelis *et al.*, 1999). Some components of such an infochemical web have been investigated here.

Plants have two major types of chemical defense against herbivores, i.e. direct and indirect defense. A direct chemical defense relates to the production of toxins or digestibility reducers which inhibit the growth of herbivores whereas indirect chemical defense relates to the emission of herbivore-induced plant volatiles that attract natural enemies of herbivores to a plant when attacked by herbivores (Dicke, 1998; reviewed in Dicke and Vet, 1999). The work reported in this thesis deals with indirect chemical defense of cassava plants through infochemically-mediated interactions in the food web. Recent studies have shown that the volatile blends emitted by a plant, are affected by herbivore damage (e.g. reviewed in Dicke, 1999). Volatile compounds produced by mechanically damaged plants may differ largely from those produced by herbivore-damaged plants in a qualitative way (Turlings *et al.*, 1990), while for other plant species the differences are mainly quantitative. Moreover, different herbivore species can induce different volatile blends, indicating that plant volatiles emitted from herbivore-damaged plants can be reliable indicators of herbivore presence and identity (Vet and Dicke, 1992; Takabayashi *et al.*, 1994). Carnivorous arthropods and parasitoids can discriminate among volatile blends when searching for their preferred prey or host (e.g. Sabelis and van de Baan, 1983; Agelopoulos and Keller, 1994; Du *et al.*, 1998; reviewed in Dicke, 1999), and prey preference can be linked to prey-related odor preference (Dicke and Groeneveld, 1986; Dicke, 1988). Several internal and external factors including food deprivation and presence of competitors are important in shaping foraging decisions of carnivorous arthropods (Janssen *et al.*, 1997, 1998; Krips *et al.*, 1999; reviewed in Dicke,

1999). When two arthropod predator species that belong to the same community share the same resources, they interact with each other, which may lead to the exclusion of the inferior competitor or to coexistence through resource partitioning. During the previous two decades, a wealth of studies has been conducted on the role of infochemicals in many plant-herbivore-predator/parasitoid systems (e.g. Vet and Dicke, 1992; Turlings *et al.*, 1995; Dicke, 1999; Dicke and Vet, 1999; Sabelis *et al.*, 1999), but relatively little was done on a system consisting of cassava plants, different spider mite and predatory mite species (but see Janssen *et al.*, 1990). Yet, information on other cassava-herbivore-carnivore interactions also exist (e.g. Bertschy *et al.*, 1997; Nadel and van Alphen, 1987; Souissi and Le Rü , 1998; van den Meiracker *et al.*, 1988).

When cassava, *Manihot esculenta* Crantz, plants are damaged by *Mononychellus tanajoa*, they emit volatile chemicals that attract the predatory mites *Typhlodromalus aripo* and *Typhlodromalus manihoti* (chapter 2). Several herbivores including the prey mite species *M. tanajoa*, *Oligonychus gossypii* and *Tetranychus urticae* are found in the cassava agroecosystem in Africa. Among these three prey mite species, *M. tanajoa* is the major pest of cassava (Yaninek *et al.*, 1989). In this study, I used two exotic biological control agents of this target pest, *T. manihoti* and *T. aripo* and the native pollen feeding predator species *Euseius fustis*.

The main objective of the research presented in this thesis was to assess how the two predatory mites *T. aripo* and *T. manihoti* use infochemicals emitted by the host plant-spider mite complex and reproduce on the three prey mite species *M. tanajoa*, *O. gossypii* and *T. urticae*. In addition, I studied how predators interact with each other and with the native predatory mite *E. fustis* on a single prey resource. I will discuss the role of *M. tanajoa*-induced cassava plant volatiles in the attraction of the two predatory mite species *T. aripo* and *T. manihoti*, followed by a discussion on how *T. aripo* and *T. manihoti* cope with and reproduce on different prey mite species. I end with a discussion of the strategies adopted by each of these predators in response to infochemicals related to conspecific and heterospecific predators.

M. tanajoa-induced cassava plant volatiles attract the predatory mites *T. aripo* and

T. manihoti

The point discussed here concerns the main source of volatiles used by *T. aripo* and *T. manihoti* during location of prey patches. The data show that *T. manihoti* and *T. aripo* are not attracted to adult female *M. tanajoa* in isolation from infested cassava plants (chapter 2), thus suggesting that *M. tanajoa* does not emit predator attractants in the absence of cassava plant. These data are similar to those reported by Sabelis and van de Baan (1983), and Sabelis *et al.* (1984): isolated *Tetranychus urticae* adult females from infested lima bean plants were not attractive to *P. persimilis* whereas lima bean plants infested by *T. urticae* strongly attracted *P. persimilis*. Mechanically wounded cassava leaves, when tested versus undamaged leaves, do not attract *T. manihoti* and *T. aripo* (chapter 2). However, when *M. tanajoa*-infested cassava leaves were offered opposite to non-infested leaves, the two predators were attracted to infested leaves, and food deprivation contributed to an increased olfactory response. Thus, the two predator species can detect nearby *M. tanajoa* patches when they are deprived of food for some hours. We show that cassava leaves infested by *M. tanajoa* are the main source of predator attractants, which in turn enable the predatory mites to locate *M. tanajoa* patches. Our data are similar to those obtained for many other plant-herbivore-carnivore systems where it has been demonstrated that plants are the emitters of predator attractants in response to herbivory. This relates to many plant species in different families (reviewed in Dicke, 1999), and most likely this is the case for cassava in response to damage by *M. tanajoa*.

Effects of different prey mite species on foraging behavior and reproductive success of the predatory mites *T. aripo* and *T. manihoti*

Feeding on prey by carnivorous arthropods in a patch cannot be considered a matter of chance. Predators are expected to be under selection pressure to feed on those prey that are most rewarding in terms of reproductive success. Predators are expected to prefer certain food types to others (Dicke *et al.*, 1989), resulting in a maximization of reproductive success. We asked whether life table parameters of *T. manihoti* and *T. aripo* when fed upon *M. tanajoa*, *O. gossypii* and *T. urticae* match their odor preference related to each of the three spider mite species. We hypothesized that prey-related odor preference is correlated with prey suitability. In chapter 4, we found that the type of food affects the female's developmental rate and fecundity. *Typhlodromalus aripo* preyed, reproduced and survived

on different spider mites such as *M. tanajoa, O. gossypii* and *T. urticae* whereas *T. manihoti* preyed, reproduced and survived on *M. tanajoa and O. gossypii* only. A rank-order hierarchy of prey suitability showed that *M. tanajoa* and *T. urticae* were more suitable prey than *O. gossypii* for *T. aripo,* while *M. tanajoa and O. gossypii* were more suitable than *T. urticae* for *T. manihoti.* *Mononychellus tanajoa* was, however, the most profitable prey for both predator species. Feeding on *M. tanajoa* resulted in faster development of pre-imaginal stages, reduction in adult female pre-ovipostion, oviposition and post-ovipositon periods, and therefore higher intrinsic rate of population increase of the predators (chapter 4). This may be caused by differences in nutritional value, quality between prey species, or consumption rates. The higher intrinsic rate of population increase is expected to lead to faster build-up of the predator population and likely rapid suppression of target prey populations.

How do predatory mites deal with information from cassava plants when infested simultaneously by key prey as well as inferior prey mites? The specificity of cues used by the predatory mites *T. manihoti* and *T. aripo* was investigated at long distance from the odor sources (chapter 3). This was studied for the same prey mites that were used in the life history analyses (chapter 4). *Typhlodromalus aripo* and *T. manihoti* were attracted to volatiles emitted from cassava leaves infested by their suitable prey *M. tanajoa,* but in comparable tests with leaves infested with either *O. gossypii* or *T. urticae* the predators were not attracted (chapters 2 and 3). An increase in the number of *O. gossypii* spider mites used for infestation resulted in attraction of *T. aripo,* but not *T. manihoti* (Chapter 3). When given a choice between volatiles from cassava leaves infested by *M. tanajoa* and cassava leaves infested by the inferior prey *O. gossypii,* the two predatory mite species preferred the volatiles emitted from *M. tanajoa*-infested cassava leaves to those from *O. gossypii*-infested leaves despite the higher densities of *O. gossypii* compared to those of *M. tanajoa.* When a choice was given between leaves infested with *M. tanajoa* and leaves infested with *T. urticae,* the majority of *T. aripo* and *T. manihoti* chose odors emitted from leaves infested with *M. tanajoa,* but this response was only significant when the density of *T. urticae* was only a quarter of that of *M. tanajoa.* Moreover, mixing odors from cassava leaves infested by *M. tanajoa* with odors from leaves infested by the inferior prey *O. gossypii* did not hamper the attraction by the two predator species. In contrast, when odors from leaves infested by *T. urticae* were mixed with odors from leaves infested with *M. tanajoa* this impeded the detection of the *M. tanajoa*-related odors. Overall, the results related to the reproductive success of predators fed different spider prey mite species, showed that *T. aripo* and *T.*

manihoti reared on *O. gossypii* and *T. urticae* have a lower reproductive success than conspecifics reared on *M. tanajoa* (chapter 4), and that cassava leaves infested by either *O. gossypii* and *T. urticae* were less attractive than those infested by *M. tanajoa* (chapter 3). This fits with our expectation that phytoseiids search for the most profitable prey species and use herbivore-induced volatiles for this. Therefore, when the two alternative prey mites are compared together with the key prey mite, prey-related odor preference and predator reproductive success are correlated.

In nature, *M. tanajoa* and *O. gossypii* can be found together on the same plant: *M. tanajoa* on foliage in the upper half of the plant and *O. gossypii* on foliage in the bottom half but they can also occur together on the same leaves. Thus, based on the current results, the presence of *M. tanajoa* in the same patch with *O. gossypii* would attract *T. manihoti* and *T. aripo* and may enhance predation on *O. gossypii*. Furthermore, cassava infested by *O. gossypii* may enhance the effectiveness of the two predator species in controlling *M. tanajoa* populations but we still need experiments in semi-natural conditions to support this hypothesis. When volatiles from *T. urticae*-infested cassava leaves were mixed with volatiles from *M. tanajoa*-infested leaves, the presence of *T. urticae* prevented *M. tanajoa* from being attacked by its natural enemies *T. manihoti* and *T. aripo*. *Tetranychus urticae* occasionally occurs on cassava but mostly feeds on alternative host plants generally found in the cassava agroecosystem. Based also on our results, *T. urticae* feeding on the same or nearby cassava plants with *M. tanajoa* may result in production of non-attractive volatile blends reaching the predators, which in turn reduce *T. aripo*'s and *T. manihoti*'s ability to efficiently locate patches of their main prey *M. tanajoa*. The extent to which this interference may affect *T. aripo*'s and *T. manihoti*'s efficiency on *M. tanajoa* requires further testing.

Infochemicals involved in intraguild interactions and niche segregation between *T. aripo* and *T. manihoti*

In their native range in the Neotropics, *T. manihoti* and *T. aripo* forage on different parts of the cassava plants: *T. manihoti* on young and old leaves whereas *T. aripo* foraged on apex (Bakker, 1993). It has been shown recently, however, that *T. aripo* forages also on young cassava leaves, but mostly during the evening and night hours while remaining in the cassava apex during the daylight hours (Onzo *et al.*, in prep.). Why do the two predators behave in this way? This is what I will discuss here. When cassava plant parts (i.e. apex, young or old

leaves) were infested by *M. tanajoa*, volatiles emitted by the different plant parts evoke different behavioral responses in the two predatory mite species: *T. aripo* preferred volatiles from apices and young leaves over old leaves infested by *M. tanajoa* whereas *T. manihoti* did not discriminate between volatiles from the three different cassava plant parts (chapter 5). The preference for young over old cassava plant tissues exhibited by *T. aripo* may be explained by intraguild interactions with *T. manihoti*. The two predators *T. aripo* and *T. manihoti* avoided each other based on volatiles from predator-invaded prey patches. However, when one patch contained *T. aripo* and the other *T. manihoti*, *T. manihoti* preferred volatiles from the patch invaded with *T. aripo*. Thus, *T. manihoti* is able to discriminate between odors from prey patches with con- and heterospecific predators (chapter 6). This suggests the existence of asymmetrical intraguild interactions between the two predators, where *T. manihoti* is the intraguild predator and *T. aripo* is the intraguild prey. Our data support those from greenhouse experiments that a population of juveniles of *T. aripo* was significantly reduced in a mixed treatment (i.e. *T. aripo* plus *T. manihoti*) compared to a treatment of only *T. aripo* under conditions of high predator: prey ratios (Onzo *et al.*, in prep.). This suggests a possible predation on juveniles of *T. aripo* by adult females of *T. manihoti*. Predation preference between con- and heterospecific intraguild prey by predatory mites has been investigated in various systems (Schausberger, 1997, 1999; Walzer and Schausberger, 1999). It might be the pressure of inter-specific competition and asymmetrical intraguild predation in the past between the two predators, that is the source of the differential selection of space or niche within cassava plant foliage (chapter 5) and *T. aripo* is likely to use volatiles from young *M. tanajoa*-infested cassava plant parts to identify the most suitable parts like the apex. *Typhodromalus aripo* uses the structure of the apex, which may provide protection against competition and intraguild predation by *T. manihoti* and also against adverse weather conditions. For the intraguild prey, the preferred foraging location should therefore be the one that provides the optimal balance between foraging returns and risk of mortality through predation (Metcalfe *et al.*, 1999). In addition to restriction of its niche (chapter 5), *T. aripo's* time allocation allows it to share resources (Onzo *et al.*, in prep.) with its competitor *T. manihoti* that can also be an intraguild predator. *Typhlodromalus aripo* is present in the apex during the day and goes down to forage on the upper leaves of the plant during the night (Onzo *et al.*, in prep.). Alternating utilization of food or energy within a habitat is a form of coexistence in animal communities (Boughey, 1973). Many animals including the scorpion *Buthus occitanus* Israelis (Skutelsky, 1996), the lizard *Lacerta monticola* Boulenger (Martín and López, 1999), and the freshwater fish *Phoxinus phoxinus* L.

(Metcalfe and Steele, 2001) modify their microhabitat or refuge use according to levels of predation risks (reviewed in Dicke and Grostal, 2001).

In conclusion, this thesis has shown that infochemicals are involved in the location of suitable prey and in intraguild interactions that mediate coexistence of the predator species *T. aripo* and *T. manihoti.* The research presented has led to a better understanding of the biology and ecology of these two exotic predatory mites that are used to control the cassava green mite in Africa (Yaninek *et al.*, 2001).

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Summary

In carnivorous arthropod communities, volatile infochemicals are of great importance in prey location and prey patch profitability. In **chapter 2** the attraction of the two predatory mites *Typhlodromalus manihoti* and *Typhlodromalus aripo* to volatile cues emitted by the host plant-spider mite complex, *Manihot esculenta – Mononychellus tanajoa*, was investigated with a Y-tube olfactometer. Our hypothesis was that *M. tanajoa* damage induces volatile cues in cassava leaves that attract *T. manihoti* and *T. aripo*. We found that females of *T. manihoti* and *T. aripo* significantly preferred odors from *M. tanajoa*-infested cassava leaves to those from non-infested leaves when they were starved for 2, 6 or 10 hours. When a choice was given between non-infested cassava leaves and either infested leaves from which *M. tanajoa* females had been removed, or infested leaves from which all *M. tanajoa* and their visible products (web, feces) had been wiped off, *T. aripo* preferred odors from both types of previously infested leaves. *Typhlodromalus manihoti* was attracted to infested leaves from which *M. tanajoa* females only had been removed. The two predators were not attracted to odors from 400 female *M. tanajoa* removed from infested plants nor to odors from mechanically wounded leaves.

In addition to the key prey species, two alternative prey mite species, *Oligonychus gossypii* and *Tetranychus urticae* also occur in the cassava agroecosystem in Africa. Different prey species are likely to infest the same cassava plant or even co-occur on the same cassava leaf. Once we know that the two predator species use spider mite-induced cassava volatiles during distant prey location and that they are attracted to odors from *M. tanajoa*-infested cassava leaves, an important question is how the two predatory mites cope with a complex of odors from plants infested with different herbivore species. This is addressed in **chapter 3**. We tested prey-related odor preference of both predatory mites either when offered a choice between cassava leaves infested with *M. tanajoa* or *O. gossypii* or when offered leaves infested with *M. tanajoa* or *T. urticae*, using a Y-tube olfactometer. We found that when *T. manihoti* and *T. aripo* were offered a choice between four non-infested leaves and four *O. gossypii*-infested leaves (100 adult females per leaf) neither of the predators was significantly attracted to odors from *O. gossypii*-infested leaves; similar attraction was found when the predators were offered *T. urticae*-infested leaves versus non-

infested leaves. However, when *M. tanajoa*-infested leaves and *O. gossypii*-infested leaves were offered simultaneously in a choice situation, *T. manihoti* and *T. aripo* preferred odors from *M. tanajoa*-infested leaves to those of *O. gossypii*-infested leaves regardless of the ratio of *M. tanajoa:O. gossypii* (e.g. 100:100; 200:100; 100:200 and 50:200). When *M. tanajoa*-infested leaves and *T. urticae*-infested leaves were offered simultaneously in a choice situation, the response of the two predators was dependent on the density of *T. urticae*. *Typhlodromalus manihoti* and *T. aripo* were attracted to odors from cassava leaves infested with both *M. tanajoa* and *O. gossypii* or to a mixture of odors from leaves infested with *M. tanajoa* and odors from leaves infested with *O. gossypii*, when compared to odors from non-infested leaves. In contrast, mixed odors from *M. tanajoa*-infested leaves and *T. urticae*-infested leaves did not yield a preference over odors from non-infested leaves.

The performance of *Typhlodromalus manihoti* and *T. aripo* feeding on the three herbivorous mite species was investigated and the results were compared to those on prey-related odor preference **(chapter 4)**. Developmental time (egg-adult) of *T. aripo* was shorter on *M. tanajoa* than on *T. urticae*, while an intermediate value was recorded for *O. gossypii*. For *T. manihoti*, developmental rate was faster on *M. tanajoa* than on *O. gossypii* and not successful on *T. urticae*. The survival rates of *T. manihoti* or *T. aripo* populations reared on the three prey species were not significantly different. For both predatory mites, the results yielded a higher intrinsic rate of population increase (r_m) and net reproduction (R_o), and a shorter generation time and doubling time on *M. tanajoa* than on *O. gossypii* and *T. urticae*. Furthermore, *T. manihoti* developed faster than *T. aripo* on either *M. tanajoa* or *O. gossypii*. Consequently, female longevity of *T. aripo* was longer than that of *T. manihoti* on these prey mite species. Based on the life table results, the rank-order hierarchy of prey suitability for each of the two predator species was as follows: *M. tanajoa* > *T. urticae* > *O. gossypii* for *T. aripo* whereas it was *M. tanajoa* > *O. gossypii* > *T. urticae* for *T. manihoti*. Prey-related odor preference matches with predator performance when the key prey is compared to the two inferior prey mite species.

Niche use by the two predators is a vital component in their evolutionary ecology. In Africa, *T. manihoti* and *T. aripo* inhabit different parts of cassava foliage. In the study reported in **chapter 5**, niche segregation by *T. manihoti* and *T. aripo* was investigated in the laboratory with an olfactometer. In response to prey feeding damage, plant tissues of different age emit volatile blends, which act as attractants for predators. *Typhlodromalus aripo*

displayed a preference for odors emitted from either *M. tanajoa*-infested apices or *M. tanajoa*-infested young leaves over *M. tanajoa*-infested old leaves, but showed equal preference for odors from infested apices and infested young leaves. *Typhlodromalus manihoti* did not discriminate between volatiles from the three *M. tanajoa*-infested cassava plant parts. The niches of the two predator species were thus partially segregated. The distribution of prey species, intraguild predation and competition are likely to play an important role in this. This suggests that the predator *T. aripo* uses differences in volatile blends released by infested cassava plant parts to restrict its fundamental niche to a realized niche, which enables coexistence with *T. manihoti*.

Carnivorous arthropods when searching for adequate food and habitat for themselves and their progeny should in the meantime avoid becoming food for other organisms. We examined by means of an olfactometer (**chapter 6**) how volatile infochemicals from prey patches with presence of competitors affect the behavior of predatory mites. We used the exotic predatory mites *T. manihoti* and *T. aripo* and the native species *Euseius fustis*. *Mononychellus tanajoa* is a common prey species for *T. manihoti* and *T. aripo* and to a much lesser extent for *E. fustis*. The results showed that the three predatory mite species are able to assess prey patch profitability. Both on leaves and apices, *T. manihoti* avoided *T. aripo*, but when a choice was offered between a patch occupied by *T. aripo* and another occupied by *T. manihoti*, *T. manihoti* preferred odors from patch with *T. aripo*. Thus, *T. manihoti* discriminates between odors from patches with con- and heterospecific competitors and prefers to visit patches with heterospecifics. *Typhlodromalus aripo* also prefers to move away from patches with an aggregation of conspecifics. *Euseius fustis* also avoided odors from patches with conspecifics or each of the heterospecifics *T. manihoti* and *T. aripo*. Under natural conditions, the three predatory mites may be involved in interspecific and/or intraspecific interactions when food becomes scarce. These results indicate that in addition to predator-prey interactions, interactions between predators should also be considered as main factors affecting population dynamics of predator and prey.

In **chapter 7**, all studied aspects in this thesis are summarized and discussed. The conclusion is that (i) volatiles from cassava infested with the key prey are important in distant prey location by *T. manihoti* and *T. aripo*, (ii) different combinations of prey species can affect interactions with predators in the cassava agroecosystem, (iii) *T. aripo* and *T. manihoti* perfom better on *M. tanajoa* than on *O. gossypii* or *T. urticae*, (iv) *T. aripo* can coexist with

T. manihoti through niche segregation and (v) presence of the native predator *E. fustis* in the cassava agroecosystem does not most likely interfere in the foraging process of the exotic predators.

Résumé

Dans les communautés d'arthropodes carnivores, les informations chimiques volatiles sont d'une importance capitale dans la localisation des proies et dans l'appréciation du profit potentiel des zones de proie. Dans le **chapitre 2**, nos investigations ont porté sur l'attraction des deux espèces d'acariens prédateurs, que sont *Typhlodromalus manihoti* et *Typhlodromalus aripo*, par les signaux volatils émis par le complexe plante hôte-acarien phytophage à savoir plant de manioc (*Manihot esculenta*)-acarien phytophage (*Mononychellus tanajoa*). Pour ce faire, nous nous sommes servis d'un olfactomètre en forme de tube en Y. Notre hypothèse de départ était que les dégâts causés par *M. tanajoa* induisent, au niveau des feuilles de manioc, des signaux volatils qui attirent *T. manihoti* et *T. aripo*. Les résultats obtenus indiquent que lorsque ces deux espèces d'acariens prédateurs ont été affamées pendant une durée de 2, 6 ou 10 heures, les femelles de *T. manihoti* et de *T. aripo* préféraient de façon significative les odeurs émanant des feuilles de manioc infestées avec *M. tanajoa* à celles émises par les feuilles de manioc non-infestées. Lorsqu'il leur était permis de choisir entre les feuilles de manioc non-infestées et celles infestées mais d'où l'on a retiré les femelles de *M. tanajoa* ou bien les feuilles infestées mais d'où l'on a retiré aussi bien les femelles de *M. tanajoa* et leurs œufs ainsi que tous les débris visibles (excréments et tissages), les femelles de *T. aripo* préféraient les odeurs provenant des deux variantes de feuilles de manioc préalablement infestées. Les femelles de *T. manihoti* n'étaient attirées que par les feuilles préalablement infestées mais d'où l'on n'a retiré que les femelles de *M. tanajoa*. Aucune des deux espèces de prédateurs n'était attirée ni par les odeurs émises par 400 femelles *M. tanajoa* isolées des feuilles de manioc infestées ni par celles émises par des feuilles mécaniquement abîmées.

En plus de l'espèce constituant la proie clé pour les deux espèces d'acariens prédateurs *T. manihoti* et *T. aripo*, deux autres espèces d'acariens phytophages qui sont des proies alternatives sont aussi rencontrées dans l'agroécosystème du manioc en Afrique. Il s'agit de *Oligonychus gossypii* et de *Tetranychus urticae*. Plusieurs espèces de proies peuvent donc infester le même plant de manioc ou mieux la même feuille de manioc. Dès lors qu'il est connu que les deux espèces d'acariens prédateurs utilisent des signaux volatils induits par l'acarien phytophage au niveau des feuilles de manioc pour le repérage à distance de leur

proie et qu'elles sont attirées par les odeurs provenant des feuilles de manioc infestées par *M. tanajoa*, la question fondamentale qui se pose est de savoir comment ces acariens prédateurs font face au complexe d'odeurs émanant de plants infestés par diverses espèces d'acariens phytophages. Le **chapitre 3** porte sur ce sujet. Nous avons testé à l'aide d'un olfactomètre, la préférence des deux acariens prédateurs quant aux substances volatiles lorsque ces derniers étaient soumis soit aux odeurs provenant des feuilles de manioc infestées avec *M. tanajoa* ou *O. gossypii*, ou des feuilles infestées avec *M. tanajoa* ou *T. urticae*. Nous avons observé que lorsque *T. manihoti* et *T. aripo* avaient le choix entre quatre feuilles de manioc non-infestées et quatre autres infestées avec *O. gossypii* (à raison de 100 femelles adultes par feuille), aucun des deux prédateurs n'était attiré de façon significative par les odeurs provenant de feuilles de manioc infestées avec *O. gossypii*. Un résultat similaire a été obtenu avec les odeurs provenant de feuilles de manioc infestées avec *T. urticae*. Néanmoins, lorsque des feuilles de manioc infestées avec *M. tanajoa* et celles infestées avec *O. gossypii* étaient simultanément offertes, *T. manihoti* et *T. aripo* avaient préféré les odeurs provenant des feuilles infestées avec *M. tanajoa* à celles provenant des feuilles infestées avec *O. gossypii*, indépendamment du ratio *M. tanajoa : O. gossypii* (e.g. 100 : 100 ; 200 : 100 ; 100 : 200 et 50 : 200). Lorsque des feuilles de manioc infestées avec *M. tanajoa* et celles infestées avec *T. urticae* étaient simultanément offertes, la réponse des deux acariens prédateurs était fonction de la densité de *T. urticae*. Les femelles de *T. manihoti* et *T. aripo* étaient attirées par les odeurs provenant de feuilles de manioc infestées à la fois avec *M. tanajoa* et *O. gossypii* ou par un mélange d'odeurs provenant de feuilles infestées avec *M. tanajoa* et d'odeurs provenant de feuilles infestées avec *O. gossypii* en comparaison avec des odeurs provenant de feuilles de manioc non-infestées. Par contre, un mélange d'odeurs provenant de feuilles infestées avec *M. tanajoa* et de feuilles infestées avec *T. urticae* n'avait suscité chez les prédateurs aucune préférence par rapport aux odeurs émanant de feuilles non-infestées.

L'aptitude de *T. manihoti* et de *T. aripo* à s'alimenter des trois espèces d'acariens herbivores a été évaluée et les résultats comparés à ceux des investigations sur la préférence aux odeurs liées à la proie (**chapitre 4**). La durée de développement (œuf-adulte) de *T. aripo* était plus courte sur *M. tanajoa* que sur *T. urticae*, alors qu'une valeur intermédiaire avait été enregistrée avec *O. gossypii*. Avec *T. manihoti*, le développement était plus rapide sur *M. tanajoa* que sur *O. gossypii* et nul sur *T. urticae*. Les taux de survie des populations de *T. manihoti* ou de *T. aripo* nourries avec les trois espèces de proies n'étaient pas significativement différents. Pour les deux espèces de prédateurs, les résultats ont abouti à un taux d'accroissement intrinsèque de la population (r_m) et un taux net de reproduction (R_o)

plus élevés et des temps de génération ou de doublement plus courts sur *M. tanajoa* que sur *O. gossypii*. En outre, *T. manihoti* s'était développé plus rapidement que *T. aripo*, aussi bien sur *M. tanajoa* que sur *O. gossypii*. En conséquence, la longévité des femelles de *T. aripo* était nettement supérieure à celle des femelles de *T. manihoti* sur ces deux espèces d'acariens proies. En se basant sur les résultats de la table de vie, l'ordre de classement hiérarchisé des proies par rapport à leur convenance pour chacune des deux espèces de prédateurs s'établissait comme suit: *M. tanajoa* > *T. urticae* > *O. gossypii* pour *T. aripo* et *M. tanajoa* > *O. gossypii* > *T. urticae* pour *T. manihoti*. La préférence pour les odeurs liées à la proie va de pair avec la performance des prédateurs lorsque l'on compare la proie clé avec les deux autres espèces d'acariens phytophages considérées comme proies alternatives.

L'usage des niches par les deux acariens prédateurs est une composante vitale de leur écologie évolutive. En Afrique, *T. manihoti* et *T. aripo* habitent différentes parties du feuillage sur le plant du manioc. Dans le **chapitre 5**, la séparation de niche entre *T. manihoti* et *T. aripo* a été examinée au laboratoire au moyen d'un olfactomètre. En réponse aux dégâts causés par l'alimentation des acariens proies, les tissus végétaux de différents âges émettent un ensemble de substances volatiles qui exercent une attraction sur les prédateurs. *Typhlodromalus aripo* a montré une préférence pour les odeurs émises soit par les apex de manioc infestés, soit par les jeunes feuilles de manioc infestées avec *M. tanajoa* par rapport aux vieilles feuilles infestées avec *M. tanajoa*. Traité dans les mêmes conditions, *Typhlodromalus manihoti* n'a fait aucune différence entre les volatiles provenant des trois différentes parties du feuillage du plant de manioc infestées avec *M. tanajoa*. Les niches des deux espèces de prédateurs sont, ainsi donc, partiellement séparées. La répartition des espèces de proie, la prédation intra-guilde et la compétition jouent vraisemblablement un rôle important dans cette ségrégation de niche. Ceci suggère que le prédateur *T. aripo* utilise les différences entre les mélanges de volatiles émis par les différentes parties végétatives infestées du plant de manioc pour réduire sa niche fondamentale; ce qui probablement rend possible la coexistence avec *T. manihoti*.

Lorsque les arthropodes carnivores sont à la recherche de proie ou d'un habitat propice pour eux-mêmes et pour leur progéniture, ils devraient au même moment éviter de devenir une proie pour d'autres organismes. Nous avons examiné au moyen d'un olfactomètre (**chapitre 6**), comment les informations chimiques volatiles provenant des zones infestées par des proies dans lesquelles se trouvent simultanément des compétiteurs affectent le comportement des acariens prédateurs. Pour cette étude nous avions utilisé les acariens prédateurs exotiques que sont *T. manihoti* et *T. aripo* et l'espèce indigène *Euseius fustis*.

Mononychellus tanajoa est une proie commune pour *T. manihoti* et *T. aripo* mais il l'est bien moins pour *E. fustis*. Les résultats ont montré que toutes les trois espèces étaient capables d'évaluer le profit potentiel de la zone infestée par les proies. Aussi bien sur les feuilles que dans les apex, *T. manihoti* évitait *T. aripo*, mais lorsque le choix était permis entre une zone occupée par *T. aripo* et une autre par *T. manihoti*, *T. manihoti* avait préféré les odeurs provenant de la zone occupée par *T. aripo*. Ainsi, *T. manihoti* fait la différence entre les odeurs provenant des zones avec un compétiteur d'une même espèce et d'une espèce différente et préfère visiter une zone où se trouve une autre espèce. *Typhlodromalus aripo* a aussi préféré fuir les zones comportant un agrégat d'individus appartenant à son espèce. *Euseius fustis* avait, lui aussi, évité les odeurs provenant des zones occupées par des individus de son espèce ou des individus de chacune des autres espèces *T. manihoti* ou *T. aripo*. Dans les conditions naturelles, les trois espèces pourraient être impliquées dans des interactions interspécifiques et/ou intraspécifiques lorsque la nourriture devient rare. Ces résultats indiquent qu'en plus des interactions entre prédateur et proie, les interactions entre prédateurs devraient être prises en considération comme étant des facteurs pouvant influer la dynamique de population du prédateur et de la proie.

Dans le **chapitre 7** se trouvent résumés et discutés tous les aspects étudiés dans cette thèse. La conclusion est que (i) les substances volatiles provenant des feuilles de manioc infestées avec la proie clé sont très importantes dans la localisation à distance de la proie par *T. manihoti* et *T. aripo*; (ii) les diverses combinaisons d'espèces de proie peuvent influer sur les interactions avec les prédateurs dans l'agroécosystème du manioc; (iii) *T. aripo* et *T. manihoti* sont plus performants sur *M. tanajoa* que sur *O. gossypii* et *T. urticae*; (iv) *T. aripo* peut coexister avec *T. manihoti* à travers une ségrégation des niches et (v) la présence de l'espèce de prédateur indigène *E. fustis* dans l'agroécosystème du manioc n'a vraisemblablement pas d'interférence avec le processus de recherche de proie par les espèces exotiques de prédateurs.

Samenvatting

Vluchtige informatiestoffen zijn voor carnivore arthropoden van groot belang voor het lokaliseren van prooien en het bepalen van prooihaard geschiktheid. Het onderwerp van **hoofdstuk 2** is aantrekking van twee roofmijtsoorten, *Typhlodromalus manihoti* en *Typhlodromalus aripo*, tot geurstoffen afgegeven door het waardplant-spintmijt complex: *Manihot esculenta – Mononychellus tanajoa*. Deze aantrekking werd bestudeerd met behulp van een Y-buis olfactometer. Onze hypothese was dat beschadiging door *M. tanajoa* de productie van vluchtige stoffen in cassavebladeren induceert en dat deze vluchtige stoffen aantrekkelijk zijn voor de roofmijten *T. manihoti* en *T. aripo*. We vonden dat *T. manihoti* en *T. aripo* vrouwtjes (gehongerd gedurende 2, 6 of 10 uur) een significante voorkeur hadden voor geuren van door *M. tanajoa* beschadigde cassavebladeren ten opzichte van onbeschadigde bladeren. Wanneer een keuze aangeboden werd tussen onbeschadigde cassavebladeren en beschadigde bladeren waarvan de *M. tanajoa* vrouwtjes verwijderd waren of beschadigde bladeren waar de *M. tanajoa* vrouwtjes en hun zichtbare producten (spinsel en uitwerpselen) afgeveegd waren, dan had *T. aripo* een voorkeur voor geuren van beide typen voorheen beschadigd blad. *Typhlodromalus manihoti* werd aangetrokken door beschadigde bladeren waarvan alleen de *M. tanajoa* vrouwtjes verwijderd waren. De twee roofmijtsoorten werden niet aangetrokken door geuren van 400 *M. tanajoa* vrouwtjes of geuren van mechanisch beschadigde cassavebladeren.

Naast de belangrijkste prooisoort komen twee alternatieve prooisoorten voor in het cassave agro-ecosysteem: de spintmijten *Oligonychus gossypii* en *Tetranychus urticae*. De verschillende prooisoorten kunnen op dezelfde plant of zelfs op hetzelfde cassaveblad voorkomen. In hoofdstuk 2 wordt aangetoond dat de twee roofmijtsoorten spintmijt-geïnduceerde plantengeurstoffen gebruiken om hun prooi van een afstand te lokaliseren en aangetrokken worden tot geuren van door *M. tanajoa* beschadigde cassavebladeren. Een belangrijke vraag is hoe *T. manihoti* en *T. aripo* omgaan met een complex van geuren van planten die beschadigd zijn door de verschillende spintmijtsoorten. Deze vraag komt in **hoofdstuk 3** aan de orde. Met behulp van een Y-buis olfactometer hebben we getest of beide roofmijtsoorten een voorkeur hebben voor cassavegeuren die geïnduceerd zijn door verschillende spintmijtsoorten. Roofmijten kregen de keuze uit cassavebladeren beschadigd door *M. tanajoa* of *O. gossypii* of de keuze uit bladeren beschadigd door *M. tanajoa* of *T.*

urticae. Als *T. manihoti* en *T. aripo* de keuze kregen uit vier onbeschadigde bladeren en vier door *O. gossypii* beschadigde cassavebladeren (100 volwassen vrouwtjes per blad), dan werden de roofmijten niet significant aangetrokken door de bladeren beschadigd door *O. gossypii*. In een vergelijkbare keuze-situatie maakten beide roofmijtsoorten ook geen onderscheid tussen door *T. urticae* beschadigde bladeren en onbeschadigde bladeren. Als de roofmijten echter de keuze kregen uit door *M. tanajoa* beschadigde en door *O. gossypii* beschadigde bladeren, dan hadden zowel *T. manihoti* en *T. aripo* voorkeur voor de bladeren die beschadigd waren door *M. tanajoa*. Deze voorkeur werd gevonden onafhankelijk van de verhouding tussen *M. tanajoa* en *O. gossypii* (b.v. 100:100; 200:100; 100:200 en 50:200). Als *M. tanajoa* beschadigde en *T. urticae* beschadigde bladeren werden aangeboden in een keuze-situatie was de voorkeur van beide roofmijtsoorten afhankelijk van de dichtheid van *T. urticae*. *Typhlodromalus manihoti* en *T. aripo* werden aangetrokken tot geuren van cassave-bladeren die door zowel *M. tanajoa* als *O. gossypii* beschadigd waren. Beide roofmijtsoorten werden ook aangetrokken tot geuren afkomstig van door *M. tanajoa* beschadigde bladeren gemengd met bladeren beschadigd door *O. gossypii*, als deze getest werden tegen de geuren van onbeschadigde bladeren. Anderzijds was een mengsel van geuren van *M. tanajoa* beschadigde bladeren en *T. urticae* beschadigde bladeren niet aantrekkelijker dan de geur van onbeschadigde cassavebladeren.

De prestatie van *Typhlodromalus manihoti* en *T. aripo* als ze zich voeden met de drie verschillende spintmijtsoorten werd onderzocht en vergeleken met de prooi-gerelateerde voorkeur voor geuren (**hoofdstuk 4**). De ontwikkelingstijd (ei tot adult) van *T. aripo* was korter op *M. tanajoa* dan op *T. urticae* en had een tussenliggende waarde op *O. gossypii*. *Typhlodromalus manihoti* had een hogere ontwikkelingssnelheid op *M. tanajoa* dan op *O. gossypii* en ontwikkeling op *T. urticae* was niet succesvol. De overleving van *T. manihoti* en *T. aripo* populaties gekweekt op de verschillende prooisoorten was niet significant verschillend. Voor beide roofmijtsoorten werd een hogere intrinsieke populatie-groeisnelheid (r_m) en netto reproductie (R_o) op *M. tanajoa* dan op *O. gossypii* en *T. urticae* gevonden. De generatietijd en populatie-verdubbelingstijd waren korter op *M. tanajoa* dan op *O. gossypii* en *T. urticae*. Verder ontwikkelde *T. manihoti* sneller dan *T. aripo* op zowel *M. tanajoa* als *O. gossypii* als prooi. De levensduur van *T. aripo* vrouwtjes was echter langer dan die van *T. manihoti* op deze prooimijtsoorten. De geschiktheids-hiërarchie van de verschillende prooisoorten werd gebaseerd op de levensgeschiedenistabellen. Voor *T. aripo* is deze hiërarchie: *M. tanajoa* > *T. urticae* > *O. gossypii* en voor *T. manihoti M. tanajoa* > *O. gossypii* > *T. urticae*. De prooi-gerelateerde voorkeur voor vluchtige stoffen komt overeen

met de hiërarchie in reproductief succes van de roofmijten wanneer de belangrijkste prooisoort vergeleken wordt met de inferieure prooisoorten.

Niche-gebruik door de twee roofmijtsoorten is een belangrijke component in evolutionair ecologisch opzicht. In Afrika komen *T. manihoti* en *T. aripo* op verschillende delen van de cassaveplant voor. In het onderzoek dat in **hoofdstuk 5** beschreven staat is niche-scheiding door *T. manihoti* en *T. aripo* onderzocht in het laboratorium met behulp van een olfactometer. In reactie op beschadiging door herbivoren produceren plantenweefsels van verschillende leeftijd vluchtige stoffen die aantrekkelijk zijn voor predatoren. *Typhlodromalus aripo* had een voorkeur voor geuren van door *M. tanajoa* beschadigde apices of door *M. tanajoa* beschadigde jonge bladeren wanneer deze getest werden tegen door *M. tanajoa* beschadigde oude cassavebladeren. *Typhlodromalus manihoti* maakte geen onderscheid tussen de vluchtige stoffen van deze drie delen van de cassaveplant wanneer deze beschadigd waren door *M. tanajoa*. De niches van de twee roofmijtsoorten waren dus gedeeltelijk gescheiden. De verdeling van de verschillende prooimijtsoorten, intragilde predatie en competitie spelen hierbij waarschijnlijk een belangrijke rol. Dit suggereert dat *T. aripo* verschillen in de mengsels van vluchtige stoffen afgegeven door de verschillende delen van de beschadigde plant gebruikt om de fundamentele niche te beperken tot een gerealiseerde niche. Deze niche-beperking maakt het samen voorkomen met *T. manihoti* mogelijk.

Wanneer carnivore arthropoden op zoek zijn naar geschikt voedsel en een geschikte leefomgeving voor zichzelf en voor hun nakomelingen, dan moeten ze tegelijkertijd voorkómen dat ze zelf voedsel voor andere organismen worden. Wij onderzochten hoe vluchtige informatiestoffen van prooihaarden waarin concurrenten aanwezig zijn het gedrag van de roofmijten beïnvloeden (**hoofdstuk 6**). Hiervoor werden de exotische roofmijtsoorten *T. manihoti* en *T. aripo* en de inheemse soort *Euseius fustis* gebruikt. *Mononychellus tanajoa* is een gangbare prooimijtsoort voor *T. manihoti* en *T. aripo* en, in veel mindere mate, ook voor *E. fustis*. De resultaten laten zien dat de drie roofmijtsoorten in staat zijn om de geschiktheid van de prooihaard te beoordelen met behulp van geurstoffen. *Typhlodromalus manihoti* vermeed *T. aripo* zowel op bladeren als apices. Als echter een keuze werd gegeven tussen geuren van een prooihaard met *T. aripo* en een prooihaard met *T. manihoti* dan had *T. manihoti* de voorkeur voor geuren van de prooihaard met *T. aripo*. *Typhlodromalus manihoti* maakte dus onderscheid tussen geuren van prooihaarden met con- en heterospecifieke concurrenten en had voorkeur voor prooihaarden met heterospecifieke roofmijten. *Typhlodromalus aripo* had ook voorkeur om geuren van prooihaarden waarin conspecifieke roofmijten aanwezig zijn te vermijden. *Euseius fustis* vermeed geuren van prooihaarden met

conspecifieke concurrenten of één van de heterospecifieke soorten *T. manihoti* en *T. aripo*. Onder natuurlijke omstandigheden kunnen de drie roofmijtsoorten met elkaar in aanraking komen in situaties waarin voedsel schaars wordt. Deze resultaten laten zien dat naast predator-prooi interacties ook interacties tussen de verschillende predatoren in beschouwing moeten worden genomen als belangrijke bepalende factoren voor de populatiedynamica van predator- en prooisoorten.

In **hoofdstuk 7** worden alle bestudeerde aspecten van dit proefschrift samengevat en bediscussieerd. De belangrijkste conclusies zijn dat (i) geurstoffen van cassave beschadigd door de belangrijkste prooisoort *M. tanajoa* belangrijk zijn voor het van een afstand lokaliseren van de prooi door de roofmijten *T. manihoti* en *T. aripo*, (ii) geurstoffen geïnduceerd door verschillende prooisoorten en combinaties van prooisoorten de interacties met predatoren in het cassave agro-ecosysteem kunnen beïnvloeden, (iii) *T. aripo* en *T. manihoti* beter presteren wanneer ze zich voeden op *M. tanajoa* dan op *O. gossypii* of *T. urticae*, (iv) *T. aripo* samen kan voorkomen met *T. manihoti* door de scheiding van niches, (v) de aanwezigheid van de inheemse roofmijt *E. fustis* in het cassave agro-ecosysteem hoogst waarschijnlijk niet met het voedselzoek-gedrag van de exotische roofmijtsoorten interfereert.

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Désiré Gnanvossou

Curriculum Vitae

The author was born on May 7, 1961 in Agnangon (Zagnanado), in the Republic of Benin (West Africa). Between 1965 and 1968 he started his primary school education at "Ecole Primaire Catholique de Zagnanado". Between 1968 and 1971 he completed his primary school education at "Ecole Primaire Protestante de Gbéto" and from 1971 to 1979 his secondary school education at "Cours Secondaire de Gbégamey-Sud" in Cotonou. He graduated in 1979 with a "Baccalaureat scientifique" degree. He joined the army for one year in 1980. In 1981 he joined the Faculty of Agricultural Sciences at the National University of Benin to study general agronomy and entomology. He graduated in December 1986 as *Ingénieur Agronome: Option Production Végétale, Protection des végétaux*. In June 1987, after an intensive course on biological control of cassava pests organized by the International Institute of Tropical Agriculture (IITA), he was selected as one of the best candidates. Thus, between August 1987 and March 2001 he was employed by IITA as a Research Assistant at the Biological Control Center for Africa, Cotonou, Republic of Benin.

In February 1998 he started the PhD-project in Entomology at Wageningen University. The results of the research are presented in this thesis. The data were collected from September 1998 to March 2001 at the Biological Control Center for Africa, IITA-Benin in cooperation with the Laboratory of Entomology, Wageningen University, The Netherlands. The final phase was undertaken at the Laboratory of Entomology, Wageningen University from April 2001 to January 2002.

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