Infochemicals in tritrophic interactions

Marcel Dicke

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Origin and function in a system consisting of predatory mites, phytophagous mites and their host plants.

Proefschrift

ter verkrijging van de graad van

doctor in de landbouwwetenschappen,

op gezag van de rector magnificus,

dr. C. C. Oosterlee,

in het openbaar te verdedigen

op vrijdag 10 juni 1988

des namiddags te vier uur in de aula

van de Landbouwuniversiteit te Wageningen.

Voorwoord

Aan de totstandkoming van dit proefschrift hebben velen een belangrijke bijdrage geleverd. Ik wil deze eerste pagina van mijn proefschrift gebruiken om hen te bedanken.

Mous Sabelis en Herman Klomp hebben het onderzoeksprojekt gestart en mijn interesse gewekt voor de populatiedynamica van roofmijten en hun prooien. Na het overlijden van Herman Klomp heeft Joop van Lenteren zijn deel van de begeleiding overgenomen. Onder de stimulerende begeleiding van Mous Sabelis en Joop van Lenteren heb ik met zeer veel plezier aan het onderzoek kunnen werken. Zowel direkt als indirekt hebben zij in belangrijke mate bijgedragen aan de totstandkoming van dit proefschrift.

Voor het organisch chemische aspekt van mijn promotieonderzoek was het geduld van Maarten Posthumus en Pim Melcher van groot belang. Uiteindelijk hebben we na veel mislukkingen een methode gevonden die het mogelijk maakte te komen tot een chemische analyse van het kasspint-kairomoon. De inzet van Teris van Beek was in een latere fase onontbeerlijk om deze analyse kompleet te maken.

Aan het onderzoek hebben veel doktoraalstudenten en werkstudenten bijgedragen: Marijke de Jong, Willem Legemaat, Henk van den Berg, René Bogaers, Irma Jorritsma, Sjan van Nieuwenhuizen, Marcel Alers, Beke van Dijk, Bernie Janssen, Bob Tetrault, Hans van Bokhoven, Rob Houthuijzen, Inge van Halder. Niet al hun werk is direkt terug te vinden in dit proefschrift, maar het heeft de ontwikkeling van de ideeën mogelijk gemaakt. Bovendien was de vriendschap met Marijke de Jong en Marcel Alers van onschatbare waarde in de periode waarin de opheffingsperikelen van de vakgroep Dieroecologie zwaar wogen.

Op de vakgroep Dieroecologie stond de zorg en aandacht van Henk Snellen voor roof- en spintmijtkweken garant voor een continue stroom proefdieren. In de beginfase heeft Adrie Groeneveld een groot aandeel gehad in de uitvoering van de experimenten.

Door de hulp van A. van Frankenhuyzen en de gastvrijheid van J. Jorissen, D. Jansen en J.F. van Dijke kon iedere zomer beschikt worden over fruitspintmijten en roestmijten.

De afwerking van dit proefschrift werd verzorgd door Piet Kostense, Frederik von Planta en Rijndert de Fluiter (tekeningen, omslagontwerp), Hein Visser, Jan Bakker, Berry Geerligs en Wim van Hof (fotografie) en Marian Koopman, Ans Klunder en Hanneke Westra (typewerk).

In de afgelopen jaren heb ik ruimschoots ervaren hoe belangrijk het is om te werken binnen een vakgroep waar zowel op werkniveau als op persoonlijk niveau goede kontakten bestaan. Door deze kontakten binnen de vakgroep Entomologie heb ik mijn promotie met veel plezier kunnen afronden.

Ik had nooit aan dit onderzoek kunnen beginnen als mijn ouders mij niet de mogelijkheid hadden geboden om Biologie te studeren. De vriendschap en niet vanzelfsprekende steun van Alie en Saskia waren onontbeerlijk voor de sfeer van vertrouwen waarbinnen ik aan dit proefschrift kon werken.

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Marcel Dicke,
April 1988.

Stellingen bij het proefschrift van Marcel Dicke:
Infochemicals in tritrophic interactions: Origin and function in a system consisting of predatory mites, phytophagous mites and their host plants.

1. Chemisch-analytisch onderzoek aan informatiestoffen is onontbeerlijk voor begrip van de funktie van deze stoffen in een tritrofische kontekst.

2. Gezien de door Regev en Cone gebruikte chemische analyse methoden, de niet-reproduceerbaarheid van hun gegevens en het niet aantreffen van de beschreven sexferomonen van de kasspintmijt in de huidige studie, verdient het aanbeveling hernieuwd chemisch-analytisch werk uit te voeren alvorens over te gaan tot onderzoek naar de mogelijke funktie van deze stoffen als vluchtig kairomoon in spintmijt-roofmijt interakties.
   S. Regev & W. W. Cone, 1975. Environmental Entomology 4: 307-311.
   S. Regev & W. W. Cone, 1976. Environmental Entomology 5: 133-138.
   S. Regev & W. W. Cone, 1980, Environmental Entomology 9: 50-52.
   F. A. Gunson & R. F. N. Hutchins, 1982. Journal of Chemical Ecology 8: 785-796.
   Dit proefschrift.

3. Omdat planten de effekten van predatoren en parasieten op plaagorganismen kunnen beïnvloeden is het noodzakelijk om bij plantenveredelingsonderzoek aandacht te schenken aan tritrofische aspekten.
   P. M. Hulspas-Jordaan & J. C. van Lenteren, 1978. Mededelingen Faculteit voor Landbouwwetenschappen Rijksuniversiteit Gent 43: 431-440.
   D. J. Boethel & R. J. Eikenbary, 1986. Interactions of plant resistance and parasitoids and predators of insects. Wiley and Sons, New York.
   R. J. F. van Haren et al., 1987. Experimental and Applied Acarology 3: 115-121.
   Dit proefschrift.

4. De respons op informatiestoffen is afhankelijk van de voorgeschiedenis van de beschouwde dieren.
   L. E. M. Vet, 1983. Netherlands Journal of Zoology 33: 225-248.
   Dit proefschrift.

5. Dat de verdediging van planten tegen fytofagen dynamisch van karakter kan zijn, betekent dat de voorgeschiedenis van planten die gebruikt worden bij onderzoek aan plant-dier relaties nauwkeurig gestandaardiseerd moet worden.
   R. Karban & J. R. Carey. Science 225: 53-54.
   D. F. Rhoades, 1985. American Naturalist 125: 205-238.
   S. Harrison & R. Karban, 1986. Ecological Entomology 11: 181-188.

6. De konklusie van Hoy en Smilanick dat de roofmijt *Metaseiulus occidentalis* op een kairomoon van de spintmijt *Tetranychus urticae* reageert, is niet gerechtvaardigd op basis van de uitgevoerde experimenten.
   M. A. Hoy & J. M. Smilanick, 1981. Entomologia Experimentalis et Applicata 29: 241-253.

7. Het adsorberend vermogen van de waslaag van bladeren verdient meer aandacht bij onderzoek naar informatiestoffen.
   M. A. Altieri et al., 1981. Protection Ecology 3: 259-263.
   C. Wall et al., 1981. Entomologia Experimentalis et Applicata 30: 111-115.
   C. Wall & J. N. Perry, 1983. Entomologia Experimentalis et Applicata 33: 112-116.
   D. A. Nordlund, R. B. Chalfant & W. J. Lewis, 1984/1985. Agriculture Ecosystems and Environment 12: 127-133.
   H. Nadel & J. J. M. van Alphen, 1987. Entomologia Experimentalis et Applicata 45: 181-186.

8. Beleidsmakers die alleen letten op de hoeveelheid publikaties van wetenschappelijk onder- zoekers behoeven bijscholing op het gebied van natuurlijke selektietheorie.

9. De inhoudelijke diskussie over wetenschappelijke publikaties kan bevorderd worden door het opnemen van een korte reaktie van de door het tijdschrift aangewezen recensenten, direkt achter de desbetreffende publikatie.

10. Door de belangrijke rol die natuurlijke vijanden bij de bestrijding van plagen spelen, verdienen ze uit de anonimiteit gehaald te worden en van een Nederlandse naam voorzien te worden.

11. Teleurgestelde idealisten zouden zich moeten verdiepen in de wordingsgeschiedenis van een kathedraal.
      D. Macaulay, 1974. De Kathedraal. Het verhaal van de bouw. Ploegsma, Amsterdam.
      C. F. Beyers Naudé & D. Sölle, 1985. Hoop voor geloof. Ten Have/IKON, Baarn.

12. De toenemende bezuinigingsdruk op universiteiten maakt kunstzinnig werk van de teken- afdelingen van de Landbouwuniversiteit extra belangrijk.

13. Zelfs niet-acarologen staan met mijten op en gaan ermee naar bed.
      J. D. Lang & M. S. Mulla, 1971. Environmental Entomology 7: 121-127.
      J. E. M. H. van Bronswijk, 1986. Experimental and Applied Acarology 2: 231-238.

14. De aanduiding paranimf is verwarrend.

CONTENTS

De hoofdstukken uit dit proefschrift zijn reeds of zullen worden
gepubliceerd in wetenschappelijke tijdschriften. Daarnaast zijn de
volgende publikaties verschenen:

Hart, J.'t, Jonge, J. de, Collé, C., Dicke, M., Lenteren, J.C. van &
     Ramakers, P. (1978). Host selection, host discrimination and
     functional response of Aphidius matricariae Haliday (Hymenoptera:
     Braconidae), a parasite of the green peach aphid, Myzus persicae
     (Sulz.). Med. Fac. Landbouww. Rijksuniv. Gent, 43 (2): 441-453.

Buyanovsky, G., Dicke, M. & Berwick, P. (1982). Soil environment and
     activity of soil microflora in the Negev desert. J. Arid.
     Environments 5: 13-28.

Meide, P.H. van der, Vijgenboom, E., Dicke, M. & Bosch, L. (1982).
     Regulation of the expression of tuf A and tuf B, the two genes
     coding for the elongation factor Ef-Tu in Escherichia coli. FEBS
     Letters 139: 325-330.

Dicke, M., Lenteren, J.C. van, Boskamp, G.J.F. & Dongen-Van Leeuwen, E.
     van (1984). Chemical stimuli in host-habitat location by Leptopilina
     heterotoma (Thomson) (Hymenoptera: Eucoilidae), a parasite of
     Drosophila. J. Chem. Ecol. 10: 695-712.

Dicke, M., Lenteren, J.C. van, Boskamp, G.J.F. & Voorst, R. van (1985).
     Intensification and prolongation of host searching in Leptopilina
     heterotoma (Thomson) (Hymenoptera: Eucoilidae) through a kairomone
     produced by Drosophila melanogaster. J. Chem. Ecol. 11: 125-136.

Dicke, M. & De Jong, M. (1986). Prey preference of predatory mites:
     Electrophoretic analysis of the diet of Typhlodromus pyri Scheuten
     and Amblyseius finlandicus (Oudemans), collected in Dutch orchards.
     Bull. O.I.L.B./S.R.O.P. IX: 62-67.

Dicke, M. (1988). Microbial Allelochemicals Affecting the Behavior of
     Insects, Mites, Nematodes, and Protozoa in Different Trophic Levels.
     In: P. Barbosa (ed.), Novel Aspects of Insect-Plant Interactions.
     John Wiley and Sons, New York (in press).

Dicke, M. & Burrough, P.A. (1988). Using fractal dimensions for
     characterizing tortuosity of animal trails. Physiol. Entomol.
     (in press).

INFORMATIESTOFFEN IN TRITROFISCHE INTERAKTIES: Oorsprong en funktie in een systeem dat bestaat uit roofmijten, fytofage mijten en hun waardplanten.

1a. Inleiding en samenvatting

Wat zijn informatiestoffen?

Chemische verbindingen spelen een belangrijke rol in interakties tussen organismen. Sommige van deze stoffen zijn voordelig (b.v. voedingsstoffen), of nadelig (b.v. giftige stoffen) voor een organisme. Andere stoffen zijn voor- of nadelig op een indirekte manier: door middel van een gedragsreaktie die ze te weeg brengen. In dit laatste geval spreken we van informatiestoffen (stoffen die, in de natuurlijke kontekst, informatie overbrengen in een interaktie tussen twee individuen, waarbij in de ontvanger een gedrags- of fysiologische reaktie te weeg wordt gebracht die voordelig is voor beide individuen of voor slechts één van beiden; hoofdstuk 2). Op een evolutionaire tijdschaal wordt het lot van een informatiestof bepaald door de selektiedruk op ieder van de twee organismen uit de interaktie. De selektiedruk wordt bepaald door de kosten en baten van alle interakties van een organisme waarbij de informatiestof betrokken is. Om pragmatische redenen wordt een kosten-baten analyse echter telkens opnieuw gemaakt voor iedere interaktie tussen twee organismen. Zodoende wordt de kosten-baten analyse beperkt tot het kleinst mogelijke aantal organismen, waardoor ze zo eenvoudig mogelijk blijft. Het gevolg is dat de informatiestof voor iedere interaktie opnieuw benoemd wordt, in overeenstemming met de gemaakte kosten-baten analyse (hoofdstuk 2; zie ook Nordlund en Lewis, 1976). Bovendien weerspiegelt de klassifikatie ook of de beschouwde organismen soortgenoten zijn of niet. Dit resulteert in de terminologie zoals weergegeven in Figuur 1.1 en Tabel 1.1 (zie ook hoofdstuk 2). De struktuur en termen zijn gebaseerd op die van signaalstoffen. Echter de informatiestofterminologie verschilt van die van signaalstoffen in twee opzichten (hoofdstuk 2):
    (1) Terminologie van informatiestoffen heeft betrekking op verbindingen die informatie overbrengen, terwijl signaalstofterminologie daarnaast ook giftige stoffen beschouwt (Whittaker en Feeny, 1971; Nordlund en Lewis,

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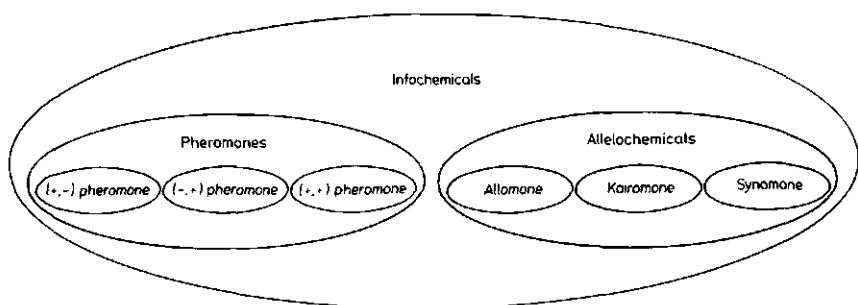
Figuur 1.1: Struktuur van informatiestof-terminologie. Voor definities, zie Tabel 1.1.

Tabel 1.1: Terminologie van informatiestoffen.

INFORMATIESTOF: een chemische verbinding die, in de natuurlijke kontekst informatie overdraagt in een interaktie tussen twee individuen, waarbij in de ontvanger een gedrags- of fysiologische reaktie teweeg wordt gebracht die van voordeel is voor één van de twee organismen uit de interaktie of voor beiden.

FEROMOON: Een informatiestof die een rol speelt in een interaktie tussen twee organismen van dezelfde soort, die voordelig is voor het organisme dat gerelateerd is aan de feromoonbron ((+,-)feromoon), of voor de ontvanger ((-,+)feromoon) of voor allebei ((+,+)feromoon).

ALLELOCHEMICALIE: Een informatiestof die een rol speelt in een interaktie tussen twee individuen van verschillende soorten.

ALLOMOON: Een allelochemicalie dat essentieel is in de biologie van een organisme (organisme 1) en dat, als het een organisme van een andere soort (organisme 2) bereikt, in de ontvanger een gedrags- of fysiologische reaktie opwekt die van adaptief voordeel is voor organisme 1 en niet voor organisme 2.

KAIROMOON: Een allelochemicalie dat essentieel is in de biologie van een organisme (organisme 1) en dat, als het een organisme van een andere soort (organisme 2) bereikt, in de ontvanger een gedrags- of fysiologische reaktie opwekt die van adaptief voordeel is voor organisme 2 en niet voor organisme 1.

SYNOMOON: Een allelochemicalie dat essentieel is in de biologie van een organisme (organisme 1) en dat, als het een organisme van een andere soort (organisme 2) bereikt, in de ontvanger een gedrags- of fysiologische reaktie opwekt die van adaptief voordeel is voor zowel organisme 1 als organisme 2.

2

1976; Nordlund, 1981). In sommige gevallen brengen giftige stoffen en voedingsstoffen informatie over. In die gevallen worden de betrokken verbindingen geklassificeerd als informatiestoffen als hun rol als informatiedrager in ogenschouw genomen wordt. Als giftigheids- of voedingsaspekten beschouwd worden, worden deze verbindingen niet geklassificeerd als informatiestoffen, maar als respektievelijk giffen en nutriënten.

(2) Naast het kosten-baten kriterium is signaalstofterminologie tevens gebaseerd op de oorsprong van de stoffen. Hoewel kennis van de oorsprong belangrijk is voor een begrip van de interaktie tussen twee organismen, kan het zeer moeilijk zijn om de exacte oorsprong op te helderen (b.v. Brand et al., 1975; hoofdstuk 4). Daarom kan toepassing van het oorsprongskriterium leiden tot dubbelzinnigheden. Omdat het kosten-baten kriterium op zichzelf goed en bruikbaar is, is de terminologie van informatiestoffen gebaseerd op dit kriterium alleen.

Informatiestoffen in tritrofische systemen.

Informatiestoffen spelen een rol in interakties tussen opeenvolgende trofische niveaus (schakels uit een voedselketen): b.v. plant-planteneter, plantenetend insekt-insektenetend insekt, zie Figuur 1.2 (b.v. Nordlund et al., 1981; Visser, 1986). Bovendien kunnen informatiestoffen ook een rol spelen in interakties tussen andere trofische niveaus (b.v. plant-insektenetend insekt; Figuur 1.2) (Price, 1981). Voor begrip van de selektiedruk op een organisme, als gevolg van een informatiestof is het
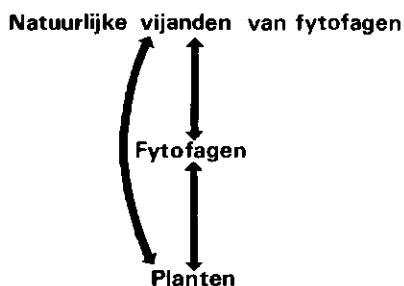
Figuur 1.2: Interakties in een tritrofisch systeem.

daarom noodzakelijk dat alle betrokken trofische niveaus in ogenschouw genomen worden. Dit heeft tot gevolg dat bij het bestuderen van interakties tussen planteneters en hun predatoren de betrokkenheid van op z'n minst het eerste trofische niveau, de plant, ook aan bod moet komen.

Het tritrofische systeem van deze studie: roofmijten, fytofage mijten en hun waardplanten.

Het fytofaag(planteneter)-predator systeem dat het meest uitvoerig is bestudeerd bestaat uit fytofage mijten en roofmijten die voorkomen in Nederlandse boomgaarden. In Figuur 1.3a,b staan de meest algemene fytofage mijten afgebeeld die als plaagorganismen in Nederlandse appelboomgaarden voorkomen: de appelroestmijt, Aculus schlechtendali (Nalepa) en de fruit-spintmijt, Panonychus ulmi (Koch) (Van de Vrie, 1973; Van Epenhuijsen, 1981; Gruys, 1982).

In Nederlandse boomgaarden komen diverse soorten roofmijten voor. De meest algemene zijn Typhlodromus pyri Scheuten (Figuur 1.3c), Amblyseius finlandicus (Oudemans) en A. potentillae (Garman) (McMurtry & Van de Vrie, 1973; Overmeer, 1981; Gruys, 1982). Deze drie soorten leven allemaal van fruitspintmijten en roestmijten, evenals van andere voedselbronnen, zoals stuifmeel van diverse plantesoorten (Overmeer, 1981; Kropczynska, 1970; Overmeer, 1985b).

In dit systeem, bestaande uit twee soorten fytofage mijten en drie soorten roofmijten (Figuur 1.4a) is onderzoek verricht naar prooivoorkeur van de roofmijten. De optimaal foerageertheorie voorspelt dat natuurlijke selektie die predatoren bevoordeelt die een voorkeur hebben voor prooien die uitgedrukt in reproduktief succes het meest opleveren (Krebs, 1978). Reproduktief succes wordt onder andere bepaald door ontwikkelingsduur, eilegsnelheid, mortaliteit gedurende de ontwikkeling en kwaliteit van de nakomelingen. Elk van deze komponenten kan afhangen van de gekonsumeerde prooien. Als een eerste stap bij het analyseren van de selektiekrachten die prooivoorkeur van roofmijten in het genoemde systeem kunnen hebben beïnvloed, heb ik onderzocht of prooipreferentie in overeenstemming is met het daaruit volgende reproduktief succes. Als prooivoorkeur niet op deze meest eenvoudige manier verklaard kan worden, zullen andere verklaringen gezocht moeten worden (zie verderop).

4
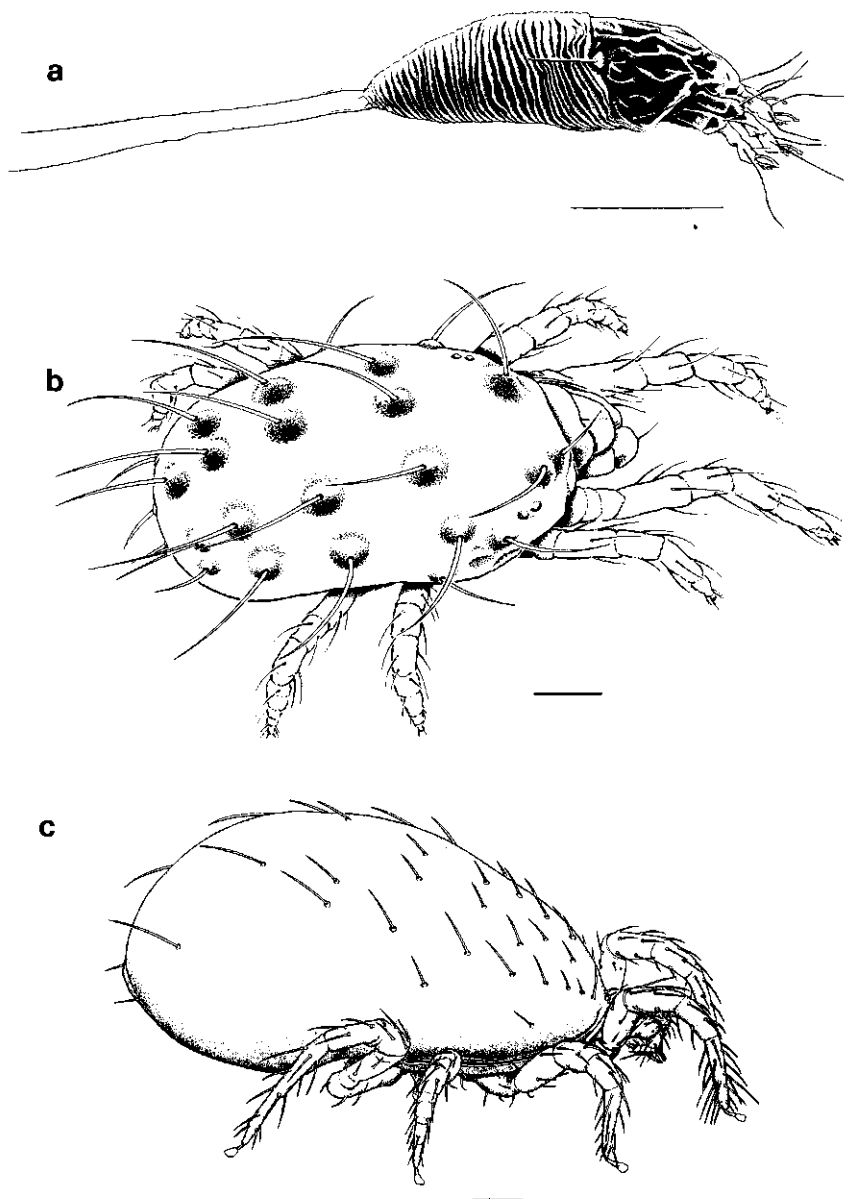
Figuur 1.3: Meest algemene fytofage mijten in Nederlandse appelboomgaarden.
(a) <u>Aculus</u> <u>schlechtendali</u> (Nalepa), volwassen protogyne vrouwtje; (b)
<u>Panonychus</u> <u>ulmi</u> (Koch), volwassen vrouwtje; (c) <u>Typhlodromus</u> <u>pyri</u> Scheuten,
volwassen vrouwtje.
Streepjes geven een lengte van 50 um aan.

Spelen informatiestoffen een rol in prooivoorkeur?

Kairomonen (Tabel 1.1, Figuur 1.1) kunnen predatoren informeren over de aanwezigheid en identiteit van prooien (Greany en Hagen, 1981). Daardoor kunnen deze stoffen van invloed zijn op forageerbeslissingen met betrekking tot de plek waar gezocht wordt, hoe lang gezocht wordt op een bepaalde plek, welke prooien geaccepteerd worden en wanneer verplaatsing met behulp van de wind aangevangen wordt (hoofdstuk 3). Bestudering van de reaktie op kairomonen kan derhalve een beeld geven van prooipreferentie. Echter, de konklusie aangaande prooipreferentie moet beperkt blijven tot de forageerfase die bestudeerd werd. De relatieve kosten verbonden aan het vinden van individuen van elk van beide prooisoorten kunnen afhangen van de forageerfase. Voor het verkrijgen van een goed beeld van prooipreferentie, moeten verschillende forageerfasen bestudeerd worden. Als dit gebeurt in onafhankelijke experimenten worden komplementaire konklusies verkregen. In deze studie is prooipreferentie bepaald op drie onafhankelijke wijzen. Er werden twee laboratoriumanalyses uitgevoerd:
- Bestudering van de reaktie op vluchtige kairomonen. Dit betreft beslissingen van de roofmijten zonder dat kontakt gemaakt wordt met prooien, zoals het geval is na beëindiging van een luchtreis of na uitmoording van een prooihaard.
- Bestudering van predatiesnelheden onder omstandigheden die alleen verschillen in prooiaanbod. Dit betreft acceptatie/verwerpings beslissingen in de fase dat kontakten met prooien plaatsvinden.

In aanvulling op de laboratoriumanalyses van prooivoorkeur, is ook een analyse uitgevoerd onder veldomstandigheden:
- Dit werd gedaan door de voedselsamenstelling te bepalen van roofmijten die in het veld verzameld werden. Met behulp van elektroforese werd de aanwezigheid van prooi-eiwitten in de darm van deze roofmijten kwalitatief bepaald.

Spintmijtkairomonen in een tritrofische kontekst.

Roofmijten onderscheiden schone planten van planten die door spintmijten geïnfekteerd zijn door middel van een vluchtig kairomoon (Sabelis en Van de Baan, 1983). Dit kairomoon lijkt een produkt te zijn van

6

a

Amblyseius potentillae
Amblyseius finlandicus   Roofmijten
Typhlodromus pyri

Panonychus ulmi
Aculus schlechtendali   Fytofage mijten

Appelboom   Waardplant

b

Phytoseiulus persimilis   Roofmijt

Tetranychus urticae   Fytofage mijt

Lima boneplant   Waardplant
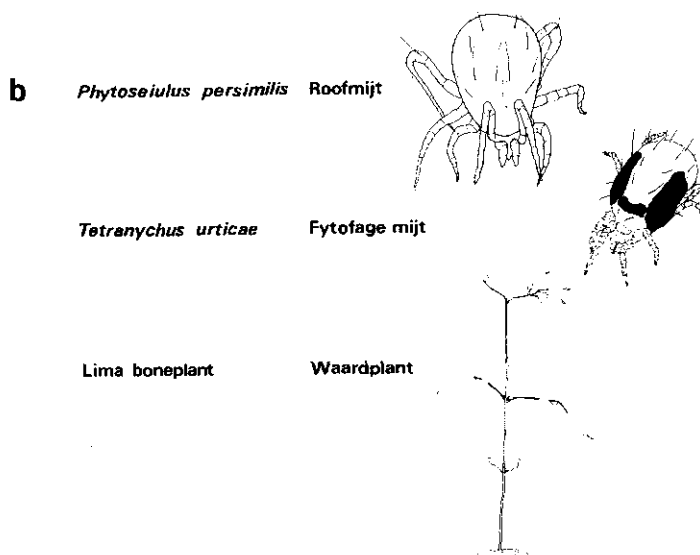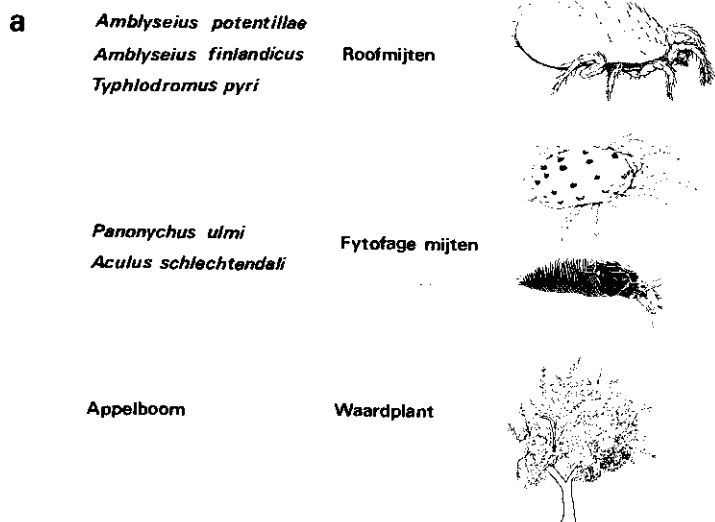
Figuur 1.4: Tritrofisch systemen uit dit proefschrift: (a) 3 roofmijten, 2 fytofage mijten en appelbomen; (b) 1 roofmijt, 1 fytofage mijt en boneplanten.

de interaktie tussen spintmijt en plant: na verwijdering van spintmijten van een geïnfekteerde plant blijft de plant gedurende enkele uren aantrekkelijk voor roofmijten, terwijl de geïsoleerde spintmijten niet meer aantrekkelijk zijn (Sabelis en Van de Baan, 1983; Sabelis et al., 1984a). Huidige gegevens over spintmijt-roofmijt interakties kunnen geen verklaring geven voor de rol van deze informatiestof in de biologie van de spintmijt (zie hoofdstuk 3 voor een overzicht). Het kairomoon zou bijvoorbeeld een onvermijdelijk bijprodukt kunnen zijn van de beschadiging van de plant door de spintmijt. Ook zou het een onontbeerlijke funktie in de biologie van de spintmijt kunnen hebben. Bovendien kan ook de plant betrokken zijn bij produktie van de informatiestof. Om te komen tot een opheldering van de rol van deze vluchtige informatiestof, is het nodig om zijn effekten te bestuderen in interakties tussen plant en spintmijt, tussen plant en roofmijt en tussen spintmijten van één soort. Om dit mogelijk te maken moet allereerst de chemische samenstelling van de informatiestof opgehelderd worden.

Dit werd bestudeerd voor een tritrofisch systeem van Lima boneplanten, de kasspintmijt, <u>Tetranychus</u> <u>urticae</u> Koch, en de roofmijt <u>Phytoseiulus</u> <u>persimilis</u> Athias-Henriot (Figuur 1.4b). De keuze voor dit systeem werd bepaald door praktische overwegingen. De plant en de spintmijt kunnen het hele jaar door gekweekt worden. Daardoor is dit systeem beter geschikt voor het ontwikkelen van een methode om de samenstelling van spintmijtkairomonen op te helderen dan een systeem waarin de plant meerjarig is.

Oorsprong en funktie van kasspintkairomoon in een tritrofisch systeem.

Spintmijten onderscheiden schone planten van planten die door soortgenoten geïnfekteerd zijn door middel van een vluchtige informatiestof (hoofdstuk 4). De spintmijt loopt weg van bladeren die zwaar geïnfekteerd zijn. Deze reaktie is voordelig voor zowel spintmijten op het geïnfekteerde blad als voor de spintmijten die zich hier niet vestigen: een toename van voedselkompetitie wordt voorkomen, zie Wrensch en Young (1978). Bovendien mijdt de weglopende spintmijt een plek die een verhoogd risiko draagt om door roofmijten te worden ontdekt (Sabelis en Van de Baan, 1983).

Om deze redenen wordt de informatiestof in deze interaktie tussen spintmijten van dezelfde soort een (+,+)dispersie-feromoon genoemd.

Biologische gegevens suggereren dat dit feromoon (ten minste gedeeltelijk)
identiek is aan het vluchtige kaïromoon waarop roofmijten reageren
(hoofdstuk 4).

De vluchtige stoffen die vrijkomen van planten die geïnfekteerd zijn
met spintmijten zijn geïdentificeerd en daaropvolgende gedragswaarnemingen
hebben geleid tot opheldering van vier kaïromoonkomponenten die de roofmijt
P. persimilis aantrekken: linalool (3,7-dimethyl-1,6-octadiene-3-ol),
methyl salicylaat, (E)- β-ocimene (3,7-dimethyl-1,3(E),6-octatriene) en
4,8-dimethyl-1,3(E),7-nonatriene. De struktuur van deze verbindingen is
weergegeven in Figuur 1.5. Op z'n minst twee van deze stoffen (linalool en
methyl salicylaat) zijn ook komponenten van een kaïromoon in een interaktie
tussen kasspint en de roofmijt A. potentillae (gekweekt op tuinboonpollen;
zie verderop) (hoofdstuk 4). Literatuurgegevens over de gedragsrespons van
kasspint laten zien dat één van deze komponenten (linalool) ook een
bestanddeel is van het (+,+)dispersie-feromoon (Dabrowski en Rodriguez,
1971).

4,8-dimethyl-1,3(E),7-nonatriene   (E)-β-Ocimene

Linalool   Methyl salicylate
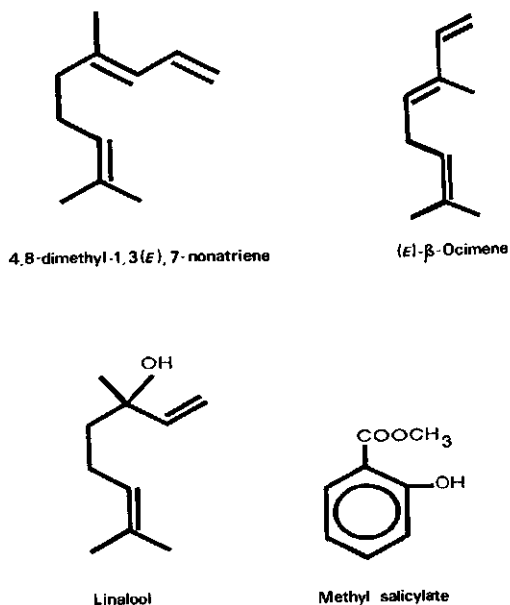
Figuur 1.5: Struktuur van kaïromoon- en feromoon-komponenten afkomstig van
Lima boneplanten die geïnfekteerd zijn door T. urticae.

Alle geïdentificeerde kairomoonkomponenten zijn bekend uit het plantenrijk. Dit suggereert dat de plant betrokken is bij de produktie van de informatiestof. Het is echter geen bewijs: de verbindingen kunnen bijvoorbeeld ook ontstaan als afbraakprodukt van een planteverbinding na injektie van speeksel door de spintmijt. Onderzoek naar bijvoorbeeld de plaats en het tijdstip van produktie en naar de mogelijke opslag van een voorloper zijn nodig als vervolg om te komen tot een opheldering van de rol van de plant in de produktie van het kairomoon. Veronderstel echter dat de spintmijt de informatiestof produceert als dispersie-feromoon. In dat geval is het niet duidelijk waarom het feromoon vluchtig zou moeten zijn. Vluchtige stoffen vergroten het risiko om ontdekt te worden door predatoren veel meer dan niet-vluchtige stoffen. Ontdekking door predatoren leidt onvermijdelijk tot plaatselijke uitroeiing van de spintmijten (Sabelis en Van der Meer, 1986). Het lijkt daarom waarschijnlijker dat de vluchtige verbindingen door de plant worden geproduceerd en dat de spintmijten er het beste van maken door deze stoffen te gebruiken als informatie over plaatsen waar ze zich beter niet kunnen vestigen. Voor een beter begrip van de evolutie van vluchtige stoffen die door planten geproduceerd worden na beschadiging door fytofagen, is het essentieel om te bepalen hoe deze stoffen worden geproduceerd, hoe veel de produktie kost en wat de baten zijn uitgedrukt in een verlaagde kans op beschadiging door fytofagen.

Betrokkenheid van vluchtige kairomonen in prooipreferentie van roofmijten.

De reaktie van T. pyri en A. potentillae op vluchtige kairomonen is afhankelijk van het dieet waarop de roofmijten gekweekt worden. Als ze gekweekt worden op een carotenoid-arm dieet reageren deze roofmijten op kairomonen van meer prooisoorten dan wanneer ze gekweekt worden op een carotenoid-rijk dieet (hoofdstukken 6,7 en 8). Carotenoiden zijn onontbeerlijk voor diapause induktie in A. potentillae (Overmeer, 1985a). De funktie van deze stoffen voor T. pyri is nog onbekend (hoofdstuk 8). Alle prooisoorten waarop carotenoid-behoeftige A. potentillae en T. pyri reageren kunnen het carotenoidtekort opheffen. Als A. potentillae en T. pyri gekweekt zijn op een carotenoid-rijk dieet reageren ze alleen op het kairomoon van P. ulmi. Bovenstaande gold voor roofmijten die 20 uur gehongerd waren. Ook langduriger hongeren van roofmijten die op een

carotenoïd-rijk dieet gekweekt zijn, vergroot het aantal prooisoorten
waarop gereageerd wordt. Bestudering van de respons op vluchtige kaïromonen
laat zien dat A. potentillae en T. pyri (al dan niet carotenoïd-behoeftig)
fruitspint verkiezen boven appelroestmijt (hoofdstukken 6,7 en 8) en dat
A. finlandicus de tegenovergestelde voorkeur heeft (hoofdstuk 11).

Dit komt overeen met konklusies uit predatie-experimenten uitgevoerd
bij verschillende kombinaties van prooiaanbod (hoofdstukken 9 en 11). De
waargenomen predatiesnelheden bij gemengd prooi-aanbod werden vergeleken
met waarden uit een model waarin parameters verwerkt zijn die geschat zijn
in experimenten met iedere prooisoort afzonderlijk. Amblyseius potentillae
en T. pyri aten meer fruitspint en A. finlandicus at meer appelroestmijt
dan voorspeld werd door het model. Dit verschil tussen voorspelde en
waargenomen predatiesnelheden kan niet verklaard worden door een
gedragsverandering van de prooien, veroorzaakt doordat ze samen aangeboden
worden, noch door een verandering in loopgedrag van de roofmijt. Daarom
wijzen deze experimenten uit dat A. potentillae en T. pyri fruitspint
prefereren en dat A. finlandicus appelroestmijt verkiest, uitgedrukt in een
verandering van acceptatie/verwerpings ratio ('succesratio').

Voor de analyse van prooivoorkeur onder veldomstandigheden werd T. pyri
verzameld in een appelboomgaard van bladeren waarop de fruitspint-
appelroestmijt verhouding sterk uiteenliep. De meeste roofmijten bevatten
fruitspint esterase, terwijl roestmijt esterase in slechts een kleine
fraktie aanwezig was (hoofdstuk 10). In A. finlandicus werden roestmijt-
esterase en fruitspintesterase even vaak gevonden. De fruitspint-roestmijt
verhouding op de bladeren waarvan A. finlandicus werd verzameld liep veel
minder uiteen dan het geval was bij T. pyri. Uit deze gegevens met
betrekking tot A. finlandicus kan geen definitieve konklusie over
prooivoorkeur getrokken worden, maar ze zijn zeer zeker niet in tegenspraak
met de konklusies uit de laboratoriumexperimenten (hoofdstuk 11). Voor A.
potentillae zijn geen veldgegevens beschikbaar.

Het feit dat de prooivoorkeur-konklusies uit deze onafhankelijke
analyses met elkaar in overeenstemming zijn voor iedere roofmijtsoort,
betekent dat deze konklusies goed gefundeerd zijn.

Prooipreferentie en reproduktief succes van roofmijten in een boomgaard
systeem met twee soorten fytofage mijten.

   De studie van het reproduktief succes van de drie roofmijtsoorten, op
hetzij fruitspint, hetzij appelroestmijt als voedingsbron laat zien dat
A. finlandicus de beste prooisoort selekteert, uitgedrukt in reproduktief
succes. Deze roofmijtsoort ondervindt ernstige mortaliteit in het
larvestadium als het voedsel uit fruitspintmijten bestaat, maar niet als ze
van roestmijten eten. Als gevolg hiervan is de intrinsieke groeisnelheid
veel groter op een dieet van appelroestmijten (hoofdstuk 12).
   Ook voor A. potentillae en T. pyri is appelroestmijt een betere prooi
dan fruitspint: de ontwikkelingsduur is geringer als de roofmijten eten van
roestmijten, terwijl de eilegsnelheid en mortaliteit niet afhankelijk zijn
van deze prooisoorten (hoofdstuk 12). Voor A. potentillae is P. ulmi aan
het eind van het seizoen mogelijk toch een betere prooisoort vanwege het
verschil in effekt op diapause induktie. Op basis van huidige gegevens kan
de optimale prooikeuze theorie de prooivoorkeur van A. potentillae en
T. pyri dus niet bevredigend voorspellen. Toekomstig onderzoek zal zich
onder andere moeten richten op (1) het mogelijke effekt van konkurrentie
tussen prooisoorten op prooibeschikbaarheid, (2) het mogelijke effekt van
konkurrentie tussen predatorsoorten op prooibeschikbaarheid en (3) een
mogelijke wijziging van prooivoorkeur in de loop van het seizoen.
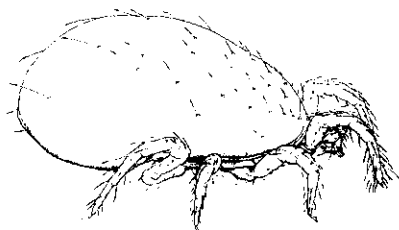
INFOCHEMICALS IN TRITROPHIC INTERACTIONS: Origin and function in a system
consisting of predatory mites, phytophagous mites and their host plants.

1b. Introduction and summary

What are infochemicals?

   Chemical compounds play an important role in interactions between
organisms. Some of these chemicals are to the benefit (e.g. nutrients) or
detriment (e.g. toxins) of an organism. Others are of benefit or detriment
in an indirect way: through the behavioural response they elicit. The
latter chemicals are termed infochemicals (chemicals that, in the natural
context, convey <u>information</u> in an interaction between two individuals,
evoking in the receiver a behavioural or physiological response that is
adaptive to either one of the interactants or both; chapter 2). On an
evolutionary time scale, the fate of an infochemical depends on selection
pressures on each interactant. Selection pressure is determined by costs
and benefits which result from all interactions of an organism in which the
infochemical is involved. Yet, for pragmatic reasons, to analyse the
function of an infochemical in the biology of an organism, a cost-benefit
analysis is made for each interaction between two organisms separately. In
this way the cost-benefit analysis is restricted to the smallest number of
interactants possible, which ensures its simplicity. Consequently, for each
interaction the infochemical is classified according to the corresponding
costs and benefits for the two interactants (chapter 2; cf. Nordlund and
Lewis, 1976). Moreover, classification also reflects whether the
interaction under consideration is between conspecifics or between
individuals of different species. This resulted in the terminology
represented in Figure 1.1 and Table 1.1 (cf. chapter 2). Its structure and
terms are based on those of semiochemicals. However, infochemical
terminology differs from semiochemical terminology in two respects
(chapter 2):
   (1) Infochemical terminology regards compounds that convey information,
whereas semiochemical terminology in addition also includes toxins
(Whittaker and Feeny, 1971; Nordlund and Lewis, 1976; Nordlund, 1981). In
some instances toxins or nutrients may convey information. If that is the

Infochemicals

Pheromones

Allelochemicals

(+,-) pheromone | (-,+) pheromone | (+,+) pheromone

Allomone | Kairomone | Synomone
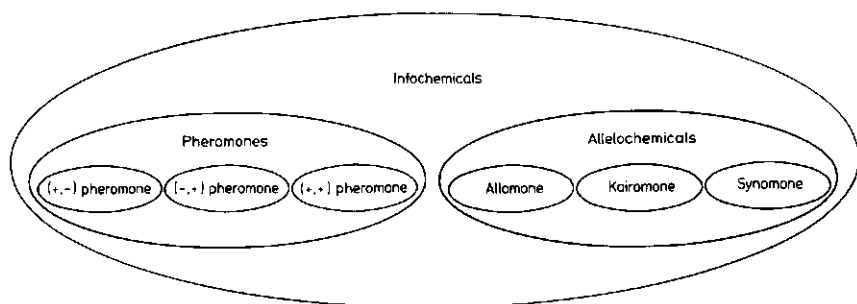
Figure 1.1: Structure of infochemical terminology. For definitions of terms
see Table 1.1.

Table 1.1: Infochemical terminology.

INFOCHEMICAL: A chemical that, in the natural context, conveys information
in an interaction between two individuals, evoking in the receiver a
behavioural or physiological response that is adaptive to either one of the
interactants or to both.

PHEROMONE: An infochemical that mediates an interaction between organisms
of the same species whereby the benefit is to the origin-related organism
((+,-)pheromone), to the receiver ((-,+)pheromone), or to both
((+,+)pheromone).

ALLELOCHEMICAL: An infochemical that mediates an interaction between two
individuals that belong to different species.

ALLOMONE: An allelochemical that is pertinent to the biology of an organism
(organism 1) and that, when it contacts an individual of another species
(organism 2) evokes in the receiver a behavioural or physiological response
that is adaptively favourable to organism 1, but not to organism 2.

KAIROMONE: An allelochemical that is pertinent to the biology of an
organism (organism 1) and that, when it contacts an individual of another
species (organism 2) evokes in the receiver a behavioural or physiological
response that is adaptively favourable to organism 2 but not to organism 1.

SYNOMONE: An allelochemical that is pertinent to the biology of an organism
(organism 1) and that, when it contacts an individual of another species
(organism 2) evokes in the receiver a behavioural or physiological response
that is adaptively favourable to both organism 1 and 2.

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case, these toxins and nutrients are classified as infochemicals when their role as information carrier is considered. When poisonous or nutritious aspects are considered, they are not classified as infochemicals, but as toxins and nutrients respectively.

(2) Semiochemical terminology is based on origin of the compounds, in addition to the cost-benefit analysis. Although knowledge of the origin is important to understand the interaction between two organisms, it may be very difficult to elucidate the origin (e.g. Brand et al., 1975; chapter 4). Therefore, application of the origin criterion may lead to ambiguities. Because the cost-benefit criterion by itself is good and useful, infochemical terminology is based on that criterion alone.

Infochemicals in tritrophic systems.

Infochemicals play a role in interactions between consecutive trophic levels (e.g plant-herbivore, phytophagous insect-entomophagous insect; Figure 1.2) (e.g. Nordlund et al., 1981; Visser, 1986). Moreover, infochemicals may also mediate interactions between other trophic levels (e.g. plant-entomophagous insect; Figure 1.2) (Price, 1981). Therefore, to understand the selection pressure on an organism, as a result of an infochemical, all trophic levels involved should be regarded. As a consequence, investigations of infochemicals in interactions between herbivores and their predators should also regard involvement of at least the first trophic level, the plant.

Natural enemies of phytophages

Phytophages
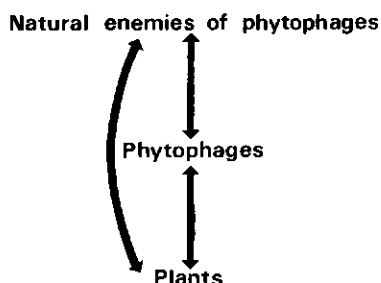
Plants

Figure 1.2: Interactions in a tritrophic system.

The tritrophic system of this study: predatory mites, phytophagous mites
and their host plants.

The herbivore-predator system investigated most extensively in this
thesis consists of phytophagous mites and predatory mites that occur in
Dutch orchards. Figure 1.3a,b depicts the two most abundant phytophagous mites
that occur as pest organisms in Dutch apple orchards: the apple rust mite,
Aculus schlechtendali (Nalepa), and the European red spider mite,
Panonychus ulmi (Koch) (Van de Vrie, 1973; Van Epenhuijsen, 1981; Gruys,
1982).

Several species of predatory mites occur in Dutch orchards. The most
abundant of these are Typhlodromus pyri Scheuten (Figure 1.3c), Amblyseius
finlandicus (Oudemans) and A. potentillae (Garman) (McMurtry & Van de Vrie,
1973; Overmeer, 1981; Gruys, 1982). All three species feed on P. ulmi and
A. schlechtendali, as well as on other food sources such as several pollens
(Overmeer, 1981; Kropczynska, 1970; Overmeer, 1985).

In this system consisting of two phytophagous prey species and three
predator species (Figure 1.4a), prey preference of the predators was
investigated. Optimal foraging theory predicts that natural selection
favours predators preferring prey species that are most profitable in terms
of reproductive success (Krebs, 1978). Reproductive success is determined,
among others, by development time, oviposition rate, mortality during
development and offspring quality. Each of these components can be affected
by the prey species consumed. As a first step in analysing which selection
pressures may have moulded prey preference of the predatory mites in the
system outlined above, I have tested whether prey preference is matched by
the associated reproductive success. If this most simple explanation for
prey preference does not hold, other explanations should be considered (see
below).

Do infochemicals play a role in prey preference ?

Kairomones (Table 1.1, Figure 1.1) may inform predators on presence and
identity of prey (Greany and Hagen, 1981) and thereby affect foraging
decisions, such as where to search, how long to search at a specific site,
which prey to accept and when to disperse on air currents (chapter 3).

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Figure 1.3: Most common phytophagous mites in Dutch apple orchards. (a)
Aculus schlechtendali (Nalepa), adult protogyne female; (b) Panonychus ulmi
(Koch) adult female; (c) Typhlodromus pyri Scheuten, adult female.
Bars indicate a length of 50 um.
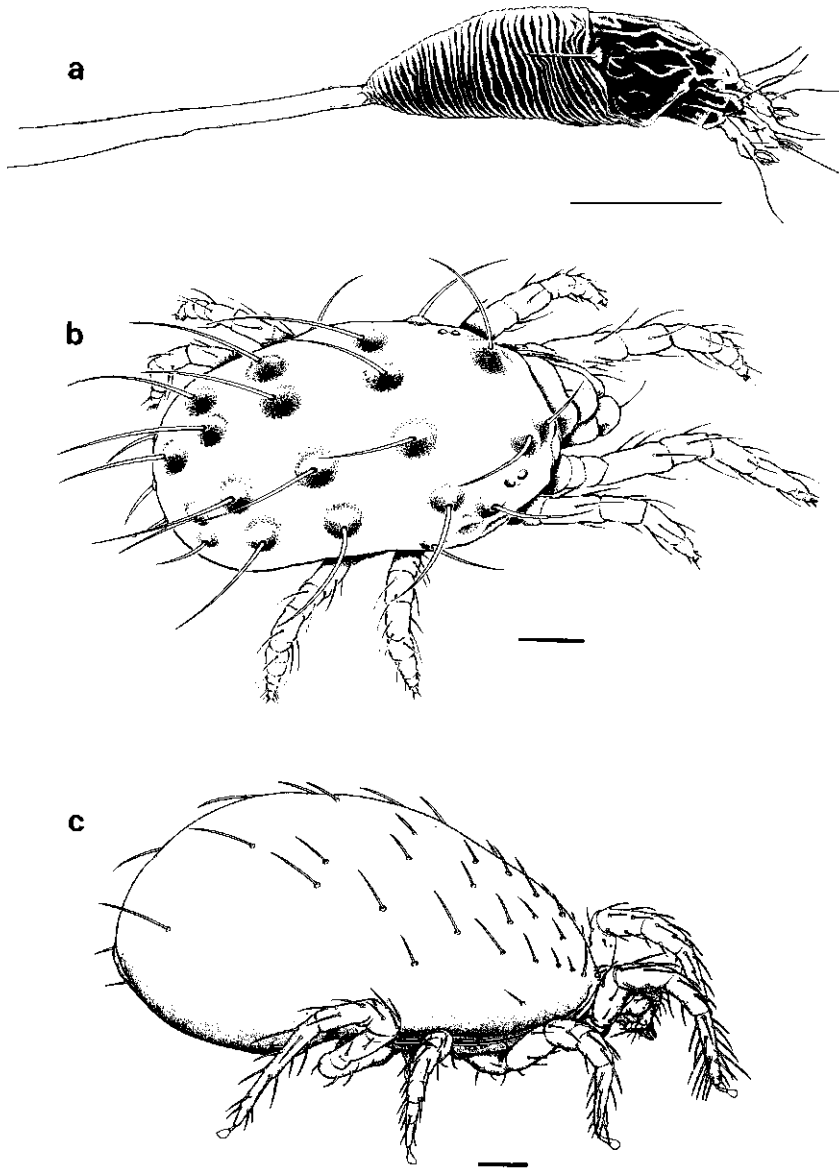
Investigation of the response to kairomones may therefore yield information on prey preference. However, the conclusion on prey preference must be restricted to the foraging phase that was studied. Relative costs involved in finding individuals of each prey species might differ for different foraging phases. Therefore, to obtain a comprehensive view of prey preference, several foraging phases should be investigated. Such analyses should be carried out independently to obtain complementary conclusions. In this study, prey preference was determined in three independent analyses. Two laboratory analyses were carried out:

- Analysis of response towards volatile kairomones. This investigation regards decisions of the predators when prey individuals are not contacted, as is the situation after termination of aerial dispersal or after eradication of a prey patch.
- Analysis of predation rates at different prey supplies. This relates to acceptance/rejection decisions during contacts with prey items.

To complement the prey preference analyses carried out in the laboratory, an investigation was made under field conditions:
- This was done by determination of diet composition by means of electrophoretic analysis of gut contents of field-collected predators.

Spider-mite kairomones in a tritrophic context.

Predatory mites distinguish plants infested by spider mites from clean plants by a volatile kairomone (e.g. Sabelis & Van de Baan, 1983). This kairomone seems to be a product of the interaction between plant and spider mites: after removal of spider mites from an infested plant, the plant remains attractive to the predators during several hours, whereas the mites alone do not remain attractive (Sabelis & Van de Baan, 1983; Sabelis et al., 1984a). Current data on spider mite - predatory mite interactions do not explain the role of this infochemical in the biology of the spider mites (cf. chapter 3 for a review). It may, for instance, be an inevitable byproduct of damage inflicted on the plant by the spider mite, and/or have an indispensable function in the biology of the spider mite. Moreover, the plant may be involved in production of the infochemical. To elucidate the role of this volatile infochemical, its effects in interactions between plant and spider mite, between plant and predatory mite and between spider

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a *Amblyseius potentillae*
    Amblyseius finlandicus   Predatory mites
    Typhlodromus pyri

    Panonychus ulmi
    Aculus schlechtendali   Phytophagous mites

    Apple tree   Host plant

b *Phytoseiulus persimilis*   Predatory mite

    Tetranychus urticae   Phytophagous mite
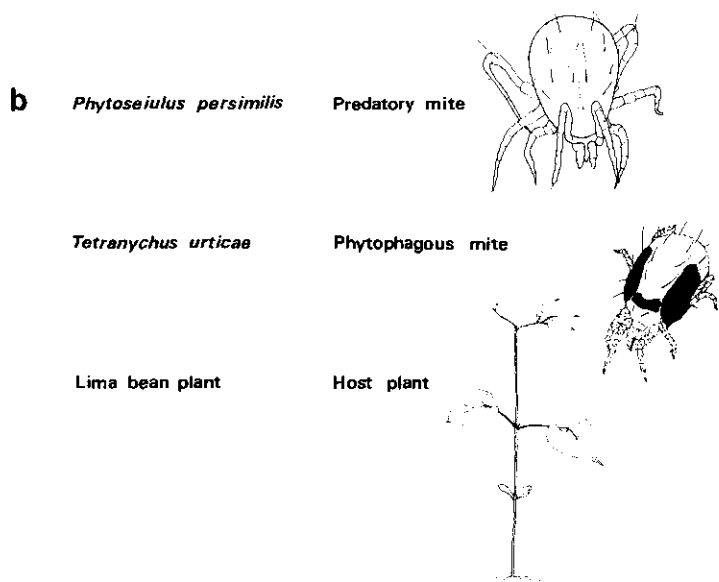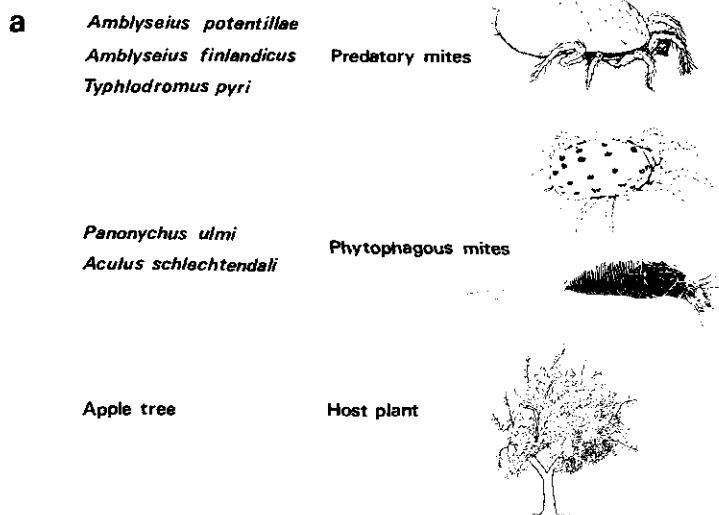
    Lima bean plant   Host plant

Figure 1.4: Tritrophic systems of this study: (a) 3 predatory mites, 2 phytophagous mites and apple trees; (b) 1 predatory mite, 1 phytophagous mite and bean plants.

mites of one species should be investigated. Before this can be done, chemical identification of the infochemical is a necessary first step.

These investigations were made for a tritrophic system consisting of Lima bean plants, the two-spotted spider mite, Tetranychus urticae Koch and the predatory mite Phytoseiulus persimilis Athias-Henriot (Figure 1.4b). This system was chosen for practical reasons. The plant and phytophagous mite can be reared throughout the year and therefore, this system is much more suitable to develop a method for the chemical analysis of spider-mite kairomones than a system in which the plant is a perennial.

Origin and function of T. urticae kairomone in a tritrophic system.

Two-spotted spider mites distinguish between a clean plant and a plant that is infested by conspecifics on the basis of a volatile infochemical (chapter 4). The spider mites move away from heavily infested leaves. This response is advantageous to spider mites on the infested leaf as well as to spider mites that avoid settling on these leaves: increased competition for food is avoided, cf. Wrensch and Young (1978). In addition, the spider mite that disperses thus avoids settling on a spot that has an increased risk of being detected by predatory mites (Sabelis and Van de Baan, 1983). Therefore, the infochemical in this interaction between conspecific spider mites is called a (+,+)dispersing pheromone. Biological evidence suggests that this pheromone is (at least partly) identical to the volatile kairomone to which predatory mites respond (chapter 4).

Volatiles emitted from plants infested by T. urticae were identified and subsequent behavioural analyses resulted in identification of four kairomone components that attract the predatory mite P. persimilis: linalool (3,7-dimethyl-1,6-octadiene-3-ol), methyl salicylate, (E)-β-ocimene (3,7-dimethyl-1,3(E),6-octatriene) and 4,8-dimethyl-1,3(E),7-nonatriene. The structure of these compounds is shown in Figure 1.5. At least two of these (linalool and methyl salicylate) are also components of a kairomone in the interaction between T. urticae and A. potentillae (when reared on V.faba pollen; see below) (chapter 4). Literature data on the behavioural response of T. urticae indicate that one of these kairomone components (linalool) is also a component of the (+,+)dispersing pheromone (Dabrowski and Rodriguez, 1971).

All identified kairomone components are well-known in the plant kingdom. This suggests that the plant is involved in production of the infochemical, but it is no proof. It may for instance be that spider-mite enzymes injected into the plant break down a plant compound. Investigation of e.g. site and moment of production and possible storage of precursors are needed as a next step to elucidate the role of the plant in kairomone production. However, suppose that it is the spider mite who produces the infochemical to serve as a dispersing pheromone. Then, it is not clear why this pheromone should necessarily consist of volatiles. As a result of the production of volatiles the spider mites incur more risks of being detected by predators than by production of non-volatile chemicals. Detection by predators inevitably leads to local extermination of spider mites (Sabelis and Van der Meer, 1986). For this reason it seems more likely that the volatiles are plant produced and that the spider mite makes the best of a bad job by using them as information to decide where not to colonize. To understand the evolution of plant-produced volatiles after herbivore attack, it is crucial to assess how they are produced, how much it costs to produce them and what the benefits are in terms of a lowered probability of herbivore attack.
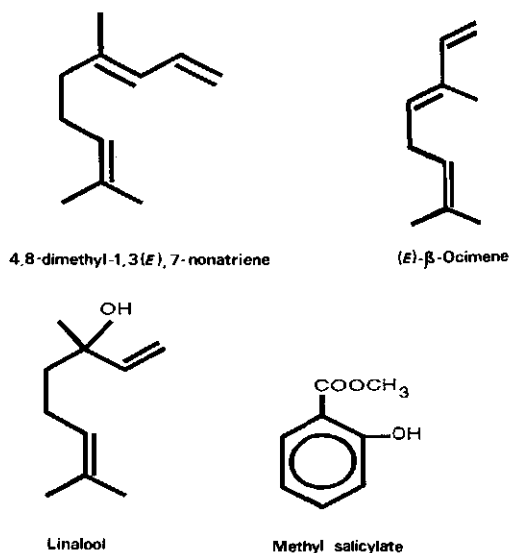

4,8-dimethyl-1,3(E),7-nonatriene   (E)-β-Ocimene

Linalool   Methyl salicylate

Figure 1.5: Structure of kairomone and pheromone components emitted from Lima bean plants infested by T. urticae.

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Involvement of volatile kairomones in prey preference of predatory mites.

The response of T. pyri and A. potentillae to volatile kairomones is dependent on the diet of the predators. When reared on a carotenoid-poor diet these predators respond to the kairomones of more prey species than when reared on a carotenoid-rich diet (chapters 6, 7 and 8). Carotenoids are indispensable to A. potentillae because of their function in diapause induction (Overmeer, 1985a). The function of these nutrients to T. pyri remains unknown (chapter 8). All prey species to whose kairomones carotenoid-deficient A. potentillae and T. pyri respond can relieve the lack of carotenoids. Carotenoid-containing A. potentillae and T. pyri only respond to the P. ulmi kairomone. The above observations were made for predators that were starved for 20 h. Longer starvation of predators reared on a carotenoid-rich diet also enlarges the number of prey species responded to. Investigations of the response to volatile kairomones indicates that A. potentillae and T. pyri (whether carotenoids are available or not) prefer P. ulmi to A. schlechtendali (chapters 6, 7 and 8) and that A. finlandicus has a reverse preference (chapter 11).

This corresponds to conclusions from predation experiments performed at different composition of prey supply (chapters 9 and 11). The observed predation rates when mixed prey supplies were offered, were compared with a model provided with parameters estimated from experiments with each of both prey species alone. Amblyseius potentillae and T. pyri fed more on P. ulmi and A. finlandicus fed more on A. schlechtendali than was predicted by the model. This difference between observed and predicted predation rates cannot be explained by a change in behaviour of the prey species as a result of being together, nor by a change in walking behaviour of the predator. Therefore, these data indicate that A. potentillae and T. pyri prefer P. ulmi and that A. finlandicus prefers A. schlechtendali, in terms of a change in acceptance/rejection ratio ('success ratio').

Analysis of prey preference under field conditions showed that most T. pyri collected from apple leaves that widely varied in P. ulmi: A. schlechtendali numbers contained P. ulmi esterase, whereas A. schlechtendali esterase was present in a minor fraction of predators (chapter 10). Rust-mite esterase and P. ulmi esterase were found equally frequent in A. finlandicus. The data for A. finlandicus, obtained over a

narrower range of prey-number ratios than for T. pyri, do not allow a
definite conclusion on prey preference. However, they certainly do not
cause rejection of the conclusion on prey preference as obtained in the
laboratory analyses (chapter 11). No field data are available for
A. potentillae.

Because the conclusions on prey preference as determined in these
independent analyses are consistent for each predator species, the
inference on prey preference is firmly established.

Prey preference and reproductive success of predatory mites in an orchard
system with two species of phytophagous prey mites.

Analysis of reproductive success of these three predator species, when
feeding on either P. ulmi or A. schlechtendali, indicates that
A. finlandicus selects the best prey species in terms of reproductive
success. This predator species suffers high larval mortality on P. ulmi,
but not on A. schlechtendali. This results in a much higher intrinsic rate
of population increase when feeding on apple rust mites (chapter 12).

Amblyseius potentillae and T. pyri would also do better by feeding
preferentially on A. schlechtendali: development times when feeding on this
prey species are shorter than when feeding on P. ulmi, whereas these prey
species do not differentially affect mortality or oviposition rate (chapter
12). For A. potentillae this may not be the case at the end of the season
because P. ulmi is a better prey species in terms of diapause induction.
Thus, on the basis of current data, optimal prey-choice theory cannot
satisfactorily predict actual prey peference of A. potentillae and T. pyri.
Future investigations should concentrate on e.g. (1) possible effect of
competition between prey species on prey availability, (2) possible effect
of competition between predator species on prey availability, and (3)
possible shift in prey preference during the season.
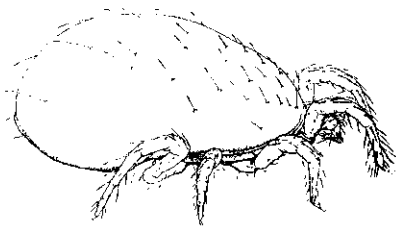
PART I. TERMINOLOGY OF INFOCHEMICALS AND A REVIEW OF THEIR POSSIBLE FUNCTIONS IN A PLANT-MITE SYSTEM.

2. Infochemical terminology: Should it be based on cost-benefit analysis rather than origin of compounds?

Marcel Dicke and Maurice W. Sabelis.

ABSTRACT

The involvement of chemicals in interactions between individual organisms has been recognized for many years. The present day terminology of these so-called semiochemicals developed over a 25 year period and the definition of each category was proposed in a specific context. As a result, the definitions of the various categories are heterogeneous.

The present definition of semiochemicals (Nordlund and Lewis, 1976) includes toxins and nutrients. We distinguish between (a) toxins and nutrients, which are themselves to the benefit or detriment of the interacting organisms, and (b) information-conveying chemicals (infochemicals) on the other hand. These latter chemicals are to the benefit or detriment through the response they elicit in the receiving organism. Toxins and nutrients may also convey information. If so, we propose to refer to these chemicals as toxin or nutrient when poisonous or nutritious aspects are considered and as infochemical when their role as information carrier is considered.

Semiochemical terminology according to Nordlund and Lewis (1976) is based to a large extent on the origin of the compounds in specific interactions. Only those interactions are regarded where one of the interactants is the producer or emitter of the chemical. However, if an infochemical plays a role in the interaction between two individuals, it is by no means certain that either of the two is the actual producer or emitter. The producer or emitter may be a different organism from the same or even from another trophic level, but is clearly associated to at least one of the interactants. Arguments are given to eliminate the origin criterion from the terminology, and to use the cost-benefit criterion as the sole determinant of infochemical subdivisions.

INTRODUCTION

The involvement of chemicals in conveying information in intra- and interspecific interactions between organisms appears to be widespread. Interactions mediated by these so-called semiochemicals (Law and Regnier, 1971; Table 2.1) can be found within and between vertebrates, inverte-

brates, plants and microorganisms (Nordlund et al., 1981; Schoonhoven, 1981; Albone, 1984; Bell and Cardé, 1984; Rhoades, 1985; Sabelis and Dicke, 1985; Sonenshine, 1985; Visser, 1986; Dicke, 1988b) in the same or different trophic levels (Price, 1981). They involve many behavioural activities or physiological responses such as searching for food or oviposition sites, locating a mate, marking a resource area, and so on.

Many terms have been proposed to describe different classes of semiochemicals (for review see Table 2.1 in Nordlund, 1981) but only a few have survived (Nordlund and Lewis, 1976). Also, the definitions of some terms have been modified. For instance, compare the definitions of allomone in Brown et al. (1970) with those in Nordlund and Lewis (1976): the latter authors modified the original definition and distinguished between allomones and synomones as two classes of the original category of allomones. Because of the many modifications in semiochemical terminology, current definitions are heterogeneous. Each definition was developed and proposed in a specific context: e.g., the term pheromone (Karlson and Lüscher, 1959; Table 2.1) was coined to distinguish this class of semiochemicals from hormones.

A summary of current semiochemical terminology (according to Nordlund and Lewis, 1976) is shown in Figure 2.1. This structure is reasonably consistent, but we think the different definitions should be based on a more explicit and common logic to make it more coherent and less ambiguous. This is the subject of the present paper.

INFOCHEMICALS, TOXINS AND NUTRIENTS

Law and Regnier (1971) defined semiochemicals as "chemical signals that transmit information between individual organisms"; the essential word being 'signals' (Greek σημειον, semeion: mark or signal). The word 'signal' presupposes that the chemical is purposefully sent to the receiver and hence that communication is taking place (Law and Regnier, 1971). However, the receiving organism commonly acts as an eavesdropper that uses chemicals from the emitter as a source of information (cf. Otte, 1974 for discussion on the terms signal, information and communication). This was recognized by Nordlund and Lewis (1976) and they therefore (Nordlund, pers.

Table 2.1: Contemporary semiochemical terminology according to Nordlund and Lewis (1976).

SEMIOCHEMICAL: Chemical involved in the chemical interactions between organisms. Nordlund and Lewis, 1976.

PHEROMONE: A substance that is secreted by an organism to the outside and causes a specific reaction in a receiving organism of the same species. Karlson and Lüscher, 1959.

ALLELOCHEMIC: Chemical significant to an organism of a species different from its source, for other reasons than food as such. Whittaker, 1970.

ALLOMONE: Chemical substance produced or acquired by an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological reaction adaptively favourable to the emitter but not to the receiver. Nordlund and Lewis, 1976.

KAIROMONE: A substance produced or acquired by, or released as the result of the activities of an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological reaction adaptively favourable to the receiver but not to the emitter. Nordlund and Lewis, 1976.

SYNOMONE: A substance produced or acquired by an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological reaction adaptively favourable to both receiver and emitter. Nordlund and Lewis, 1976.
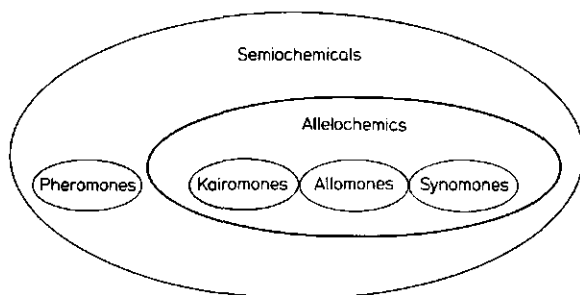
Figure 2.1: Structure of current semiochemical terminology according to Nordlund and Lewis (1976).

comm.) eliminated 'signal' from the definition, and redefined
semiochemicals as "chemicals involved in the (chemical) interactions
between organisms" (Nordlund and Lewis, 1976; Nordlund, 1981). Also the
qualification 'information transmitter' has been removed. Thus the original
definition of a semiochemical (Law and Regnier, 1971) has been broadend by
Nordlund and Lewis (1976) and Nordlund (1981) to include not only
information-transmitting chemicals, but also chemicals that function as
toxins or nutrients.

Why organisms invest in producing information transmitters is an
interesting question and requires a clear understanding of the benefits to
the producer. The evolution of their production is less obvious than that
of nutrients and toxins, that are themselves to the benefit or detriment of
the receiving organism. Organisms generally perceive toxins and nutrients
(but see Schoonhoven, 1981) and respond to them (e.g. Pasteels et al.,
1986). Thus, toxins and nutrients may also convey information. For these
reasons we propose to classify poisonous substances as toxins and
alimentary substances as nutrients when poisonous or alimentary aspects are
considered respectively, and as infochemicals (Table 2.2), when their role
as information carriers is considered. Thus, a specific chemical may be
both a toxin and an infochemical, depending on which aspect of the chemical
is being discussed. In the remaining part of this paper we discuss
infochemicals exclusively.

PHEROMONES AND ALLELOCHEMICALS

Pheromones, as originally defined by Karlson and Lüscher (1959) are
substances that are secreted by an organism to the outside that cause a
specific reaction in a receiving organism of the same species. Originally
the words 'to the outside' were the most important part of this definition,
to distinguish these chemicals from hormones. To date, for distinguishing
pheromones from allelochemicals the last four words of the pheromone
definition ('of the same species') are the most important. According to
current terminology, a pheromone mediates an interaction in which the
emitter and the receiver are conspecific, whereas in the case of an
allelochemical the 'source' and receiver belong to different species

Table 2.2: Infochemical terminology.

INFOCHEMICAL: A chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response that is adaptive to either one of the interactants or to both.

PHEROMONE: An infochemical that mediates an interaction between organisms of the same species whereby the benefit is to the origin-related organism ((+,-)pheromone), to the receiver ((-,+)pheromone), or to both ((+,+)pheromone).

ALLELOCHEMICAL: An infochemical that mediates an interaction between two individuals that belong to different species.

ALLOMONE: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2) evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 1, but not to organism 2.

KAIROMONE: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2) evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 2 but not to organism 1.

SYNOMONE: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2) evokes in the receiver a behavioural or physiological response that is adaptively favourable to both organism 1 and 2.

(Table 2.1). Allelochemicals can be subdivided into three categories: allomones, kairomones and synomones (Nordlund and Lewis, 1976). According to the current definitions, these chemicals are produced or acquired by the source organism (the 'emitter'). The distinction between the three categories is made according to which organism benefits in the interaction: the emitter (allomone), the receiver (kairomone) or both (synomone). In agreement with Weldon (1980) we feel that this is a useful criterion as knowledge of an organism's response to an infochemical necessarily brings forth questions about cost − benefit aspects.

Allomones

The term allomone has been used widely and without question. However, we feel that in many cases the chemical substances described are toxins and not infochemicals: e.g. Nordlund (1981), who regarded venoms used by rattlesnakes in prey capturing as allomones and Duffey (1976), whose review on allomones deals exclusively with toxins.

Often allomones are described as repellants, but they may also serve as attractants of prey organisms (Eberhard, 1977; Balan and Gerber, 1972; Jansson and Nordbring-Hertz, 1979).

Kairomones

There have been many discussions on the usefulness of the term kairomone (Blum, 1974; Weldon, 1980; Pasteels, 1982) but it is used widely. The main criticism has been that a chemical mediating an interaction that is detrimental to the emitter will be selected against. However, the evolutionary persistence of a kairomone depends on the benefit the emitter obtains in other interactions mediated by this chemical, relative to the detriment suffered when it acts as a kairomone (Nordlund and Lewis, 1976; Weldon 1980). For instance, the aggregation pheromone of bark beetles may function in interspecific interactions as a kairomone that attracts predators (Wood, 1982).

The term kairomone has mainly been used for chemicals mediating interactions between predators / parasites and their prey / host (e.g. Nordlund et al., 1981). Unfortunately, infochemicals mediating interactions between herbivores and their host plants have not generally been referred to as kairomones (for exceptions see e.g. Metcalf et al., 1980; Price, 1981; Schoonhoven, 1981; Lanier, 1983; Andersen and Wilkin, 1986; Dicke, 1986).

Synomones

Whittaker and Feeny (1971) classified allelochemicals that benefit the receiver as well as the emitter as both kairomones and allomones (see Table 2.1 in their publication). Nordlund and Lewis (1976) ended this confusion

and introduced the term synomone for such allelochemicals. This was a
valuable contribution to the terminology. However, application of the term
has not been satisfactory to date. Many authors who describe synomones do
not use this word (e.g. Eisner and Grant, 1981; Camazin, 1985). Others
erroneously use the repellency criterion to classify compounds as allomones
that are in fact synomones. For instance, Schoonhoven (1981) reviews plant
chemicals that repel herbivorous insects, and classifies these compounds as
allomones. However, as these allelochemicals not only benefit the plant in
reducing damage, but also benefit the insect by avoiding intake of toxins,
they should be classified as synomones.

CRITERIA IN THE PRESENT TERMINOLOGY

The present terminology (Nordlund and Lewis, 1976) uses two criteria
for classification: (1) whether the organism that is the origin of the
compound ('the secreting organism' in the pheromone defintion or 'the
source' in the allelochemical definition; Table 2.1) and the receiver are
conspecific or not; (2) whether the emitter ('producer or acquirer'), the
receiver or both benefit from the interaction that is mediated by the
compound; this cost-benefit criterion has only been applied to
allelochemicals. The origin of the compound is of central importance in
this terminology. However, in the splitting phase that heavily depends on
the origin criterion (distinction between pheromones and allelochemics)
ambiguous terms are used, such as 'secreted to the outside' or 'source'.
These terms do not clarify whether the origin produces or emits the
compound. Moreover, apart from confusion about the identity of the origin,
the mere inclusion of the origin in the splitting phase may lead to
ambiguities. This is illustrated below.

(1) Ambiguity of the origin concept causes confusion: an example of beetle
    pheromones produced by associated microorganisms.

   Bark beetles aggregate on trees, where they feed and breed on host
tissues just under the bark. The interindividual interactions in which
these beetles take part are mediated by many infochemicals (Wood, 1982;
Birch, 1984). Bark beetles that singly attack a tree may die because of
resin production by the tree. By attacking a tree communally the beetles
are more likely to overwhelm the tree's defence and colonize it. After
pioneer beetles attack a tree, conspecifics are attracted by a mixture of
beetle-released compounds and host tree compounds (see Birch, 1984, for
review). However, in some cases the beetle-released compounds are produced
by symbiotic fungi living in the beetle's mycangium (Brand et al., 1975,
1976). Therefore, not all components are pheromones (sensu stricto).
However, although it is known that bark beetle pheromones may not be
produced by the beetles but by microorganisms, the fact that the
interaction concerned is between conspecific beetles has been the decisive
factor for classifying non-beetle compounds as beetle pheromones.
   A similar situation exists in the beetle Costelytra zealandica (White)
when a volatile sex pheromone (phenol) is produced by bacteria in the
colleterial glands of the females (Henzell, 1970; Henzell and Lowe, 1970;
Hoyt et al., 1971).

(2) Ambiguities resulting from inclusion of the origin criterion in the
    terminology: plant-related origin of spider-mite kairomone and spider-
    mite pheromone.

   Spider mites feed on plants. They insert their stylets into the plant
tissue, inject saliva (Storms, 1971) and consume the predigested plant-cell
contents (Helle and Sabelis, 1985). During this process, volatile chemicals
are emitted from the plant - mite complex, which are used as infochemicals
in prey searching by spider-mite predators from the acarine family
Phytoseiidae (Sabelis and Dicke, 1985). These volatile allelochemicals are
spider-mite species specific (Sabelis and Van de Baan, 1983; Dicke and
Groeneveld, 1986) and have been referred to as kairomones by these authors.
The cost-benefit analysis was the criterion for that classification.

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Application of the origin criterion is far from clear cut as the origin of
the compounds is still poorly understood. The compounds are present on/in
the host-plant leaf, in the spider mites and their faeces (Sabelis et al.,
1984a). Previously infested leaves from which all spider mites are removed
are highly attractive to the predators, but it is unknown in what way the
leaf gets contaminated with the kairomone. The faeces on the leaf are not
sufficient to explain the attractivity of previously infested leaves
(Sabelis et al., 1984a). Presumably part of the compounds is emitted from
the host plant, but only after spider-mite damage. This mechanism that will
be called induction is an alternative to production or acquirement of an
allelochemical (cf. definitions of allomones, kairomones and synomones in
Nordlund and Lewis, 1976). Induction is a process where the actual producer
of the infochemical may not be one of the interactants but an organism that
is closely associated to one of the interactants (Figure 2.2). In this
process the inducer affects the production of the infochemical. The spider
mite may be involved actively (nutritious compounds are metabolized by the
spider mite and the waste products emanate from the plant) or passively
(the plant "recognizes" spider-mite damage and responds by producing the
specific allelochemical). Research directed to elucidating the source of
the kairomone will be of great value in better understanding the role of
the spider mite and the host plant in kairomone production/induction.

From comparison of data of Dabrowski and Rodriguez (1971), Dicke (1986)
and Dicke et al. (1988d) it can be deduced that at least one of the
kairomone components is also a component of a volatile dispersing pheromone
of the prey mite. This means that an infochemical that is emitted from the
host plant mediates the interaction between individual conspecific spider
mites. Because of its origin this infochemical would not be classified as a
pheromone (emitter and receiver are not conspecific), but when the
interacting organisms are regarded it is.

(3) Application of the origin criterion limits the number of interactions
    considered.

In the current terminology (Nordlund and Lewis, 1976) the cost-benefit
analysis determines whether an allelochemical is an allomone, kairomone, or
synomone. In a few instances, a definite classification has not been made

Figure 2.2: Illustration of 3 possible
infochemical emitting pathways.
E=Emitter;
P=Producer;
A=Acquirer;
I=Inducer;
R=Receiver.

Produced Acquired Induced
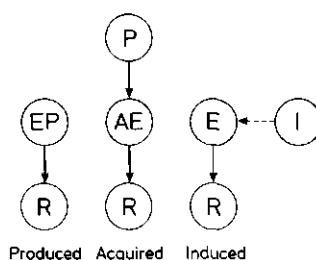
because the costs/benefits of the chemical to the emitter were not
understood (Carton, 1976; Greany et al., 1977; Dicke et al., 1984). In
these cases, fermentation products of the microbial food of dipteran larvae
are involved in host-habitat location by larval parasites. For
classification of these infochemicals the interaction between the microbes
and the wasps has been taken into account. Because nothing is known about
the costs and benefits of the allelochemical (emitter and receiver belong
to different species) no further classification can be made in the
interaction between microbe and parasitic wasp. However, when the origin
criterion is abandoned, also another interaction can be considered: that
between the microbivore and the parasitic wasp. This interaction is
mediated by the microbial infochemicals, which are pertinent to the biology
of the microbivore, and therefore a cost-benefit analysis for this
interaction is appropriate. This analysis leads to the conclusion that in
this interaction the allelochemical is a kairomone.

VALUE OF THE ORIGIN CRITERION

For most infochemicals the exact origin (producer) has not been
investigated. However, in two cases microorganisms have been implicated as
the producers of an insect pheromone (Henzell, 1970; Henzell and Lowe,
1970; Hoyt et al., 1971; Brand et al., 1975, 1976,) and in another the
evidence suggests that a plant is involved in production of a spider-mite
pheromone and a spider-mite kairomone (Dicke, 1986; Dicke et al., 1988d).
In our opinion the origin of an infochemical is not important for
classification of the compound. From an evolutionary point of view the mere

fact that an interaction is mediated by the infochemical invites a cost-benefit analysis for both interactants. We propose to eliminate the origin criterion. The role of organisms other than the producer/emitter or receiver, in the production of the infochemical is thereby included. This has often been overlooked, especially when microorganisms are involved in the production of an infochemical that mediates an interaction between two other organisms. Microorganisms are involved in infochemical production in many different interactions (for review, see Dicke, 1988b). With the increase in our knowledge of the involvement of microorganisms in interactions between other organisms (e.g. Jones, 1984), more mediations by microbes will be discovered. When microorganisms are involved in production of infochemicals that mediate interactions between other organisms, the origin criterion of infochemical terminology would complicate classification because of the intermediate microbial trophic level. In our classification every interaction mediated by an infochemical deserves a cost-benefit analysis but only if the infochemical is pertinent to the biology of one of the interactants.

NEW DEFINITIONS OF PHEROMONE, ALLELOCHEMICAL, ALLOMONE, KAIROMONE AND SYNOMONE

Pheromone

Concurrent with elimination of the origin criterion we propose to include a cost-benefit criterion. To date, no cost-benefit criterion exists for pheromones, although "benefit-to-the-species" terms have been employed. However, natural selection does not act on the species level, but on that of genes or individuals. Therefore, the cost-benefit analysis should be applied to the latter. Interacting individuals of the same species are selected for contributing a maximum genetic output to the next generation. Therefore, pheromones may not always benefit both emitter and receiver, both because of asymmetric intraspecific interactions and because of the existence of individual or kin pheromones (e.g. Greenberg, 1979; Beauchamp et al., 1985; Blaustein and O'Hara, 1986; Linsenmair, 1985; Sherman and Holmes, 1985). This was recognized by Rutowski (1981) who suggested

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restricting the term pheromone to intraspecific interactions where both emitter and receiver benefit. However, he did not suggest names for pheromones benefitting only one of the interactants. As the term pheromone has been widely accepted we suggest that its use be continued, but that it should be qualified with '(+,-)', '(-,+)' or '(+,+)' where respectively the origin-related organism, the receiver or both benefit. We therefore propose the following definitions of a pheromone: an infochemical that mediates an interaction between organisms of the same species in which the benefit is to the origin-related organism ((+,-)pheromone), to the receiver ((-,+)pheromone), or to both ((+,+)pheromone).

An example can be found in the cannibalistic snail _Fasciolaria tulipa_ L. (Snyder and Snyder, 1971). This predator produces a pheromone that may be used to distinguish different sizes of conspecifics. A snail moves towards conspecfics with a size smaller than its own and away from conspecifics with a size larger than its own. For a large receiver snail the infochemical involved is a (-,+) pheromone, as is the case for an interaction where the large snail is the emitter and a small one the receiver.

The pheromone subdivisions proposed here, based on cost-benefit analysis at the individual level, complement previous subdivisions that were made on the basis of the kind of interactions being mediated (e.g. marking, sex attraction). Thus one may distinguish e.g. (+,+), (+,-) and (-,+) marking pheromones. However, it should be noted that many of these classifications suggest that natural selection acts at the species level. For instance, the term anti-aggregation pheromone in bark beetles suggests that the colonizing bark beetles emit an anti-aggregation signal. This, however, denies the selection pressure present on individual beetles and therefore is evolutionarily inconceivable. The colonizing beetles convey information about the present beetle density. Beetles that receive this information may then decide to settle despite the emission of the pheromone or to leave in search for another tree. Their response is determined over evolutionary time by the prospects for reproduction of these alternatives (Alcock, 1982).

Allelochemical, allomone, kairomone, synomone

Analogous to the new definition of pheromones, an allelochemical is defined as an infochemical that mediates an interaction between two individuals that belong to different species. By eliminating the origin criterion and maintaining the cost-benefit analysis criterion we propose the following definitions for the allelochemical subcategories:

Allomone: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2) evokes in the receiver a behavioural and/or physiological response that is adaptively favourable to organism 1, but not to organism 2.

Kairomone: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2) evokes in the receiver a behavioural and/or physiological response that is adaptively favourable to organism 2 but not to organism 1.

Synomone: An allelochemical that is pertinent to the biology of an organism (organism 1) and that, when it contacts an individual of another species (organism 2) evokes in the receiver a behavioural and/or physiological response that is adaptively favourable to both organism 1 and 2.

Nordlund and Lewis (1976) distinguished a fourth category of allelochemicals: apneumones. These are substances emitted by non-living material that evoke in the receiver a behavioural or physiological response that is adaptively favourable to a receiving organism but detrimental to an organism of another species that may be found in or on the non-living material. This category has not been used since it was proposed, possibly because it is difficult to distinguish apneumones from allelochemicals that are produced by microorganisms on the non-living material (Dicke, 1988b). However, when the origin criterion is omitted from the terminology, this class becomes superfluous. These allelochemicals will be classified as
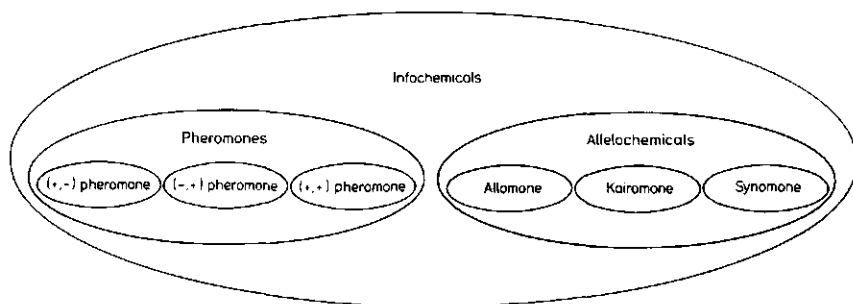
Figure 2.3: Structure of infochemical terminology.

kairomones based on the cost-benefit analysis for the interactants: the
receiving organism and the organism that may be found on or in the non-
living material. We therefore exclude apneumones from our classification
(Figure 2.3).

In all interactions mediated by infochemicals, that are known to us, at
least one of the interactants is benefitted. However, it is not
inconceivable that interactions exist where neither of the two interactants
benefit. However, a term for these interactions should await their
discovery.

CONCLUSION

The infochemical terminology that has developed to date has aided
discussion of these substances. However, phenomena are being discovered
that cannot easily be classified using current terminology. Therefore, we
have proposed alternative terminology that abandons the origin criterion
(compare terminology of Nordlund and Lewis, 1976) because this criterion
excludes ecologically significant interactions where the producer/emitter
is not one of the interacting organisms. Instead we classify infochemicals
on the basis of the costs and benefits of the interaction for both
interactants.

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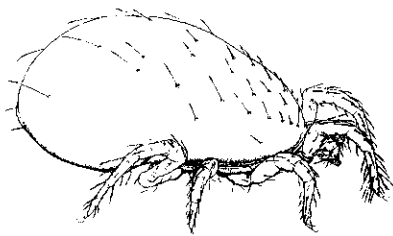
3. Infochemicals in long-range dispersal and searching behaviour of phytoseiid mites. A review.

Maurice W. Sabelis and Marcel Dicke
In: W. Helle and M.W. Sabelis (eds.) Spider Mites. Their Biology, Natural
Enemies and Control. World Crop Pests Vol 1B: 141-160 (1985).

INTRODUCTION

One of the most intriguing questions in the ecology of phytoseiid
predators is how these tiny and wingless creatures find their prey, in this
case the spider mites. To understand the problems faced by foraging
phytoseiids the distribution pattern of the spider mites should first be
taken into account. Spider mites have an intrinsic tendency to found new
colonies on leaves·close to the parental colony. In this way a cluster of
colonies arises, further referred to as a spider—mite patch. Therefore,
prey searching can be divided into three phases: searching (1) for spider—
mite patches in the habitat; (2) for spider—mite colonies within a patch;
and (3) for spider mites within a colony. The second problem imposed on
foraging phytoseiids is the transient nature of spider—mite patches.
Spider mites, such as _Tetranychus urticae_ Koch, tend to overexploit their
host plant as a food source. Hence, the spider—mite population in the patch
will crash as a result of intraspecific competition and dispersal. However,
when a predatory mite invades a spider—mite patch long before host—plant
exhaustion, it reproduces and gives rise to a rapidly growing predator
population that is capable of suppressing the spider—mite population to
very low densities. This pattern of growth and crash applies to many, if
not all, _Tetranychus_ spp. and also, to varying degrees, to other
tetranychid species. Spider mites are therefore a transient food source for
their phytoseiid predators, either because the host plant is transient
(e.g. annuals), or because the host plant becomes overexploited by the
spider mites, or because the spider mites themselves are overexploited by
their predators.

After the prey population crashes, the predators are forced to disperse

and search for new prey. If the predators thrive on spider mites only, they should find new spider-mite patches. This is the case for Phytoseiulus persimilis Athias-Henriot, a predatory mite that thrives almost exclusively on Tetranychus mites. To find new spider-mite patches a predatory mite would have to cover larger distances than possible by locomotion only. This so-called long-range dispersal is probably brought about by passive transport on wind currents. The predator may land directly in a spider-mite patch but, if not, it should start locomotory search either for suitable places to initiate another aerial voyage or for prey patches in the neighbourhood of the landing spot. An ability to locate spider-mite patches that are present close to the landing spot would be advantageous to specialized predators such as P. persimilis. Moreover, once a spider-mite patch has been invaded, an ability to find profitable prey colonies and to detect the patch and colony edges would also be advantageous. For these reasons it is expected that natural selection would favour any improvement in these abilities. But, how can these abilities be improved? Phytoseiids have long been assumed to be blind, actively wandering mites. In fact this was why generic names such as Typhlodromus (Scheuten, 1857) were coined. Ever since, prey location has been assumed to be a matter of chance (Putman, 1962; Mori and Chant, 1966; Rabbinge, 1976). Only very recently has this hypothesis been rejected once and for all, it having been shown that phytoseiids are able to perceive chemical cues produced by their spider-mite prey (Hoy and Smilanick, 1981; Hislop and Prokopy, 1981; Sabelis and Van de Baan, 1983; Sabelis et al., 1984a,b). The chemical compounds involved are called kairomones, which are defined by Brown et al. (1970) as 'Substances produced or acquired by an organism that when they contact an individual of another species in the natural context, evoke in the receiver a behavioural or physiological response that is adaptively favourable to the receiver but not to the emitter'. The role of kairomones in prey location by arthropod predators has been reviewed by Greany and Hagen (1981). The recent advances in research on the role of kairomones in phytoseiid-tetranychid interactions will receive major emphasis in this chapter.

EVIDENCE REQUIRED TO DEMONSTRATE THE INVOLVEMENT OF KAIROMONES

The kairomones involved in prey searching can be divided into 2 classes: (1) contact and (2) volatile kairomones. Contact kairomones evoke a behavioural response in the predator after direct physical contact in solid or liquid form. Volatile kairomones on the other hand are perceived by the predator without contacting the source. The difference is arbitrary, as a contact kairomone can also evaporate and may serve as a volatile kairomone. Moreover, the physiological processes of perceiving contact kairomones by taste or volatile kairomones by smell are similar, except for the threshold concentration, which is usually lower for the perception of volatiles. What evidence is required to demonstrate that kairomones are involved in prey searching by phytoseiid predators? To begin with, the bioassays required to make inferences about the involvement of contact or volatile kairomones are different.

(1) Contact kairomones can be demonstrated to play a role by using bioassays that involve observation of behaviour after the predator contacts a treated substrate (e.g. a leaf disc or a disc of filter paper). However, volatile kairomones emitted from the substrate may be perceived simultaneously, which hampers a straightforward interpretation of the behaviour observed. The bioassay should therefore be combined with one specifically designed to elucidate the involvement of volatile kairomones. If the latter assay does not reveal a response, contact kairomones are likely to be involved. However, if a response to volatiles is found, it is either the volatile that also acts as a contact kairomone, or there are two types of kairomones with very different physical properties.

(2) Volatile kairomones can be demonstrated to be present by using several types of olfactometers (see Kennedy, 1977a,b). The main principle is that the predator does not contact the kairomone source. However, it is possible that the volatile is not perceived until after it has been adsorbed by the substrate on which the predator is walking. This possibility can be ruled out if the predator ceases to respond shortly after the remote kairomone source has been removed.

The existence of either type of kairomone can be inferred when (by comparison with an appropriate control) a behavioural response is recorded in the predator to:

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(a) spider mites with host plant, or spider mites alone (meaningful only in the case of a volatile), or to a previously infested host plant from which the spider mites have been removed;

(b) one of the above items if this response differs from the response to the same items that have been washed with an appropriate solvent (provided that no other explanations can be given for the differential response);

(c) a substrate (e.g. non-infested leaf or artificial material) treated with an extract of a spider-mite infested host plant, the spider mites or their residues (faeces, silk, exuviae);

(d) a volatile extracted from adsorbent material that has previously been placed in an air stream blown over spider-mite infested plants;

(e) a substrate treated with a purified extract;

(f) a substrate treated with an identified chemical in synthetic form.

The above treatments ((a)-(f)) are ranked in order of the increasing information on the existence and chemical nature of the kairomone. Using this methodological scheme it is worthwhile evaluating the current status of the evidence that phytoseiid predators make use of kairomones when searching for spider mites. Hoy and Smilanick (1981) used two-choice discs in experiments with the predator Typhlodromus occidentalis Nesbitt and the two-spotted spider mite T. urticae. On the basis of evidence classified above under (1b) they suggest that there is a contact kairomone present on infested leaf surface or silk. Using water as solvent they washed leaf disc halves that had been infested by spider mites which had been removed immediately prior to the washing. They combined these washed disc halves with unwashed infested leaf disc halves or with clean half-discs to form two-choice discs. Half-an-hour after introduction significantly more predators were present on the unwashed infested halves than on those that had been washed. This might indicate that a kairomone had been washed away, but another explanation might be that the webbing structure may have been altered by the washing procedure (even though this washing procedure was qualified as 'gentle') or that the physical properties of the silk (e.g. stickiness) and other residues are changed by washing. Hence, extraction of a chemical is not proven, as no additional trials were carried out in which the kairomone was transferred via the water to a clean leaf disc (the evidence classified above uner (1c). The latter type of evidence has been

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presented by Hislop et al. (1978) and Hislop and Prokopy (1981). Using the solvent methanol they extracted silk and associated residues of T. urticae. This extract elicited more frequent returns of the predator Amblyseius fallacis (Garman) to a treated filter paper disc than to discs treated with methanol only. The kairomone was not soluble in chloroform, hexane or water (Hislop et al., 1978). This evidence belongs to category (1c) above, and is more conclusive than that of Hoy and Smilanick. It should also be stressed that the chemical is not proven to be a contact kairomone, because the bioassays were not combined with tests specifically designed to elucidate responses to volatile kairomones. The chemicals are, however, likely to be non-volatile because the response to extracts of several-days-old silk was hardly different from that to fresh extracts.

The existence of volatile kairomones that are prey-species specific has been demonstrated by Sabelis and Van de Baan (1983). In a Y-tube olfactometer they recorded a positive response from the predators P. persimilis and T. occidentalis to odour coming from leaves infested with T. urticae, whereas Amblyseius potentillae (Garman) and Amblyseius finlandicus (Oudemans) showed a positive response to odour of leaves infested with Panonychus ulmi (Koch) (evidence classified under (2a); response is absent directly after removal of odour source). Neither predator showed such a response to the other spider-mite species. Using the same olfactometer Sabelis et al. (1984a) showed that females of P. persimilis walked towards filter paper discs treated with a dichloromethane extract of leaves previously infested by spider mites but freed from these mites and most of their web just prior to extraction (evidence classified under (2c)). The kairomone could not be transferred to the filter paper discs when the solvent was ethanol, acetone or pentane and is therefore likely to be different from the methanol-soluble kairomone extracted by Hislop et al. (1978) (this kairomone was not extracted by chloroform). The kairomone appeared to be present on the leaf surface and in much smaller amounts in the faeces and the prey eggs. It did not evaporate from the web and was not emitted by spider-mite females that had been separated from their host plant. The latter result implies that the compound is different from the volatile kairomone of T. urticae to which the anthocorid predator Anthocoris nemorum L. responds in a petri-dish olfactometer with 2 odour-inlets. This is because the latter predator shows a positive response to

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host plants infested by T. urticae as well as to the spider mites separated from their host plants (Mpakagiannis, 1982).

It will be clear that still more evidence is needed to complete the proof that kairomones are involved in prey location by phytoseiid predators. Chemical identification of the kairomone(s) is of major importance, but it is also necessary (1) to find the kairomone source, (2) to elucidate kairomone synthesis, (3) to locate the predator's chemosensors and (4) to carry out electrophysiological studies using extracts from prey with or without host plant or synthetic kairomones. Some first attempts to find the source of the volatile kairomone have been reported (Sabelis et al., 1984a). The kairomone can be produced by all active stages of the spider mite when feeding and it can be extracted from pulverized adult females using dichloromethane as a solvent. The latter result does not prove that the kairomone is produced directly by the spider mite, as it may be present in the plant food consumed. It is unlikely to be merely a product of the plant since the predator does not respond to clean leaves of the host plant or to mechanically damaged leaves (Sabelis and Van de Baan, 1983). Moreover, as the kairomone is prey-species specific and the mouthparts of Panonychus species and Tetranychus species are morphologically very similar, mechanical damage resulting from stylet piercing is unlikely to be the only cause of kairomone production. Hence, the spider mite may be directly responsible for the kairomone, or the plant may produce it in response to being attacked. As long as the kairomone remains unidentified, it is difficult to ascertain its synthesis, but it is possible to investigate which sensors have morphological structures that allow for contact chemoreception or chemoreception of volatiles. Some chemosensors have been located by Jagers op Akkerhuis et al. (1985). They followed up the suggestion made by Jackson and Ford (1973) that chemoreceptors are most likely to be present on top of the pedipalps and on the tarsi of the first legs (which are moved around like the antennae of parasitic wasps). According to Jackson (1974) the tarsi of the first legs have several long pointed setae and a group of small, peg-like setae in between. Jagers op Akkerhuis et al. (1985) argue that these small setae are unlikely to be mechanoreceptors or contact mechanoreceptors because of the presence of long setae nearby (Fig. 3.1 a). Using transmission electron microscopy it was found that the short setae are multiply innervated and that their thin

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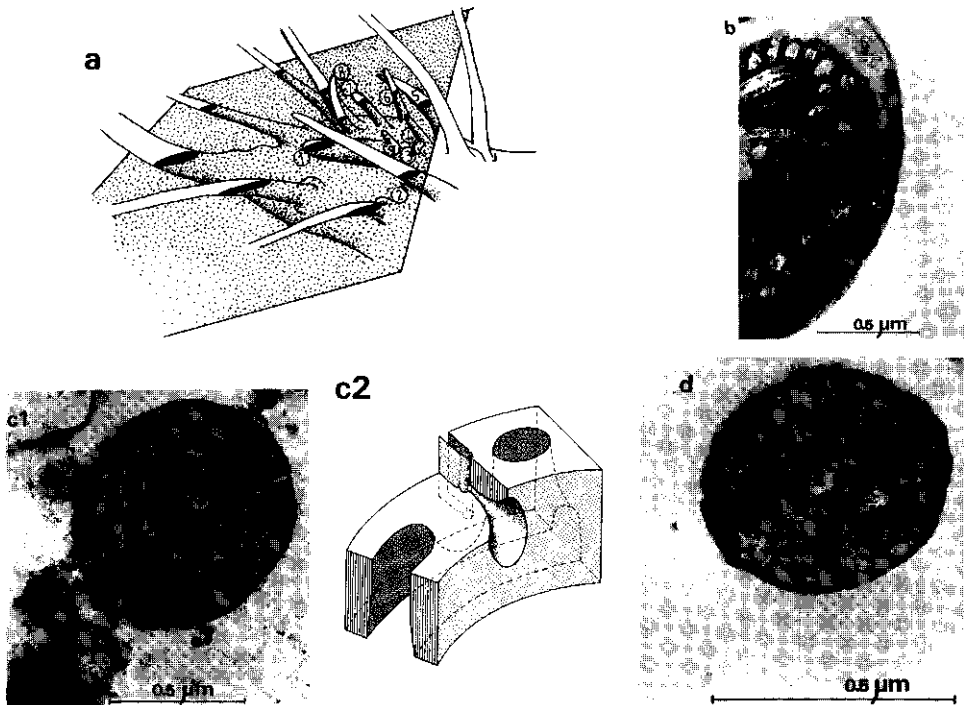
Figure 3.1 (a) Chaetal pattern on the dorsal field of the first tarsi of Phytoseiulus persimilis, showing the small peg-like setae in between the long setae and the cross-sectional plane of the TEM micrographs in (b), (c1) and (d). (b) Part of the cross-section of a multi-innervated seta with a single wall and funnel-shaped pores. Starting from the outer side of the wall, pore width is 0.06 um, then the pore narrows to 0.037 um and at the inner side pore width is enlarged to 0.125 um. (c1) Cross-section of a double-walled seta with spoke canals (0.023 um diameter) in the first wall and cone-shaped connections to the inner fluid through the second wall. (c2) Diagram of the pores shown in (c1) showing that the spoke canals are supposed to be part of a longitudinal slit along the shaft wall. Therefore the cross-sections contain only now and then connections between the slit and the small end of the cone-shaped canal. (d) Cross-section of a seta with mushroom-shaped spoke canals in the first wall and an incomplete second wall. Diameter of the canals is ca. 0.008 um. (Jagers op Akkerhuis et al., 1985)

walls contain either funnel-shaped pores in single-wall setae or slit-pores with cone-shaped connections in the second wall to the inner fluid, or spoke canals with a mushroom-shaped connection to the inner fluid in setae with an incomplete second wall (Fig. 3.1 b-d). This result provides

morphological evidence for the presence of chemosensors that may be
involved in the perception of the volatile kairomones. Transmission
electron microscopy also revealed the location of contact chemosensors on
top of the pedipalps. These setae were found to be innervated, but no pores
could be detected in their wall shaft. It could be that a few pores were
present on top of the setae. This was indicated by the presence of 'blobs
of material' on top of these setae. These blobs are presumed to be extruded
through pores and because these were found exclusively on top of the
pedipalpal setae, they are thought to indicate the terminal porous area of
a contact chemosensor (Jackson, 1974; Jagers op Akkerhuis et al., 1985).
Future studies may indicate other chemosensors elsewhere on the soma, but
if smell-sensors are located exclusively on the first legs and taste
sensors on the pedipalps, this would provide a breakthrough for
electrophysiological and behavioural studies on the differential role of
volatile and contact semiochemicals. This is because Egan (1976)
demonstrated that electrophysiological responses can be derived from an
electrode inserted into the first leg of a parasitic mite.

LONG-RANGE DISPERSAL

Phytoseiid mites cannot cover large distances by locomotion. In
greenhouses locomotory dispersal is probably their only option. Hence, to
determine the distances that can be covered by walking it is relevant to
consider the greenhouse situation. Van de Vrie (1985a) found females of P.
persimilis on greenhouse chrysanthemums 15 m from the location where these
mites had been released a week before. Because this mite is one of the most
agile and active phytoseiids, this result may well serve to indicate the
maximum dispersal capacity by locomotion. Hence, to reach places hundreds
of metres away the predators would have to use other modes of dispersal.
At present, there are only a few reports of phytoseiids being
transported by organisms with a higher dispersal capacity than the
phytoseiid mites. This phenomenon is called phoresy and has been
extensively reviewed by Binns (1982). Treat (1969) occasionally found
specimens of A. potentillae and Typhlodromus tiliarum Oudemans on the
abdomens of some noctuid moths. Amblyseius spp. have also been found on

ceratopogonid flies (Grogan and Navai, 1975) and aphids. Krantz (1973) reported an interesting case of Amblyseius aberrans Oudemans hitchhiking on the filbert aphid. At no time were mites observed on wingless filbert aphids. Mature female predators repeatedly attempted to board alate aphids and, if successful, they congregated on the dorsum of the thorax and the anterior abdominal tergites of the aphid. The aphids were hardly affected by the mites, as they continued to feed and could easily fly to other leaves. Krantz did not study the circumstances that induce boarding and detachment of the mites.

Another means of long-range displacement is passive dispersal on air currents. Johnson and Croft (1979, 1981) and Hoy (1982) obtained evidence that A. fallacis and T. occidentalis are aerial planktors, by succeeding in trapping them on greased plates even at considerable distances from the orchards inhabited by the predatory mites. Hoy (1982) released an OP-carbaryl resistant strain of T. occidentalis at one spot in a large almond orchard. After 1 year OP-carbaryl resistant predators were found throughout the 32 ha almond orchard. She considers it to be unlikely that the predators would have dispersed so far within a year solely by walking. Because many T. occidentalis were trapped on greased plates in and around the orchard, aerial dispersal is the most likely explanation of the spread of this predator strain. Hoy et al. (1985) crudely estimated that 100–150 million predators left an 18 ha almond orchard over one season.

Because phytoseiid females have much higher food demands than immatures and males (see e.g. Sabelis, 1985b), they reach the lower subsistence threshold earlier and are therefore likely to be the most frequent aeronauts of all life stages. Indeed, Hoy (1982) reports that all the predators she trapped on sticky panels were females: no males or immatures were recovered. Johnson and Croft (1979) report that most predators trapped were adult females (53%), but males (18%) and immatures (29%) were also captured. Of course, when food is absent, it is better to start long-range dispersal, but it is doubtful whether aerial dispersal of immatures or males is effective in terms of future reproduction (e.g. because of low chances of finding mates). If not too dehydrated, the adult females will be capable of producing offspring on a food source discovered after aerial dispersal. This is because females usually mate soon after their last moult and retain fecundity even after periods of severe starvation (see Sabelis, 1985c).

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How far predatory mites disperse on air currents is not well known. Johnson and Croft (1979, 1981) found that the number of predators trapped at 4, 8, 19, 42 and 72 m from the border of an apple orchard decreased exponentially. They conclude that predators could disperse over 72 m during a 1-month period. Hoy et al., (1985) report that predators were trapped on greased plates at 200 m distance from an almond orchard. Sabelis (unpublished data, 1982) measured the terminal rate of fall of T. occidentalis which was equal to ca. 0.5 m/s. Hence, if the wind is laminar and constant with height above the soil (no friction), dispersal distance can be calculated from the product of wind speed and height of take-off, divided by the terminal rate of fall. In the extreme case that a predator disperses from the top of a 10 m tree and the wind speed is high, e.g. 10 m/s, the distance dispersed by the mite would be 200 m. Generally, however, the height of take-off and the wind speed will be lower, and friction by the vegetation and the soil will cause the wind speed to decrease with decreasing height above the soil surface. Hence, given that Hoy et al. (1985) believe that dispersal distances may well be greater than 200 m, turbulent air movement and convection are probably of great importance in determining the distances a phytoseiid can disperse in the field.

Abiotic and biotic factors are involved in the initiation of aerial dispersal. According to wind tunnel experiments carried out by Johnson and Croft (1976) wind speed should exceed 0.5 m/s for the predators to take off. Hoy et al., (1985) observed that most aerial movements occurred between 16.00 and 22.00 h, when relative humidity and wind speed increased and temperature decreased. Among the biotic factors prey density is of crucial importance. Johnson and Croft (1981) found that as prey in apple trees became limiting to predator reproduction, predator dispersal on air currents and via the trunks into the ground cover increased thereby increasing the predator density in the weeds beneath the trees. Behavioural studies in a wind tunnel revealed a specific behaviour of adult predators (male and female), the frequency of which increased with the period of starvation preceding the experiment (Johnson and Croft, 1976). This behaviour was suspected to be involved in the initiation of aerial dispersal of A. fallacis (Johnson and Croft, 1976) and T. occidentalis (Field, 1981). When wind speed exceeded 0.5 m/s, the predators altered their behaviour from a random search movement to a directional movement

toward the edge of the experimental arena, where they terminated all forward locomotion. Subsequently, they began to orientate to the air flow and eventually assumed a standing posture by lifting the first and second pair of legs and the anterior part of the body above the substrate and supported the body with the third and fourth pair of legs. Recently, a similar behaviour has been described by Washburn and Washburn (1984) from observations on the wingless first instars of a coccid. They argue that crawlers in the standing posture experience more drag than those in walking posture because of the greater fluid momentum higher in the boundary layer. To become airborne, crawlers and phytoseiids apparently must generate enough drag to tear their tarsi from the substrate. Sabelis, Van Velden and Huisman (unpublished data, 1983) also observed the same dispersal behaviour of A. fallacis in wind tunnel experiments provided with time-lapse video equipment which enabled continuous observation over a period of 10 h with 5 recordings per second. It was found that the standing posture was not essential to achieve take-off; just before take-off the predators were frequently in a normal position with all walking legs in contact with the substrate. Moreover, similar studies of P. persimilis females revealed that these predators never exhibited the standing posture (Sabelis and Afman, 1984). They may lift their soma by stretching their legs prior to take-off, but this phenomenon could also be interpreted as the simple effect of the wind drag pushing the soma away from the substrate. This suggests that there may be alternative ways to exert control over take-off, e.g. by control over the position of the claws or by walking towards the edge of a leaf, where they are more exposed to the wind current. Hence, there are probably several ways to achieve take-off and perhaps the standing posture is functional only under particular conditions, which still have to be elucidated. The most important quenstion to be solved in future is why A. fallacis females maintain the standing posture for long periods (ranging from several minutes to 1 h) even in strong wind currents, when the main function of this posture is only to experience more drag in order to become airborne.

Although aerial dispersal is a passive method of transport, the take-off to an aerial voyage need not be so. Clear proof that aerial dispersal is initiated actively by phytoseiid mites is given by Sabelis and Afman (1984). They found that when starved for 1 day at 25°C, ca. 75% of P.

persimilis females dispersed from a leaf in a wind tunnel. However, if the
leaf had previousy been exploited by spider mites and the spider mites plus
all webbing had been removed just prior to the experiment, less than 10% of
the starved predator females dispersed. No reduction in take-offs occurred
when the exploited leaves were left unused until 1 day had elapsed since
the removal of the spider mites. This suggests that a kairomone had acted
to suppress aerial dispersal, even when the predators were starved. This
convincingly demonstrates that the starved female predators are not
abiotically dislodged from the leaves, but that they can decide when to
start aerial dispersal. As the effect of the kairomone rapidly dissipated
after the spider mites had been removed, these chemicals act as information
on prey availability. Thus, if the kairomone is perceived, it is more
beneficial to the predator to postpone aerial dispersal and continue to
search for spider mites.

SEARCHING FOR SPIDER-MITE PATCHES

Once in the air, the predator is at the mercy of the wind and will soon
land at one place or another. The mite cannot decide when or where to land,
but it may be able to fall at a faster rate (e.g. by contracting the legs)
in response to perception of volatile kairomones in the air. This needs
further investigation. Landing may take place either on a spider-mite patch
or in the kairomone-odour plume emitted by a spider-mite patch, or
elsewhere. The latter is probably the most common event as the fraction of
leaf area covered by spider-mite patches in a large environment will be
small. In that case the predator can either search for a suitable place to
start another aerial voyage or can start searching for prey in the
neighbourhood of the landing spot. When given the choice, females of at
least some phytoseiid species prefer to search on hairy leaves (Overmeer
and Van Zon, 1984), where they may have some advantage of being protected
against rain and wind. Recent investigations (Sabelis, Gunther, Schippers
and Van der Weel, unpublished data) have shown that starved females of P.
persimilis take their cue from wind direction and walk upwind. Whenever
they enter an odour plume emitted by an upwind spider-mite patch, they may
be stimulated to search for the source in several ways. According to the

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investigations mentioned above, the anemotactic response of the starved predators is intensified when the kairomone is perceived by smell. Whereas this response could help the predator move towards the odour source, chemotactic reactions could help it to keep moving along the plume. Under natural conditions an odour plume coming from a spider-mite patch close to the ground where the air flow is gentle, may retain steep tranverse gradients for several decimetres downwind. Sabelis et al. (1984b) found that starved females of P. persimilis are capable of turning back after passing a steep odour gradient of the kairomone. Most of the return-tracks could be described as right-about turns, bringing the predator from the odour-free zone back into the odour plume. The turns are triggered by temporal comparison of odour concentrations and were enacted by a self-steered change of course, i.e. not by orientation on the odour gradient itself. The ability of phytoseiids to orient on a gradual decrease in odour concentration has not yet been investigated. Another effect of kairomone perception by the predator is likely to be suppression of take-off for aerial dispersal. Sabelis and Afman (1984) found such an effect when kairomones were present on the leaf surface, and this implies that take-off behaviour may also be suppressed when kairomones are in the air. The latter response, however, needs experimental corroboration. In conclusion, positive anemotactic and chemotactic reactions combined with suppression of take-off increase the predator's chances of finding an upwind, odour-emitting spider-mite patch. The maximum distance of prey-patch location has not yet been determined, but there is little doubt that it must exceed 1 m. One of the most important questions to be solved in future is how the mites avoid being trapped in the dead-end-streets (cul-de-sacs) of a heterogeneous vegetation, while searching for a patch. It may be that the predator deposits pheromones to mark the leaf area searched and avoids recrossing these marked areas. First indications of the existence of marking pheromones have been reported by Hislop and Prokopy (1981). These marks are easily discovered at a second visit to a plant because phytoseiid mites search along the shoots, stems, leaf ribs and leaf edges. In this way a much smaller area of the plant is scanned by the predators and consequently the chance of detecting previously deposited marks is higher. The thigmotactic searching behaviour has been termed edge-oriented searching by Sabelis (1981). The predators repeatedly return to the leaf

edge or rib while walking beside these plant structures. Because female
spider mites usually found their colonies close to these structures, the
edge-oriented walk promotes the efficiency of searching for spider-mite
colonies on a plant. In Fig. 3.2 some characteristic walking patterns of A.
potentillae on rose leaves are shown. That orientation on edges is
essential to this behaviour is further corroborated by the walking patterns
presented in Fig. 3.3, which were recorded on rose leaves provided with
incisions or acentric circular holes. Apparently the predators deflect
their walking path to the side where they last detected an edge or a rib.

In future the ability of phytoseiids to locate prey patches should be
investigated in the field. This could be done by comparing the number of
invasions into trees with different levels of infestation by spider mites.
For example, Johnson and Croft (1979, 1981) found that colonization of
apple trees by A. fallacis early in the growing season occurred to an
important extent by ambulatory dispersal from the ground cover via the
trunks into the trees. They stated that colonization of the trees depended
on the density of phytophagous mites. Whether this phenomenon was the result
of predators being arrested once they arrived in the prey patch or of pre-
dators being attracted to it from a distance, remains to be elucidated.
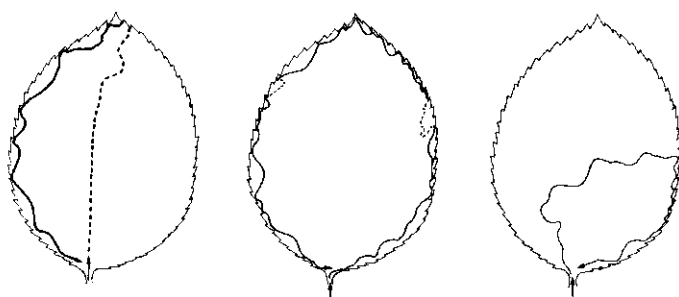
Figure 3.2. Edge-oriented walking patterns of Amblyseius potentillae on a
rose leaf (cv. Sonia). Drawn lines represent paths on the upperside of the
leaf, whereas the dotted lines are the paths on the lower side of the
leaves. If the predator invades the leaf at its lower side it usually
starts following the main rib, but near its end the rib becomes thinner
which is probably the reason why the predator meanders until it reaches the
leaf edge (leaf at the left). If the predator invades via the upperside of
the leaf, it usually follows the edges and not the main rib, being a less
pronounced structure (leaf in the middle). Occasionally, the predator
invades the leaf by roaming over the leaf surface until it reaches the edge
(leaf at the right). (Sabelis, unpublished data, 1978).

SEARCHING FOR SPIDER MITES IN THE COLONIZED LEAF AREA

After invading a leaf area colonized by spider mites the predator has
to find these prey mites. Within the webbed leaf area of a colony of two-
spotted spider mites, prey location is probably a matter of chance. Sabelis
(1981) found only minor differences between the rate of predator-prey
contact within a colony and that calculated with a model based on the
assumption of random search until physical contact between predator and
prey. This corroborated the postulate of Jackson and Ford (1973) who stated
that P. persimilis detects its prey only after chance contact and that
contact chemoreceptors enable the predator to identify its prey. The latter
suggestion was derived from predation experiments with a simultaneous
supply of water-washed and unwashed eggs of T. urticae. The water-washed
eggs appeared to run much less risk of being consumed than the unwashed.
Hence, there may be kairomones involved in the phase of acceptance.

If prey searching is random with respect to the positions of the spider
mites in the colonized leaf area, then protraction of the sojourn time in
this area is the only solution to finding more prey. Schmidt (1976, 1977)
was the first to study the influence of the components of colonies of T.
urticae on the arrestment of the predatory mite P. persimilis. The
components distinguished were eggs, fresh and old web, exuviae and other
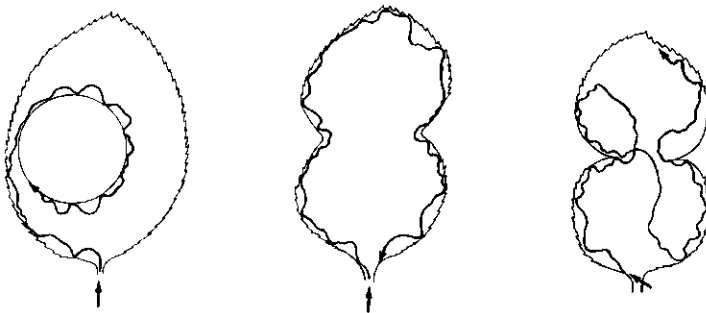residues left behind after colonization. Schmidt found that only webbing

Figure 3.3. Walking paths of Amblyseius potentillae on a leaf with an
acentric circular hole and on two leaves, one with a broad angle incision
and one with a sharp angle incision (Sabelis, unpublished data, 1978).

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influences the time spent by the predator on the infested part of a group
of bean leaves. She did not find significant differences between leaves
with fresh and old webbing, but did not analyse further whether the
arrestment by the web resulted from behaviour elicited by chemoreception or
from the more daunting topography of the web labyrinth, or more simply from
the fact that the three-dimensional structure of the web enlarges the space
for locomotion (relative to the two-dimensional surface of the leaf area
that is colonized). Since Schmidt's contribution more work has been done to
study cues that influence the arrestment of the predator in the webbed area
of a spider-mite colony. Hislop and Prokopy (1981) emphasized the chemical
properties of T. urticae silk and associated faeces to be of importance for
the arrestment of A. fallacis. They showed that both fresh and 14-day-old
silk elicit a reduced speed, longer halts, and, thus, also a longer sojourn
on silk-treated filter paper discs. The time between visits to the disc may
also have decreased, but this effect was not significant. On filter paper
discs treated with a methanol extract of the silk the time between visits
was significantly reduced, but the speed, the halts and, thus, also the
time spent per visit did not change significantly, compared with the
results for control discs. The physical and chemical factors that reduced
speed and prolonged halts on silk-treated discs were not analysed further.
Sabelis (1981) recorded the walking behaviour of different phytoseiid
species on webbed and unwebbed leaves using video equipment. In the webbed
area the walking speed is reduced and the walking pattern is very tortuous.
The tortuosity of the walking paths in the web hardly changed after contact
with prey. However, on unwebbed substrates increased tortuosity immediately
after prey contact has been reported by Eveleigh and Chant (1982a) for 2
phytoseiid species, P. persimilis and Amblyseius degenerans (Berlese).
Although tortuous paths will certainly lengthen the time a predator spends
in the webbed area, they do not provide an explanation for the long sojourn
times observed in webbed areas with abundant spider mites. Sabelis (1981)
analysed walking patterns of predatory mites in the webbed area. A model
provided with parameters obtained from the walking pattern analysis
predicted the sojourn time correctly in the case of prey being absent, but
the predictions were far too low in the case of prey eggs being abundant in
the webbed area. Sabelis (1981) hypothesized that the predators are able to
detect the edges of the webbed area and turn back as long as foraging

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provides sufficient prey. This would explain why sojourn times cannot be predicted from the walking patterns within the webbed area. To date there is evidence that predatory mites do turn back in response to loss of a kairomone stimulus. Hislop and Prokopy (1981) found that the time between return visits to paper discs treated with a methanol extract of the silk (and associated faeces) was significantly shorter than the time intervals between visits to methanol-treated paper discs. Sabelis et al. (1984b) found a similar response to loss of the stimulus of the volatile kairomone emitted from bean leaves infested by two-spotted spider mites. Females of P. persimilis turned back after passing a steep odour gradient of the kairomone (Fig. 3.4). The frequency of returns was significantly greater than measured in the control experiments. Most likely a spider-mite colony is enveloped in a cloud of spider-mite odour with a steep gradient at the edge of the colony, which, if crossed by the predator, may help it to return to the colony.

Among the factors that influence the time spent in a spider-mite colony, prey density seems to be the most important. Sabelis (1981), Ryoo (1982) and Bernstein (1984) found that the residence time increases with prey density. Ryoo (1982) additionally showed that this period did not solely depend on prey density, but also on the presence of webbing. In the absence of webbing the residence time of P. persimilis was found to be

Figure 3.4. A walking path of a female Phytoseiulus persimilis on a fine mesh gauze that had an air stream approaching the predator from below. Within the circle indicated by the dotted line the air stream was permeated by odour coming from bean leaves infested by Tetranychus urticae. The path starts in the centre of the circle (marked by +) (Sabelis et al., 1984b). Marks on x- and y-axis are at 1 cm intervals.

short, independent of the number of prey eggs per unit of leaf area.
Eveleigh and Chant (1982b,c,d) found that females of P. persimilis tended
to concentrate foraging in areas with the highest prey densities and
avoided ovipositing in areas with few prey. Another predatory mite, A.
degenerans, however, did not show these tendencies. This predator
distributed its search efforts randomly between areas with different prey
densities. Eveleigh and Chant explain this response by emphasizing that A.
degenerans is less specifically tied to two-spotted spider mites as prey
than is P. persimilis. Amblyseius degenerans feeds and reproduces on non-
prey foods such as pollen, and is even more fecund when fed with a
combination of pollen and spider mites. It would be interesting to compare
the responses of the two phytoseiid species to both the volatile and
contact kairomones of two-spotted spider mites.

When predatory mites tend to aggregate in areas with high prey density,
predator density in these areas will increase, which may lead to
interference between the predators. Fernando and Hassell (1980) studied the
changes in the rate of dispersal of P. persimilis females as a function of
the latter's density. They found that the proportion of predators leaving
increased with their density, but this effect could not be distinguished
clearly from the effect that the simultaneous decrease in prey density had
on predator dispersal. Eveleigh and Chant (1982d) also found indications
that interference limited the tendency of P. persimilis females to
aggregate in areas with high prey density. In an attempt to study the
effect of predator density on predator dispersal at a constant prey density
Bernstein (1984) showed that an increase in predator density produced an
increase in predator 'emigration'. Though his experimental design enabled a
more detailed analysis to be carried out, it is questionable whether the
prey density remained constant because ovipositing females were used as
prey and only the density of these females was kept constant. Nevertheless,
similar responses to the density of conspecific females were found for
another predatory mite, T. occidentalis (Kuchlein, 1966; Sabelis, 1981).
Again, the constancy of prey density in the latter experiments was
imperfect, but the experiments of Sabelis were carried out at very high
prey egg densities and moderate predator densities (maximally 1 predator
per cm^2 webbed area). On the basis of all evidence to date, it seems likely
that predator density is a factor influencing predator dispersal

independent of the actual prey densities. How the predators detect the presence of conspecifics, has not yet been analysed in detail. Kuchlein (unpublished data, 1978) did not find important changes in foraging behaviour during the time spent on a leaf disc that had high densities of prey and predators. There is an indication that detection of conspecifics is achieved by perception of a pheromone. Hislop and Prokopy (1981) observed shorter sojourn times on paper discs that had previously been visited by conspecific predators. Discs treated with a methanol extract were visited for shorter periods than discs treated with methanol alone, but the effect was not statistically significant.

SEARCHING FOR SPIDER-MITE COLONIES WITHIN A PATCH

Because spider mites colonize leaves only, there is a considerable part of the host plant without prey, i.e. the stems, stalks and branches connected to the colonized leaves. By leaving a prey colony the predator runs some risk of walking out of a cluster of colonies that together constitute a patch. It would be advantageous to the predator to be able to detect the borders of such a patch. Because the odour concentration falls off sharply, especially on the windward side of the patch, there are steep gradients present at its borders and, as discussed before, chemotactic reactions could help the predator to return and stay in the patch. Sabelis et al. (1984b) found that steep gradients of odour elicited right-about turns of the predators that had passed the gradient. Hence, even when the predator lost track of the odour, it was capable of returning to the position it had come from. Recent investigations (Sabelis, Gunther, Schippers and Van der Weel, unpublished data, 1984) showed that well-fed females of P. persimilis walk downwind in a kairomone-free air stream, whereas they tend to walk upwind when the volatile kairomone is in the air stream. Clearly, this response enhances the ability of P. persimilis to return to a spider-mite patch, whenever it happens to move out of it.

The above results could explain the results of simulation models predicting population fluctuations of predatory mites and spider mites within the patches formed by spider mites on rose and cucumber plants (Sabelis et al., 1983; Sabelis, unpublished data, 1986). A model assuming

that all colonies of a patch can be considered as one coherent large colony from which the predators do not disperse until after complete extermination of their prey, was very successful in predicting population trends in the greenhouse. However, models that take into account dispersal from colony to colony, gave very poor predictions unless it was assumed that the cluster of colonies was bordered by some kind of a 'wall' that acts so as to deflect predators back into the patch whenever they arrive at its edge. This so-called wall may in fact very well be the steep gradient of odour that is likely to be present around a patch under greenhouse conditions. To date, there is no evidence that predators within a spider-mite patch can home in on other colonies some distance away; dispersal from colony to colony within the patch seems to be largely random. Hence, predator aggregation in areas with high prey density results from arrestment and to a much lower extent from attraction.

KAIROMONE PRODUCTION AND REPRODUCTIVE SUCCESS OF SPIDER MITES

If kairomones are only to the benefit of the receiver and not to the emitter, then why are these chemicals produced? If these cues are not of vital importance to the spider mites or involuntary products of physiological processes, then any individual not producing them would leave more offspring and, if this ability has a genetic basis, the genes carried by this mite would be favoured by natural selection. Therefore, it is to be expected that the chemical compounds that function as kairomones in predator-prey interactions, have either a clear function in the biology of spider mites or are inevitable products of vital physiological processes. Based on these evolutionary considerations it follows that the following items are good candidates as kairomones or their sources.

(1) Silk strands seem to be a product of vital importance to the reproductive success of spider mites. According to Gerson (1979, 1985) the main functions are attachment to the plant, its use as an aid in dispersal, retention of moisture, its role in sexual communication, protection against adverse weather conditions, competitors and predators. Hislop and Prokopy (1981) extracted a kairomone from the silk and associated faeces. If the chemical is an essential part of the silk strands, then the above-mentioned

benefits of the silk should outweigh the disadvantage of producing a cue for the predator. If it is in the faeces, it should be an inevitable product of egestion.

(2) Saliva secretion is involved in extra-intestinal predigestion of the content of plant parenchyma. Perhaps the volatile kairomone is formed at secretion or only after chemical reactions between the saliva enzymes and plant chemicals.

(3) Mechanical damage to the leaves is unavoidable for any phytophagous arthropod. The plant chemicals released from the wounds could function as kairomones. However, this possibility does not explain why phytoseiid predators are capable of distinguishing between odours coming from leaves infested by Panonychus species and odours coming from leaves infested by Tetranychus species, whereas these tetranychids damage the leaves with morphologically similar mouthparts.

(4) As the kairomone comprises prey-specific components, chemicals involved in intraspecific communication between spider mites should be considered. For spider mites the chemical nature of the sex pheromones of T. urticae has been investigated. These pheromones seem to be the terpenes farnesol, nerolidol and citronellol (Regev and Cone, 1975, 1976, 1980). Being volatile chemicals these terpenes may well be involved in distant prey location. The sex pheromones of other tetranychid species have not yet been identified. Apart from sex pheromones there is sound evidence for the existence of alarm pheromones (Kuwahara et al., 1975) and aggregation pheromones (Kuwahara et al., 1982) in other acarines. The role of these and other types of pheromones has not yet been studied for spider mites.

These examples constitute the best guesses as to the source of the kairomone and its relation to the biology of spider mites.

THE ROLE OF THE HOST PLANT IN CUE PRODUCTION

So far, the role of the plant in cue production has received little attention, but there are good reasons to consider this aspect more closely in future research. When spider mites have imposed selective forces strong enough for their host plants to adapt to in evolutionary time, then it is conceivable that the host plant plays a role in cue production. Suppose

that there is a host plant genotype releasing volatile chemicals upon attack by spider mites, then any predator genotype coding for a positive searching response may be favoured by natural selection when the spider mites on the host plant are sufficiently abundant to ensure the predator's reproductive success. In this way chemical communication between the host plant and the predator may evolve which is to the benefit of both the emitter and the receiver. Chemicals with such a function are called synomones (Nordlund and Lewis, 1976). Whether such chemicals play a role in the interaction between phytoseiid mites and the host plants of their phytophagous prey is still to be elucidated. That the host plant is involved in the production of the volatile kairomones is suggested by the results of experiments by Sabelis et al. (1984a). They found a positive response to spider-mite odour only when the odour came from spider mites feeding on leaves. Air blown over spider mites isolated from their host plant did not elicit a response of the phytoseiid predators. Moreover, it was shown that the kairomone could be produced only by the mobile stages, when feeding, and not by the quiescent stages. That phytoseiid predators can distinguish between the odours of different tetranychid species (Sabelis and Van de Baan, 1983) feeding on the same host plant species, does not contradict the hypothesis presented above. The specificity of the odours may originate from the response of the host plant on a particular tetranychid species. Therefore it is certainly worthwhile to consider the role of the host plant in the interaction between spider mites and their predators. This point has been stressed by Price et al. (1980) with respect to tritrophic systems in general.

PREY SELECTION AND THE PREDATOR'S REPRODUCTIVE SUCCESS

Reproductive success depends to a major extent on the rate of prey consumption and hence also on prey availability, searching behaviour and prey selection. Predators that search and select prey species so as to maximize their contribution of progeny to the next generations are favoured by natural selection, if there is a genetic basis for such foraging behaviour. If so, it can be assumed that the behavioural responses elicited by cues from their prey have evolved because they led the predator to

profitable prey species, i.e. profitable in terms of the predator's reproduction. This hypothesis can be tested as the response to kairomones and the reproductive success can be measured in independent experiments and subsequently compared to reveal any relation. Experimental tests of the above evolutionary hypothesis may have practical implications for screening phytoseiid species before field experiments on biological control are carried out. Usually, it is not possible to carry out field tests with all possible predator candidates. Hence, there is a need of methods and criteria for initial screening. If it can be proven that the olfactory response of the predators is a reliable guide to the selection of the most promising predators for use in biological control of spider mites, then screening would become more efficient, because experiments with two-choice discs and Y-tube olfactometers are not time consuming and do not require a complicated set-up.

To date, some experimental tests of the above hypothesis have been reported in the literature. Hoy and Smilanick (1981) found that T. occidentalis was arrested by residues of Tetranychus and Eotetranychus species. Cues of Panonychus species had a significant but less pronounced effect on arrestment of this phytoseiid predator (as also reported by Hislop et al. (1978) for A. fallacis). The same holds for the effect of cues of Bryobia species on T. occidentalis. Previous conditioning of T. occidentalis by rearing stock cultures on P. ulmi instead of on T. urticae did not affect the results. Thus, the differences in responses to P. ulmi and T. urticae cues appear to be inherent rather than influenced by previous exposures to prey residues during development and young adulthood. Hoy and Smilanick state that the responses elicited by the cues of different prey species parallel the relative effectiveness of this predator against the above prey species in the field. The authors did not, however, determine reproductive success of T. occidentalis on these prey species. Another test has been reported by Sabelis and Van de Baan (1983). They found that hungry females of A. potentillae and A. finlandicus were attracted by volatile kairomones emitted by P. ulmi feeding on apple leaves, but not by T. urticae feeding on either apple or bean leaves, nor by Tetranychus viennensis Zacher feeding on apple leaves. This was a surprising result because they used A. potentillae that had been reared for several years on T. urticae (but perhaps not surprising in view of the

results of the conditioning experiments carried out by Hoy and Smilanick).
Moreover, the developmental and ovipositional rates of A. potentillae on
both tetranychid species do not differ in any conspicuous way (Overmeer,
1981, Sabelis, 1981). They key to the problem lies in the effect of webbing
on predation and hence on reproductive success. Both the rearing method and
the life history experiments that these authors performed with T. urticae
as prey, were set up so as to minimize the negative effects of webbing. The
presence of webbing would have hampered the searching behaviour of A.
potentillae to an important extent and hence also its life-history
parameters, such as development, mortality and reproduction. Sabelis (1981)
found that the webbing caused predation success to be lower than on
substrates without webbing. Moreover, females of A. potentillae were
sometimes observed to stick to the silken threads with their dorsum and
they tended to avoid the webbed parts of a leaf, when given the choice.
Almost no numerical response of A. potentillae to increasing densities of
T. urticae has been observed (Sabelis and Van de Vrie, 1979; Sabelis,
unpublished data). Hence, because reproductive success on P. ulmi is high
as this spider mite does not produce a complex web like T. urticae, it does
make sense that A. potentillae prefers P. ulmi relative to T. urticae.
Sabelis and Van de Baan (1983) argue that the same explanation holds for
the differential response of A. finlandicus, a predator that is also
hampered by the web of Tetranychus mites.

Sabelis and Van de Baan (1983) also found that satiated females of P.
persimilis respond to kairomones emitted by plants infested by T. urticae,
but not to kairomones emitted by plants infested by P. ulmi. Recent
investigations showed additionally that starved females responded to both
spider-mite species when offerend against the alternative of non-infested
plant leaves, but when these prey species were offered simultaneously in
the Y-tube olfactometer, the starved females clearly preferred to walk
towards the arm containing air blown over the leaves infested by T. urticae
(Sabelis and Groeneveld, unpublished data). Because the predators used in
the olfactometer experiments had no previous experience with P. ulmi as
prey, it can be concluded that P. persimilis has an innate behavioural
response to prey odours. Moreover, these responses have a hierarchical
structure: T. urticae is preferred to P. ulmi. This preferential response
parallels the reproductive success of P. persimilis on these prey species.

According to Sabelis (1981) the predation success of this predator against all stages of T. urticae is much higher in the web produced by this prey, than on leaves without web, other things being equal. As P. ulmi does not produce a complex web, it is to be expected that predation success on this spider mite will be low. This hypothesis was not rejected by predation experiments. Females of P. persimilis did not reproduce when adult females of P. ulmi and their eggs were offered as prey. They even died after a few days despite the abundance of this prey. However, larvae and nymphs of P. ulmi were suitable prey stages for P. persimilis; the predators succeeded in attacking and feeding on these prey stages and they did also produce eggs, but the rate of reproduction by the predator was found to be considerably lower on P. ulmi than on T. urticae. It can therefore be concluded that the predator perceives odours from different spider mite species and responds to these kairomones so as to maximize its chances at reproductive success.

FUTURE PROSPECTS

That phytoseiid predators select prey species so as to maximize their reproductive success is supported by some evidence from recent investigations. Though this hypothesis clearly makes sense, uncritical acceptance should be avoided, especially when much more decisive tests are conceivable. To clarify this point we present some of the many questions to be solved in the future.

(1) Why do spider-mite species betray themselves by producing odours? Are these volatile chemicals necessary byproducts? Could their production be avoided? Are spider mites capable of 'masking' their odour (e.g. by continuously changing the chemical composition)? (Note that this would render the predators less capable of finding their prey despite the prey's profitability.)

(2) Are there tetranychid species which are chemically protected against predators by (slowly acting) systemic poisons present in their tissues and, if so, do the odours emitted by these prey species elicit averse responses of the predators?

(3) What is the role of plant-produced odours in eliciting the

searching response of the predators? Are these odours continuously produced or only after the plant is damaged by spider mites?

(4) Is the predator's response to prey odours really a heritable trait? If so, is there any genetic variation with respect to the trait? How fast do the gene frequencies change in environments with prey species compositions that are different from those under the current field conditions? (Think of mass rearings on artificial diets or on prey species that are irrelevant in the field or consider differences in prey species composition between locations. Indications for intraspecific variability in olfactory responses to prey odours have been reported by Dicke (1984).)

(5) Are the prey choices assessed in laboratory experiments representative of the prey choices made under field circumstances? (Electrophoresis of field-sampled phytoseiids may be a way to analyse this; see Solomon et al., 1985)

(6) Does prey selection by phytoseiid predators generally lead to maximum reproductive success? (Other acarine predator-prey interactions than those discussed in this chapter should also be analysed before inferences can be made about the generality of the maximization principle.)

Experimental analysis of the above questions will prove to be a fertile area of basic research that may also be of practical importance.
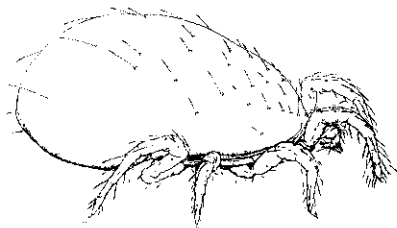
PART II. ORIGIN(S) AND FUNCTION(S) OF INFOCHEMICALS IN A PREDATOR-PREY-PLANT SYSTEM: *Phytoseiulus persimilis, Tetranychus urticae* AND *Phaseolus lunatus.*

4. Volatile spider-mite pheromone and host-plant kairomone, involved in spaced-out gregariousness in the spider mite *Tetranychus urticae*.

Marcel Dicke

ABSTRACT

From the host plant-spider mite complex <u>Phaseolus lunatus</u>-<u>Tetranychus urticae</u> Koch a volatile chemical is emitted that acts as a kairomone for the predatory mite <u>Phytoseiulus persimilis</u> (Sabelis et al. 1984a). This kairomone is apparently a byproduct of a vital physiological process and/or it has a function in the biology of the spider mite as well.

The spider mite-host plant complex also emits a volatile spider-mite dispersing pheromone. This is shown in the present study where spider mites were introduced into an odour patch on a horizontal screen in a vertical airflow olfactometer. When spider-mite infested leaves of Lima bean are offered, the spider mites walk mainly straight and soon reach the edge of the screen. On the other hand, when clean Lima bean leaves are offered, the mites walk tortuously most of the time and reach the edge of the screen much later. Artificially damaged plants elicit the same response as undamaged plants. Differences in spider-mite behaviour are observed in the vertical airflow olfactometer when odour of either clean or spider-mite infested leaves is offered. A comparison of the behaviour in these two situations with that when no odour was offered suggests that Lima bean leaves emit a volatile kairomone that activates T. urticae and makes them return after losing the stimulus. A Y-tube olfactometer experiment confirms the existence of this kairomone.

At a low ratio of dispersing pheromone to plant kairomone the spider mites behave as if only kairomone is present, walking mainly tortuously. At a high ratio they disperse. No aggregation-pheromonal effect is observed.

The possibility that the spider-mite dispersing pheromone acts as a kairomone for <u>P.persimilis</u> is discussed.

INTRODUCTION

The phytophagous spider mite <u>Tetranychus urticae</u> Koch (Acarina: Tetranychidae) has a clustered distribution: female spider mites and their progeny form colonies in a self-produced web. The number of spider mites in a new colony increases exponentially and the colony spreads over the host-plant leaf. Teneral females that develop in these colonies disperse, and

establish new colonies nearby (Hussey and Parr,1963). An infested host plant therefore contains many spider-mite colonies.

Many phytophagous insects are known to have an optimal density range (for review, see Prokopy, 1981). In black bean aphids, adults originating from an initial nymphal population of 8 per plant reproduced more rapidly than adults originating from lower or higher densities (Way and Banks, 1967). Apparently a small aggregation of aphids is better able to direct nutrients to its leaf than is a single individual, as a result of combined feeding activities (Way and Cammell, 1970). On the other hand, too large an aggregation causes a local shortage of food (Way and Banks, 1967).

A similar situation may exist for T. urticae. Storms (1971) supplied bean plants with ^{32}P-phosphate and demonstrated that a local mite infestation caused an increased transport of phosphates to the infested leaf, probably because the mites injected saliva into the host plant. However, although a clustered distribution may have advantages, too high a density results in a reduced developmental rate, survival, ♀:♂ sex ratio (Wrensch and Young, 1978) and fecundity (Attiah and Boudreaux, 1964; Wrensch and Young, 1975) in Tetranychus species. In the trade-off between the two effects it will, at some stage, become of selective advantage to leave the colony and establish a new one.

Tetranychus urticae is a prey species for many species of predatory mites. According to recent research, chemical cues play an important role in prey searching by predatory mites (for review see Sabelis and Dicke, 1985). Sabelis and Van de Baan (1983) found that a spider-mite-species-specific volatile kairomone, emitted from host plants infested by T. urticae, is used in prey location by the predatory mite Phytoseiulus persimilis Athias-Henriot. Sabelis et al. (1984a) found that (a) all active stages contribute to the kairomone production, (b) the kairomone is produced during feeding, (c) the kairomone is mainly present on the leaf surface and to a minor extent in the spider mite faeces and (d) the predator does not respond to host plants on which spider mites have been present for 0-3 h. Sabelis and Van de Baan (1983) found that after spider mites had been removed from the host plant, the leaves remained attractive to the predator for at least 3 h. Thus, the kairomone is a product of the interaction between the host plant and T. urticae.

The semiochemical is used by P. persimilis in prey location, but it may

have an important function in the biology of the spider mite as well. Otherwise it is expected that selection would have acted against kairomone production. The predators may cue in to several spider-mite related chemicals but as a result of natural selection, only a response to a chemical with an indispensable function for the prey will last for a long time (on an evolutionary timescale). Such a dual role of semiochemicals has been found in several interactions of parasite and host (e.g. Lewis et al., 1982). We may hypothesize about intraspecific functions of the T. urticae kairomone: it could be an inevitable excretion product, or a byproduct of an enzymatic reaction following injection of saliva into the host plant, or it could be a spider-mite pheromone, whose chemical structure may be identical to that of the aforementioned two spider-mite products. To date, only sex pheromones have been reported in the Tetranychidae. Penman and Cone (1972,1974) give evidence that male T. urticae orient themselves towards quiescent deutonymph females. Regev and Cone (1975,1976,1980) presented indications that three terpenoids isolated from quiescent deutonymph females act as sex pheromones for T. urticae.

Aggregation pheromones or pheromones that influence the spatial distribution by causing animals to disperse from presently or potentially overcrowded food sources have not yet been reported for spider mites. The latter pheromones have been called epideictic pheromones (Prokopy, 1981) and comprise e.g. oviposition deterring pheromones and marking pheromones. In a recent review of these pheromones the term 'epideictic pheromone' was not used anymore but was replaced by terms such as 'dispersing' or 'spacing signals' (Prokopy et al., 1984). As the word 'epideictic' is not very informative, I prefer to use the terms 'dispersing pheromone' and 'spacing pheromone'. A dispersing pheromone makes individuals leave the pheromone source, whereas a spacing pheromone also somehow restrains that dispersal, thereby keeping the individuals at a certain distance from each other. Such pheromones may influence several types of behaviour, such as oviposition-site location, host- or prey-searching or food-plant location. Although T. urticae has an aggregated distribution, it may produce a dispersing or a spacing pheromone. To describe such spaced aggregations Kennedy and Crawley (1967) introduced the term 'spaced-out gregariousness'. They studied the behaviour of the aphid Drepanosiphum platanoides (Schrank) and showed that both aggregative and repellent responses existed, together resulting in a

certain degree of aggregation. The only stimuli mentioned for this aphid were tactile. In T. urticae both an aggregation pheromone and a dispersing or a spacing pheromone could influence spatial distribution. Bernstein (1984) demonstrated a positive relationship between the proportion of T. urticae females that left a host plant and the time during which spider mites had been present on that host plant. However, it cannot be concluded from his experiments whether this phenomenon was the result of a dispersing pheromone or of the deterioration of the host plant. In this paper I present the results of a search for an intraspecific function of the volatiles that are emitted from host-plant leaves infested by T. urticae, and to which P. persimilis responds. The behaviour was studied of T. urticae females that were offered volatiles of the substrate from which the kairomone is emitted: bean leaves infested by conspecific mites.

MATERIAL AND METHODS

Spider mites.

Spider mites were reared on Lima bean (Phaseolus lunatus L.) at 20-30°C, 50-70% r.h. under continuous fluorescent light that was added to the natural daylight regime. In all experiments the behaviour of well-fed females in the oviposition phase was studied. These were taken with a fine brush from heavily infested green leaves and immediately transferred to the olfactometer.

Olfactometers.

The objective was to elucidate the possible pheromonal function for T. urticae of the spider-mite kairomone that is used in prey location by P. persimilis. The expected types of spider-mite response towards odour of spider-mite infested leaves were movement towards the odour source, movement away from the odour source, or no net spatial redistribution at all. These three types of response will be called attraction, repellency and arrestment respectively. Different bioassays can be used to study these three types of behaviour. Either a vertical airflow olfactometer or a Y-tube olfactometer may be used to test for attraction (see Sabelis et al., 1984b, and Sabelis and Van de Baan, 1983, respectively). The latter

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apparatus, however, is not suitable for the study of arrestment or repellency, as in these cases the semiochemicals involved may prevent the mite from walking upwind. A vertical airflow olfactometer offers a suitable bioassay when the semiochemicals involved elicit attraction, arrestment or repellency. Therefore, to study whether aggregation pheromones or dispersing or spacing pheromones are emitted from a host plant - spider mite complex, a vertical airflow olfactometer was used. A Y-tube olfactometer was used to ascertain whether clean Lima bean leaves emit a volatile chemical that functions as kairomone for T. urticae.

The olfactometers were used in a climate room ($26 \pm 1°C$, 60 ± 10 % r.h.). The air stream in the olfactometers was generated by a suction cleaner outside the climate room.

Vertical airflow olfactometer (Figure 4.1).

A vertical airstream flowed through a horizontal screen (24 x 24 cm, gauge 0.2 mm) surrounded by a glass observation box. A spider mite could be introduced onto the centre of the screen through a small hole in one of the walls of the observation box. The spider mite was allowed to walk off the brush and from that moment on, its walking path was plotted (with cross-lines at 10 s intervals) on graph paper. This was facilitated by a grid that was drawn on the screen as well as on the graph paper.

In the airstream (speed 0.3 - 0.4 m/s) a cylinder with odour-emitting objects can be placed, which creates a circular odour field (diameter 4 cm) with a steep odour gradient in the centre of the screen (Figure 4.2). The olfactometer is described in more detail by Sabelis et al.(1984b).
The spider mites were observed under six treatments:
(1) airstream without odour,
(2) airstream with odour of 7 clean Lima bean leaves (surface area ca. 20 cm^2) in the central odour field,
(3) airstream with odour of 7 Lima bean leaves infested by all stages of T. urticae (ca. 15 adult females, 200 juveniles and 1000 eggs per leaf) in the central odour field,
(4) airstream with odour of 7 clean Lima bean leaves that had been damaged 1 h before the experiment started, by rubbing their underside with Carborundum on a pad of wet cotton,
(5) airstream with odour of 7 clean Lima bean leaves that had been damaged

22 h before the start of the experiment, as described under (4), and

(6) airstream with odour of 7 clean and 7 infested Lima bean leaves in the central odour field.

In each treatment the leaves were used in the olfactometer for about 1 h. Forty spider mites were observed individually in each treatment. An observation ended when the spider mite reached the edge of the screen or when it had been present on the screen for 15 min. A maximum of five spider mites was observed per day to minimize the risk of pheromone contamination on the screen. No trend could be seen in the behaviour of these five spider mites and thus there is no indication of interference from a pheromone that might have been deposited on the screen. After every observation the screen was brushed, to remove the silk threads that the spider mites produce continuously while walking (Saitô, 1977).

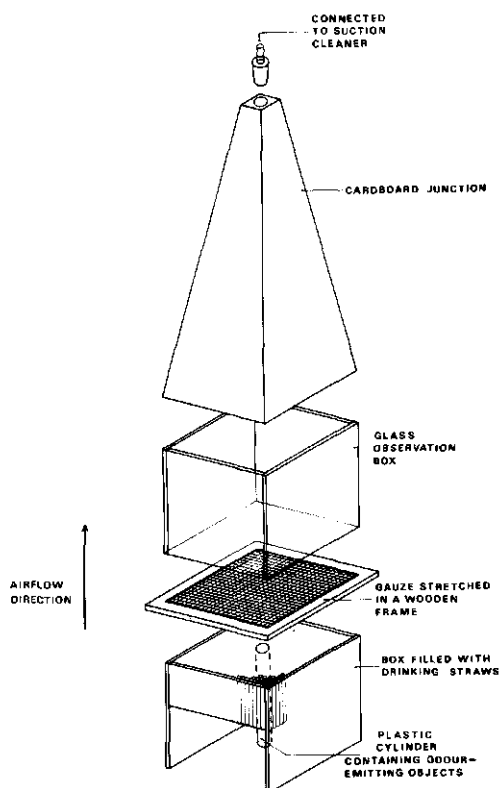The following parameters were determined to characterize the walking

Figure 4.1:
Vertical airflow olfactometer
(Sabelis et al., 1984b).

path of each spider mite:

(1) The number of returns to the central circle: a return was scored when the spider mite that had left the central circle re-entered it.

(2) The time spent in the olfactometer: the time until the mite reached the edge of the screen. In the case where the mite did not reach the edge of the screen within 15 min. this time period was scored for the mite.

(3) The percentage of time spent walking more or less straight: the percentage of 10 s periods in which the mite walked without recrossing its path or turning through angles of more than 90 degrees. Although this walking pattern is not straight (sensu stricto), the term 'straight' is used for convenience to characterize it.

(4) The percentage of time spent walking tortuously: the percentage of 10 s periods in which the mite recrossed its own path and frequently turned through angles of more than 90 degrees.

(5) The percentage of time spent standing still: the percentage of 10 s periods in which the mite remained stationary.

Y-tube olfactometer.

The Y-tube olfactometer consisted of a Y-shaped glass tube with an iron wire in the centre. At the end of both arms of the olfactometer odour-emitting objects could be placed in PVC cages. Air was sucked out at the base of the tube. The air speed in both arms was measured with a hot wire anemometer and standardized at 0.7 ± 0.1 m/s by inserting dry cotton wool in the inlets of the airstream and/or changing the total airflow by means of a valve. In this olfactometer an experiment was carried out to study the response of the spider mites to odour coming from clean Lima bean leaves. In one arm 7 small Lima bean leaves were offered. Both arms were provided with wet cotton wool so that the humidity would be equal. Sixty spider mites were observed individually until they reached the far end of one of the arms. After each observation the iron wire was brushed to remove silk. After each series of ten observations the PVC cages were uncoupled and connected to the opposite arm of the olfactometer. For further details of the olfactometer or the experimental procedure, see Sabelis and Van de Baan (1983).

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Statistical methods.

The results of the observations in the vertical airflow olfactometer were subjected to an analysis of variance to determine whether the six values (corresponding to the six treatments) of any parameter that was measured were drawn from the same population. If the result was negative, the Tukey test (Snedecor, 1957) was applied to detect the significant differences (0.05). To obtain normal distributions the time spent in the olfactometer was transformed to its reciprocal multiplied by the maximum experimental time (900 s), and the number of returns was transformed to its square root.

The Y-tube olfactometer results were analysed with a sign-test under the null hypothesis that the probabilities of spider mites walking into either arm, were equal. Given the null hypothesis and the number of spider mites reaching the far end of either arm the critical level can be determined by use of the binomial distribution. When the critical level was less than 0.05, the null hypothesis was rejected.

RESULTS

After time-consuming observations on 240 spider mites and analysis of their walking patterns, the parameters characterizing the spider mite behaviour in the vertical airflow olfactometer were extracted (Table 4.1a). The associated analysis of variance (Table 4.1b) shows that the null hypothesis, that the values obtained in different treatments originate from the same population is rejected for all variates. The significant differences between two means, according to the Tukey test, are specified in Table 4.1a. Characteristic walking patterns of spider mites in the six treatments are presented in Figure 4.2. Figure 4.3 shows the time allocation by the spider mites under different treatments.

Table 4.1a: Walking pattern characteristics of female T. urticae in vertical airflow
olfactometer, when different odours are offered in central circle. Forty females were
observed per treatment. Figures in same column that are followed by the same letter do
not differ significantly from each other (α= 0.05, Tukey test). For more complete
descriptions of walking pattern characteristics see 'Material and Methods'.

Treatment	Mean time in olfactometer (s)	Mean number of returns to central circle	Percentage of time spent:		
			walking straight	walking tortuously	standing still
1. No odour	730a	0.9a	22a	47a	31ac
2. Clean bean leaves	680a	3.3bc	37a	52a	12b
3. Infested bean leaves	440b	1.6ac	72b	21b	7b
4. Artificially damaged bean leaves (1 h)	590a	2.7bc	30a	56a	14b
5. Artificially damaged bean leaves (24 h)	560ab	2.2ac	40a	41ab	19bc
6. Clean plus infested bean leaves	560a	1.7ac	36a	50a	14b

Table 4.1b: Analysis of variance.

Walking pattern characteristic	F-value	Critical value
Maximal experimental time divided by residence time in olfactometer	7.2	<0.001
Square root of number of returns to central circle	3.2	<0.01
Mean percentage of time walking straight	13.7	<0.001
Mean percentage of time walking tortuously	7.7	<0.001
Mean percentage of time standing still	7.8	<0.001

Response to odour of clean leaves versus response to odour of infested leaves.

The spider-mite behaviour showed large differences between treatments 2 (clean leaves) and 3 (spider-mite-infested leaves) (Table 4.1a, Figure 4.2 parts 2 and 3). When the odour of spider-mite-infested leaves was offered, the females spent an average of 440 s in the olfactometer, walking straight most of the time (72%) (Figure 4.2 part 3). When the odour of clean leaves was present, however, the mites stayed significantly longer in the olfactometer (680 s) as they spent most of the time (52%) walking tortuously and only 37% of the time walking straight (Figure 4.2 part 2). These behavioural differences imply that the spider mites distinguish between the odour of clean and spider-mite infested leaves. This may be the result of (1) a positive response to odour of clean leaves and a neutral response to odour of spider-mite infested leaves, (2) a neutral response to odour of clean leaves and a negative one to odour of infested leaves, or (3) a positive response to odour of clean leaves and a negative response to odour of infested leaves. To distinguish between these possibilities, the behaviour of the spider mites in treatments 2 and 3 should be compared with that in treatment 1, in which no odour was present.

Response to odour-free airstream versus either response to odour of clean leaves or to odour of infested leaves.

The response of the spider mites in an odour-free airstream (Table 4.1a, Figure 4.2 part 1) is characterized by a low number of returns and a high percentage of time that is spent standing still (31%). This low number of returns is not the result of each individual mite returning less frequently to the imaginary central circle. In this treatment (1), 15 spider-mite females never left the central circle during the 900 s of the experimental period (this phenomenon never occurred in the other 5 treatments !): there was a strong tendency to stay near the place of release. This may be interpreted as a tendency in the spider mites to try to regain contact with the lost resource. Sabelis et al.(1984b) found the same phenomenon in well-fed females of the predatory mite P. persimilis. When hungry (i.e. taken from a place without food), the predators did not
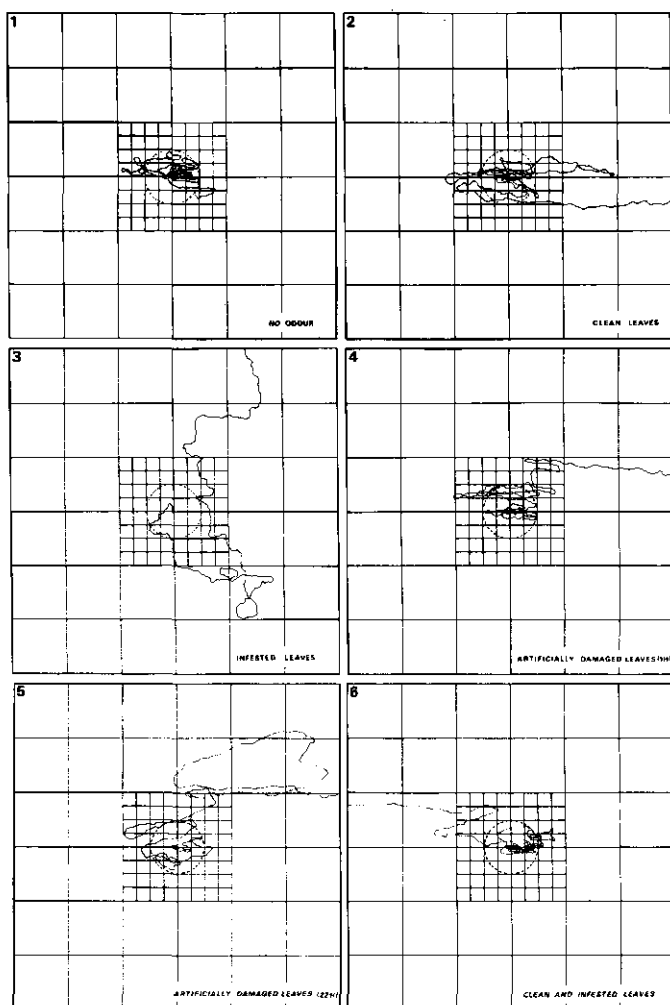
Figure 4.2: Characteristic walking patterns of female T. urticae in
vertical airflow olfactometer.
a) no odour in central circle
b) odour of 7 clean Lima-bean-leaves in central circle
c) odour of 7 spider-mite infested Lima-bean-leaves in central circle.
d) odour of 7 clean Lima-bean-leaves, artificially damaged 1 h before the
    experiment, in the central circle
e) odour of 7 clean Lima-bean-leaves, artificially damaged 22 h before the
    experiment, in the central circle
f) odour of 7 clean and 7 spider-mite infested Lima-bean-leaves in central
    circle.

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show this tendency to stay at the place of release when an odour-free airstream was offered.

Comparison of the response of T. urticae females when no odour was present with the response to odour of clean bean leaves in the central circle suggests that the odour of bean plants activates the spider mites (less time is spent standing still). After leaving the central circle they also returned more frequently when odour of clean leaves was present in the central circle. This behaviour could have been incited by a plant-produced kairomone. The existence of such a semiochemical is shown by the experiment in the Y-tube olfactometer (Table 4.2): the spider mites preferred the arm with Lima-bean-leaf odour over the control arm. This is the first demonstration of involvement of a volatile plant kairomone in host-plant recognition by a spider mite.
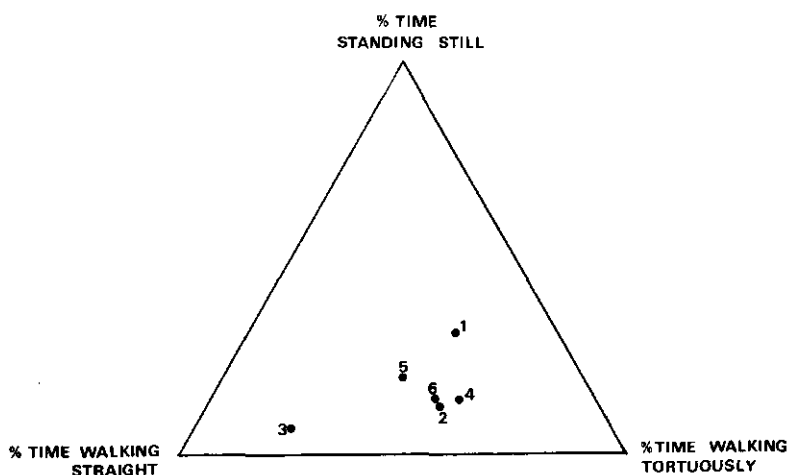
Figure 4.3: Time allocation by female T. urticae in vertical airflow olfactometer in different treatments.
1) no odour,
2) odour of 7 clean Lima bean leaves,
3) odour of 7 spider-mite infested Lima bean leaves,
4) odour of 7 Lima bean leaves artificially damaged 1 h before the start of the experiment,
5) odour of 7 Lima bean leaves artificially damaged 22 h before the start of the experiment, and
6) odour of 7 clean plus 7 spider-mite infested Lima bean leaves in central circle.

The response to the odour of spider-mite infested leaves in the vertical airflow olfactometer was distinctly different from the response when no odour was present. Time allocation was totally different in the two treatments (Table 4.1a and Figure 4.3). This resulted in a much shorter time before the mites reach the edge of the screen in the presence of odour from spider-mite infested leaves (440 s versus 730 s). The differences in behaviour of the mites under treatments 1 and 3 imply that leaves infested with T. urticae emit a dispersal-inducing odour. If this odour is produced by the spider mites it is a pheromone, but if it is produced by the plant after damage, then it is an allomone according to the present definitions of these terms (Nordlund and Lewis, 1976).

Response to odour of artificially damaged leaves.

To study whether the dispersal-inducing odour originates from the plant, I offered the spider mites artificially damaged host-plant leaves. Of course, this damage is different from spider-mite damage but a host plant infested by any other phytophage could not be used because in that case other phytophage-related allelochemicals might influence the spider-mite response. It has been shown that several methods of inflicting damage artificially will evoke the same effects (qualitatively or quantitatively) in a host plant as damage by phytophagous insects (Ryan and Green, 1974) or mites (Karban, 1985). Both recently damaged leaves (damaged 1 h before the

Table 4.2: Response of T. urticae females in Y-tube olfactometer.

Contents of arm 1	Contents of arm 2	n*	n(1)	n(2)	Critical level
7 clean Lima bean leaves plus wet cotton wool	wet cotton wool	60	40	20	0.007

* n= number of observations.
n(1), n(2)= number of mites that reached the far end of arms 1 and 2, respectively.

start of the experiment) and leaves that had been damaged 22 h before the start of the experiment were used. In both treatments the response of the spider-mite females was identical to that when the odour of clean leaves was present (Table 4.1a, Figure 4.3). There is thus no evidence that a plant-produced allomone plays a role in causing the response of T. urticae to the odour of leaves infested by conspecifics, either when short term or relatively long term physiological processes are allowed to take place in damaged leaves. It can therefore be concluded that a dispersing pheromone is emitted from T. urticae-infested bean leaves.

Response to a mixture of clean and infested leaves.

Both a plant kairomone and a spider-mite pheromone are emitted from the host plant-spider mite complex. At the offered pheromone/kairomone ratio (treatment 3) the spider mites dispersed (Table 4.1a, Figure 4.2 part 3). The question arose whether lower pheromone/kairomone ratios would evoke an aggregative or a neutral response. To study this 7 clean and 7 infested bean leaves were offered in one treatment (number 6). In this case the same amount of dispersing pheromone was offered as in treatment 3. However, this amount of pheromone was mixed with an additional amount of plant kairomone, emitted from the clean leaves. Surprisingly this first trial already resulted in a spider-mite response that did not differ significantly from the response to clean bean leaves. Hence, after reducing the ratio of spider-mite pheromone to plant kairomone, the spider mites behaved as if only plant kairomone was present. The ratio of spider-mite pheromone to plant kairomone apparently informs the spider mites about the level of infestation. At this low pheromone/kairomone ratio no aggregative response was observed in T. urticae (compare the behaviour in treatments 2 and 6 (Table 4.1a, Figure 4.2 parts 2 and 6, and Figure 4.3)) and thus the data give no evidence for aggregation pheromonal activity of a low dispersing pheromone/plant kairomone ratio.

DISCUSSION

Volatile spider-mite dispersing pheromone.

Compared with other spider mites, e.g. Panonychus ulmi (Koch), the two-
spotted spider mite has a distribution that is more aggregated. Some
advantages of an aggregated life style may lie in communal defence,
increased nutrient flow to the infested leaf, or increased chances of
finding a mate. Disadvantages include increased chances of detection by
predators, or rapid food depletion locally. The present study did not find
evidence for a volatile aggregation pheromone. Aggregation behaviour is
probably mediated by other substances, for example silk, or a non-volatile
pheromone. On the other hand, this study shows that a dispersing pheromone
is emitted from T. urticae-infested leaves. Because artificially damaged
plants do not emit an allomone, the origin of the dispersing pheromone is
either the spider mite or the interaction between spider mites and host
plant. In both cases spider mites are involved in pheromone production,
either directly or indirectly. In the latter case the definition of a
pheromone (a substance, secreted by an organism to the outside, that causes
a specific reaction in a receiving organism of the same species; Nordlund
and Lewis, 1976) may not cover what is called here a dispersing pheromone.
However, the word 'secreted' should be interpreted as 'produced or
acquired', words that are used in the definition of a kairomone to describe
the origin of this semiochemical (Nordlund and Lewis, 1976).

It is assumed that the dispersing pheromone is of advantage to the
individuals that produce it. Otherwise it could be expected that natural
selection would act against production of the compound. An intriguing
question is why T. urticae uses a volatile dispersing pheromone. Volatiles
may inform distant predators about spider-mite presence. Other, less risky,
information sources for the spider mite can be thought to induce dispersal,
e.g. physical contact with conspecifics, silk, faeces or a contact
pheromone. Any spider-mite individual that does not secrete (sensu lato)
the volatile dispersing pheromone but uses another source of information
about the profitability of the food source, is expected to be favoured by
natural selection if this characteristic is genetically determined.
However, as the dispersing pheromone is volatile, two hypothetical

situations can be envisaged: (1) The spider mite can control secretion (sensu lato) of the semiochemical and the advantages of the volatile dispersing pheromone, e.g. creation of an uninfested territory around the colony, are more important than the disadvantages of possibly attracting predators; or (2) the spider mite cannot control secretion (sensu lato) of the volatile dispersing pheromone. The plant controls secretion (sensu lato) after recognizing the damage specific to that specific spider-mite species. For the plant such a semiochemical offers no advantages when it only functions to disperse spider mites over short distances, but attraction of predators may be more important to the plant and nearby genetically related conspecific plants. In this second case, for the spider mite the semiochemical would be an inevitable byproduct of damaging a host plant. As its secretion cannot be prevented, the spider mite makes the best of a bad job by using the compound as dispersing pheromone.

At present a choice cannot be made between the above hypotheses, but elucidation of the chemical structure of the semiochemical may help to ascertain the role of the plant.

Are spider-mite dispersing pheromone and spider-mite kairomone identical?

The predatory mite P. persimilis uses a volatile semiochemical as kairomone that evaporates from a host plant infested with T. urticae. Are there any indications that this kairomone is identical to the volatile dispersing pheromone of T. urticae?

Sabelis et al. (1984a) elucidated the origin of the spider mite kairomone to a large extent. However, as interaction of host plant and spider mite is needed for kairomone production, they could not draw a definite conclusion on the origin. Sabelis and Van de Baan (1983) argue that the kairomone is not a compound that originates from host-plant damage as P. persimilis is attracted to leaves infested with T. urticae, but not to those infested with P. ulmi. As the mouthparts of these spider mites are very similar, the type of damage they cause will also be similar.

Even less is known about the origin of the spider-mite dispersing pheromone. To gain insight into whether the dispersing pheromone acts as a kairomone for P. persimilis, more knowledge is needed about the origin of the dispersing pheromone. Nevertheless there is some evidence that the

dispersing pheromone acts as a kairomone for P. persimilis: (1) both
semiochemicals are volatiles; (2) neither the spider-mite kairomone (Dicke,
unpublished results) nor the dispersing pheromone is emitted from
artificially damaged leaves; (3) all feeding stages of T. urticae produce
the kairomone (Sabelis et al. 1984a) and both semiochemicals are used as an
indicator of spider mite density (Sabelis and Van de Baan, 1983; this
paper). Thus, if the spider-mite kairomone and the dispersing pheromone
were identical, all spider-mite stages would contribute to the dispersing
pheromone. However, only elucidation of the chemical structure of both the
spider-mite dispersing pheromone and the spider-mite kairomone can
definitely reveal whether this pheromone or some of its components act as
kairomone for the predator. This will be the emphasis of future research.

Volatile plant kairomone.

Spider mites disperse passively on wind currents and probably cannot
control where they land. After dispersal a spider mite has to find a
suitable host plant and the sense of smell can give information at some
distance from the plant. This study presents evidence for the existence of
a volatile kairomone produced by the host plant P. lunatus. Spider-mite
females are activated by this kairomone and if they stray out of the
kairomone-odour field they return. These responses of the spider mite may
be important in distant host-plant location. The activation of the spider
mite after it has entered a kairomone odour plume and its returning after
it has strayed out of the odour plume may be valuable responses that enable
the mites to track the odour plume to its source, the host plant.

Volatile kairomones and food selection by spider mites.

The two-spotted spider mite is a polyphagous phytophage and therefore
one may wonder whether the volatile kairomone is an indicator of any
potential host plant or whether it is specific to the host plant Lima bean.
Visser (1979) demonstrated that the monophagous Colorado beetle,
Leptinotarsa decemlineata Say, shows electroantennogram responses to
several leaf aldehydes and alcohols constituting a 'general green leaf
volatile complex'. At least some of these compounds have also been isolated

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from bean plants (P. vulgaris) (Matthew & Galliard, 1978). Matthew &
Galliard (1978) and Visser et al. (1979) isolated these compounds after
steam distillation of the plant material, i.e. after destroying the plant
tissue, but a few of these compounds have also been found after trapping
volatiles emitted by intact plants of several species (Buttery et al. 1982,
1984, 1985; Buttery and Ling, 1984; delRosario et al., 1984); every plant
species emits a specific mixture of volatiles, and thus phytophages may
discriminate between host plants on the basis of these mixtures. The theory
on optimal foraging postulates that animals search for food so as to
maximize reproductive success (Pyke et al.,1977; Krebs, 1978; Krebs and
McCleery, 1984). According to this hypothesis T. urticae will select those
host plants that offer the best prospects for reproductive success. It
would be interesting to know whether volatile plant kairomones enable the
polyphagous T. urticae to distinguish between different host plants.
Volatile kairomones are known to be used by other polyphagous mites to
distinguish between prey species: Dicke & Groeneveld (1986) showed that a
polyphagous predatory mite can distinguish between kairomones from
different prey species and that this predator shows a hierarchy of
kairomone preference.

ACKNOWLEDGEMENTS

5. Isolation and identification of some components of a volatile kairomone of the two spotted-spider mite, *Tetranychus urticae*, with respect to two predatory mite species.

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ABSTRACT

   A volatile kairomone emitted from Lima bean plants infested with the spider mite <u>Tetranychus urticae</u> Koch, was collected on Tenax-TA, and analysed with a gas chromatograph / mass spectrometer via a thermodesorption / cold trapping technique. The response of two species of phytoseiid predators towards the identified chemicals was tested in a Y-tube olfactometer. Four of the tested compounds, linalool (3,7-dimethyl-1,6-octadiene-3-ol) , (<u>E</u>)- β -ocimene ((<u>E</u>)-3,7-dimethyl-1,3,6-octatriene), 4,8-dimethyl-1,3(<u>E</u>),7-nonatriene and methyl salicylate attracted females of <u>Phytoseiulus persimilis</u> Athias-Henriot. Linalool and methyl salicylate attracted females of <u>Amblyseius potentillae</u> (Garman), whereas no definite conclusion on the effect of (<u>E</u>)- β -ocimene on this predatory mite species could be drawn. The response of <u>A. potentillae</u> to linalool and methyl salicylate was affected by the rearing diet of the predators, in the same way as was reported for the response of this predator species to the natural kairomone blend (Dicke et al., 1986): when reared on a carotenoid-containing diet the predators responded to the volatile kairomone of <u>T. urticae,</u> but when reared on a carotenoid-deficient diet they did not.
   The response of predators to one major component emitted from spider-mite infested leaves, 4,8,12-trimethyl-1,3(<u>E</u>),7(<u>E</u>),11-tridecatetraene, has not been investigated yet.
   Based on the present study and literature data on the response of <u>T. urticae</u> to infochemicals, it is concluded that the kairomone component linalool is also a component of a volatile spider-mite dispersing pheromone.

INTRODUCTION

   Since the first report on volatile kairomones (<u>sensu</u> Dicke and Sabelis, 1988) in interactions between phytophagous mites and predatory mites (Sabelis and Van de Baan, 1983), a lot of biological information has been obtained on many aspects of these acarine infochemicals. Volatile kairomones have been reported from 13 acarine prey-predator interactions (Sabelis and Van de Baan, 1983; Sabelis and Dicke, 1985; Dicke and Groeneveld, 1986; Dicke et al., 1986; Dong and Chant, 1986; Dicke, 1988a;

Dicke et al., 1988b). Studies have concentrated on the effect of predator condition on the response (Sabelis and Van de Baan, 1983; Dicke and Groeneveld, 1986; Dicke et al., 1986; Dong and Chant, 1986; Dicke, 1988a), the type of behaviour elicited in the predator by the kairomone (Sabelis et al., 1984b), kairomone preference (Dicke and Groeneveld, 1986; Dicke, 1988a) and isolation of the compounds and study of their origin (Sabelis et al., 1984a).

As a result of the increased biological knowledge on volatile kairomones in spider mite – predatory mite interactions, questions have arisen that can only be answered by identification of the chemicals involved. Some of these questions are:

(1) Do different predator species, that all respond to the same prey species, use the same chemicals as a source of information? The phytoseiid species Phytoseiulus persimilis Athias-Henriot, Typhlodromus occidentalis (Nesbitt), Amblyseius potentillae (Garman) and Typhlodromus pyri Scheuten, all respond to a volatile kairomone of the two-spotted spider mite, Tetranychus urticae Koch (Sabelis and Van de Baan, 1983; Dicke et al., 1986; Dicke, 1988a). To the former two predator species, T. urticae is a valuable prey species, but the latter two are severely hindered by the dense web structure of this prey, which reduces the reproductive success of these predator species (Sabelis, 1981; Overmeer, 1981; McMurtry et al., 1970). This difference in profitability is reflected in the response of the predators to a volatile kairomone of T. urticae. Phytoseiulus persimilis and T. occidentalis respond to the kairomone when satiated or starved for 24 h respectively (Sabelis and Van de Baan, 1983), but severe quantitative or qualitative food deficiencies are needed to demonstrate the response of A. potentillae and T. pyri to the volatile kairomone of T. urticae (Dicke et al., 1986; Dicke, 1988a; Dicke, unpublished data). It is interesting to know whether the different effects of T. urticae on reproductive success of these four phytoseiid species are also reflected in differences in the chemical information used by the predators.

(2) Does a phytoseiid that responds to a volatile kairomone of two or more prey mite species use the same or different compounds as a source of information in these situations? Knowledge on this aspect will be of great value for determination of the species specificity of volatile kairomones of acarines. Satiated females of P. persimilis respond to a volatile

kairomone of T. urticae (Sabelis and Van de Baan, 1983; Sabelis et al., 1984a) and Tetranychus pacificus McGregor (Dong and Chant, 1986), but not to a volatile kairomone of the spider mite Panonychus ulmi (Koch) (Sabelis and Van de Baan, 1983). Therefore, the kairomone seems to be genus specific at least. The question remains whether predators are able to distinguish between different prey species of the same genus on the basis of volatile kairomones? This question is directly linked to the issue of the origin of the kairomone. An interaction of the host plant and the spider mite is a prerequisite for demonstration of a response by P. persimilis (Sabelis et al., 1984a; Dicke, unpublished data). The kairomone is present on previously infested leaves from which all spider mites, exuviae and webbing have been removed (Sabelis et al., 1984a). This indicates that the kairomone may have been produced by the plant and/or that the kairomone has been produced by the spider mites and deposited on the plant. Chemical identification of the kairomone may help in developing new procedures to reveal the origin of the kairomone and the role of the plant and the spider mite in its production.

(3) What is the function of the volatile kairomone to the prey mite itself? The predators may cue in on any spider-mite related chemical, but as a result of natural selection, only a response to a chemical that is indispensable or inevitable to the prey mite, will last long on an evolutionary time scale. Dicke (1986) demonstrated that a spider-mite dispersing pheromone is emitted from leaves infested with T. urticae. He suggested that the kairomone and the dispersing pheromone may be (partly) identical. However, only identification of the chemicals involved in the intraspecific and interspecific interactions may reveal whether the kairomone and the dispersing pheromone have components in common.

Here we report on the isolation, by collection of airborne chemicals, and identification of components of the volatile T. urticae kairomone.

METHODS AND MATERIALS

Mites.

    The two-spotted spider mite (T. urticae) was reared on Lima bean plants
(Phaseolus lunatus L.) at 20-30°C, 50-70 % r.h. under continuous
fluorescent light that was added to the natural daylight regime.

    The predatory mite P. persimilis was obtained from Koppert BV. (Berkel
en Rodenrijs, The Netherlands), 6 years ago. It was reared at c. 20°C on
Lima bean leaves infested with T. urticae, either on wet cotton wool or on
clay flower pots that were put upside down in a water basin.

    In contrast to P. persimilis, A. potentillae is severely hindered by
the dense webbing of T. urticae that is present on infested Lima bean
leaves (Sabelis, 1981), and therefore, this predator species was confined
on plastic plates (McMurtry and Scriven, 1965; Overmeer, 1985c) onto which
spider mites were brushed. This rearing method circumvents the adverse
effects of the web. This predator population which has been reared on
T. urticae in the laboratory for more than 10 years will be referred to as
A. potentillae (Tu). In addition, a separate A. potentillae population,
originating from the above-mentioned rearing was fed with Vicia faba pollen
on plastic plates. This latter culture which has been maintained for c. 6
years will be referred to as A. potentillae (Vf). Both A. potentillae
populations were obtained from A.Q. van Zon and W.P.J. Overmeer (University
of Amsterdam; cf Overmeer, 1981), in 1982.

Isolation of kairomone.

    Twenty Lima bean plants, with 2-3 leaves per plant, were cut and
immediately placed in a 5 l glass jar with 1 cm of freshly distilled water
at the bottom. Care was taken not to damage the plants except for cutting
the stem. Three plant treatments were used: clean, infested with spider
mites or damaged mechanically by rubbing the leaves with carborundum on a
wet cotton wool pad. A pyrex glass tube (161 x 6.4 mm (OD), ID 3 mm) with
90 mg of Tenax-TA in the center, held in place by two plugs of silanized
glass wool, was connected to the outlet of the jar, and a charcoal filter
to the inlet. Air was sucked through the system with a membrane pump at c.
15 ml/min, for 1 - 60 min.

    The collected volatiles were released from the adsorbent by heating in

a Thermodesorption Cold Trap Unit (Chrompack, Middelburg, The Netherlands) at 250°C for 3 min, with a N2 flow of 20 ml/min. The desorbed compounds were collected in a cold trap (SIL5CB coated fused silica capillary) at -100°C. Flash heating of the cold trap provides sharp injection of the compounds into the capillary column of the gas chromatograph to which the cold trap is connected.

This desorption procedure is inapplicable when thermolabile compounds are present. This was not the case here: no qualitative differences were observed between the chemicals identified with thermodesorption and those identified after solvent extraction of Tenax on which volatiles were collected from plants infested with T. urticae (Van Bokhoven, Van Beek and Dicke, unpublished data). Thermodesorption was used here because it provides a sensitive analysis method: the total of the desorbed volatiles is injected into the GC.

Analysis.

The thermodesorbed volatiles that were collected on Tenax-TA were analysed by GC/MS using a Chrompack SIL19CB column (25 m x 0.25 mm ID). The column temperature was held at ambient for 2 min, then at 40°C for 4 min, and then programmed to 280°C at a rate of 4°C/min. Electron impact ionisation was carried out at 70 eV on a VG MM7070F mass spectrometer (resolving power 1000).

Separation of (Z) and (E)- β-ocimene.

Synthetic β-ocimene (3,7-dimethyl-1,3,6-octatriene) could only be obtained as a mixture of the (Z) and (E) isomers, which were separated by preparative GC on a 9.8 % Carbowax on Chromosorb W-HP 100/120 column (200 x 0.2 cm), with a TCD detector. Column temperature was 80°C.

The desired isomer was collected in a capillary tube which was cooled in dry ice. The ratio of the two isomers in the collected fractions was determined on the same column.

Bioassay.

To determine whether extracts or synthetic chemicals had kairomonal activity, a Y-tube olfactometer was used. A glass Y-shaped tube held an iron wire in the centre. At the end of both arms of the olfactometer filter

paper with test or control solutions was placed in PVC cages. Air was sucked out at the base of the tube and led to the outside of the climate room in which the olfactometer was positioned. The air speed in both arms of the olfactometer was measured with a hot wire anemometer and standardized at 0.7 ± 0.1 m/s by adding dry cotton wool in the PVC cages and/or changing the total airflow speed with a valve. Female predators were placed on the wire in the basal tube and observed individually. When the predator walked upwind and reached the far end of one of the arms, the experiment was terminated. A sign test was used to analyse the results ($\alpha = 0.05$). The experiments were performed at $25 \pm 1°C$ and $60 \pm 10\%$ r.h.

Synthetic chemicals were dissolved in dichloromethane (β-ocimene, linalool (3,7-dimethyl-1,6-octadiene-3-ol) and methyl salicylate), paraffin oil (1-octen-3-ol, ($\underline{Z}$)-3-hexen-1-ol, ($\underline{Z}$)-3-hexen-1-yl acetate, 4,8-dimethyl-1,3,7-nonatriene) or hexane (β-ocimene). Aliquots of 0.1 ml of diluted chemicals were poured onto a piece of filter paper outside the climate room and transferred to the olfactometer immediately. A filter paper with 0.1 ml solvent served as control. In these tests, new filter papers were prepared for each predator tested. All chemicals were obtained from Aldrich except for ($\underline{Z}$)-3-hexen-1-yl acetate which was a Roth product, β-ocimene which was obtained as an isomer mixture from International Flavor and Fragrances Industries as well as a mixture which was obtained from Dr. J.H. Tumlinson (USDA, Gainesville, Florida.), and 4,8-dimethyl-1,3,7-nonatriene isomers which were synthesized by R.M. Peperzak, H.J.M. Gijsen and Ae. de Groot (Agricultural University Wageningen, Department of Organic Chemistry) (procedure of Maurer et al., 1986). All chemicals, except β-ocimene, were >98% pure.

Only female predators were tested in the olfactometer. In the case of P. persimilis, they were well-fed, but unless stated otherwise, A. potentillae females were tested after a 20 h-period of starvation at 25°C in a plastic tube.

RESULTS

Collection of kairomone and identification.

The suitability of Tenax-TA to collect the volatile kairomone of
T. urticae was demonstrated by Van Bokhoven, Dicke and Van Beek
(unpublished data) in an olfactometer with satiated females of P.
persimilis. By GC/MS analysis of the mixture of volatiles collected from
spider-mite infested plants on Tenax-TA, many components could be
distinguished (Table 5.1, Figure 5.1). Only two of these (2-butanone and 1-
octen-3-ol) have been found in samples collected from clean intact bean
plants (Table 5.1). Some others, such as the general green leaf volatiles
(Z)-3-hexen-1-ol and (Z)-3-hexen-1-yl acetate are expected to be emittted
by Lima bean plants, but if so, the quantities were very small, compared to
the amounts of these compounds that were obtained from spider-mite infested
bean plants. An analysis of the volatiles released by artificially damaged
plants revealed several compounds that are also emitted by spider-mite
infested plants (Table 5.1). Thus, emission of these compounds from spider-
mite infested plants presumably is the result of mechanical damage of the
plant. The other volatiles emitted from T. urticae-infested bean plants are
caused by spider-mite activities or are emitted from the spider spider
mites.

Apart from volatiles emitted from the plants other compounds were also
present in the samples, mainly lower alkanes (C_5-C_{12}) and aromatic
hydrocarbons. These compounds were also present when the charcoal filter at
the inlet of the system was displaced by a Tenax filter. However, they were
absent when a regularly pretreated Tenax-TA containing tube that had not
been exposed to the set-up for collection of volatiles, was thermodesorbed.
These compounds are ubiquitous air-pollutants, probably originating from
evaporated or incompletely combusted automobile fuel. Their concentration
in air in built-up areas may range from 5 ~ >100 ng/l; Grob and Grob, 1972)
and thus were also present in the glass jar used for sampling.

All identifications mentioned in Table 5.1 are based at least on a
comparison of obtained mass spectra with reference spectra. Moreover, for
characterisation of (E)- β-ocimene this was confirmed by determining mass
spectra and retention times of synthetic β-ocimene isomers under similar

conditions as was done for the spider mite compound (Figure 5.2). The relative intensities of ions with m/z of 79 and 80 and ions with m/z of 90 and 91 as well as the relative intensity of M+ (136) are important for distinguishing between the two isomers (Figure 5.2a,b). These investigations confirmed the identification of the spider-mite related compound as (E)-β-ocimene. Identification of 4,8-dimethyl-1,3(E),7-nonatriene and 4,8,12-trimethyl-1,3(E),7(E),11-tridecatetraene was recently established on the basis of NMR, retention times and mass spectra (Van Beek, Dicke and Posthumus, unpublished data).

One compound in the sample obtained from T. urticae-infested plants could not be identified yet (Table 5.1, Figure 5.1).

Bioassay of identified components.

The effect on the predatory mite P. persimilis of those identified chemicals that were available synthetically was tested in the Y-tube
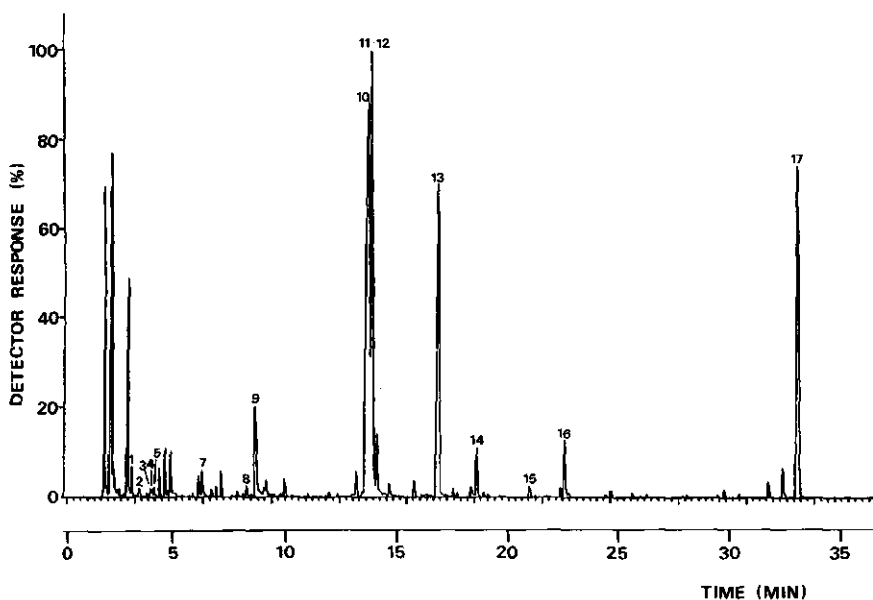

Figure 5.1: Gaschromatogram of volatiles from spider-mite infested bean plants, collected on Tenax-TA. Peak numbers correspond to numbers given in Table 5.1. Most unlabeled peaks are atmospheric or instrumental background peaks.

Table 5.1: Volatiles collected from intact, artificially damaged, and T. urticae infested Lima bean plants and their function as kairomone for the phytoseiid mites P. persimilis or A. potentillae reared on V.faba pollen (Vf) or T. urticae (Tu) or as dispersing pheromone for the spider mite T. urticae[1].

Peak number	Tentative identification	Identified in			Component of		
		T. urticae infested plants	artificially damaged plants	undamaged plants	Kairomone of		Dispersing pheromone of T. urticae
					P. persimilis (Vf)	A. potentillae (Tu)	
1	2-Butanone	+	+	+			
2	2-Methyl-propan-1-ol	+	-	-			
3	1-Butanol	+	-	-			
4	3-Pentanone	+	-	-			
5	1-Penten-3-ol	+	-	-			
6	Hexanal	-	+	+			
7	Unidentified (MW=98)	+	+	-			
8	2-Hexenal	+	+	+			
9	(Z)-3-Hexen-1-ol	+	+	-	-	-	
10	(Z)-3-Hexen-1-yl acetate	+	+	-	-	-	
11	1-Octen-3-ol	+	+	+	-	?	
12	(E)-β-Ocimene	+	-	-	+	?	
13	4,8-Dimethyl-1,3(E),7-nonatriene	+	-	-	+		
14	Linalool	+	-	-	+	+	
15	(Z)-3-Hexen-1-yl butyrate	+	+	-		-	+
16	Methyl salicylate	+	-	-	+	+	
17	4,8,12-Trimethyl-1,3(E),7(E),11-tridecatetraene	+	-	-			-

? = uncertain because pure isomer was not tested.

1) P. persimilis were tested as satiated females, Amblyseius potentillae as females that were starved during 24 h at 25°C. Data on T. urticae are based on the data of Dabrowski and Rodriguez (1971), Dabrowski et al. (1976) and Dicke (1986).

olfactometer. Figure 5.3 shows that linalool is attractive to P. persimilis
females at a wide dose range, whereas methyl salicylate proved to be
attractive when offered at an amount of 20 ug. The other three compounds
tested did not attract the predators. These latter compounds had been
identified both in samples obtained from artificially damaged and spider-
mite infested plants.

The data for ocimene are presented in Figure 5.4. β-Ocimene was
obtained as mixtures of the (E) and (Z) isomers at ratios of 90:10 and
70:30. Gas chromatography on 3% OV-17 showed that some minor compounds were
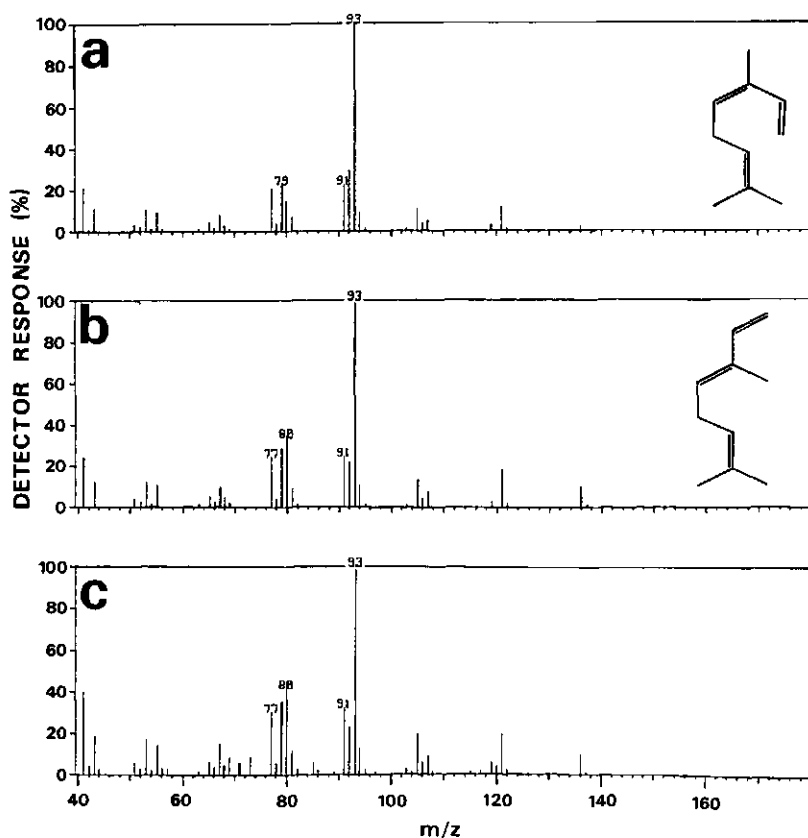

Figure 5.2: Mass spectra of β-ocimene isomers:
(a) (Z)- β-ocimene
(b) (E)- β-ocimene
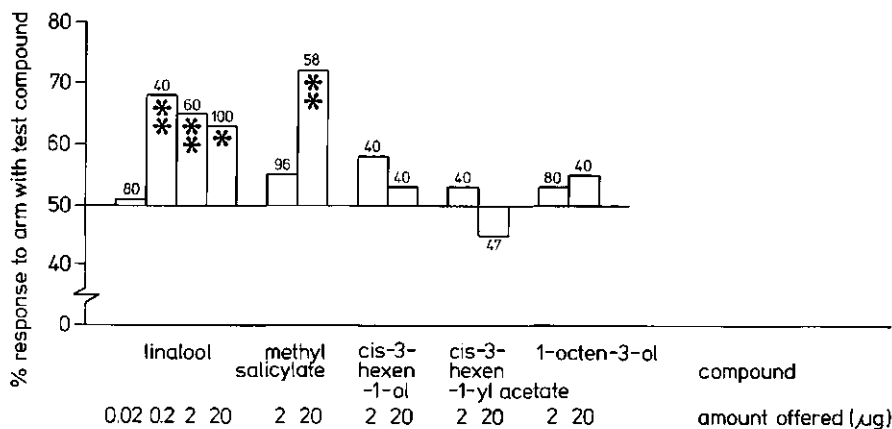(c) component collected from T. urticae-infested Lima bean plants.

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Figure 5.3: Final choice of P. persimilis females in Y-tube olfactometer to various doses of synthetic chemicals identified after collection of volatiles emitted from T. urticae-infested Lima bean plants. Numbers under and above columns indicate the number of predators tested.

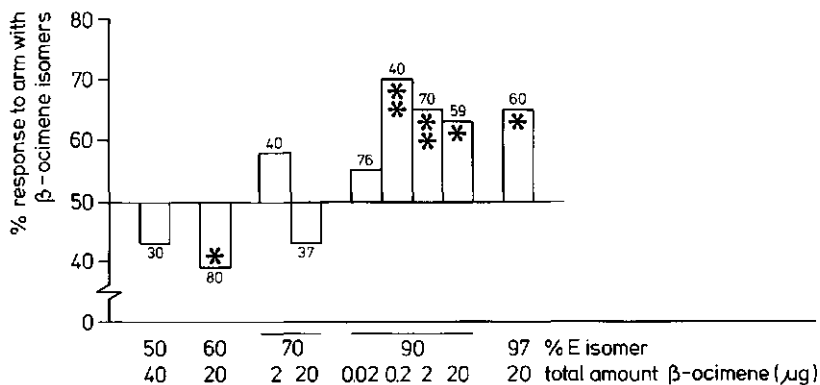*** = P < 0.001
** = 0.001 < P < 0.01
*   = 0.01 < P < 0.05

Figure 5.4: Final choice of P. persimilis females in Y-tube olfactometer to β-ocimene, offered at various (E):(Z) ratios, and various doses. Numbers under and above columns indicate the number of predators tested. For explanation of asterisks, see Figure 5.3.

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also present. When tested in the olfactometer only the 90:10 (E)/(Z)
β-ocimene mixture proved attractive to P. persimilis, either when 20,
2 or 0.2 ug were offered. When offered at an amount of 0.02 ug no response
was observed. The difference in response of P. persimilis to the two
β-ocimene samples could be caused by the impurities or by the
difference in (E)/(Z) ratio. To investigate this, (E):(Z) ratios of 50:50
and 60:40 were prepared from the 90:10 sample and a 97:3 (E):(Z) ratio from
the 70:30 sample. The 97:3 sample was attractive, but the 50:50 sample was
not. The 60:40 (E):(Z) ratio was even repellent. These data show that (Z)-
β-ocimene reduced the attractivity of (E)-β-ocimene to P. persimilis.

A recent experiment with the methyl terpene 4,8-dimethyl-1,3(E),7-
nonatriene shows that 20 ug of this compound is also attractive to
P. persimilis, whereas the (Z)-isomer is not (Figure 5.5). No data are
available on the response to the other methyl terpene, 4,8,12-trimethyl-
1,3(E),7(E),11-tridecatetraene, yet. Structures of the active kairomone
components are shown in Figure 5.6.

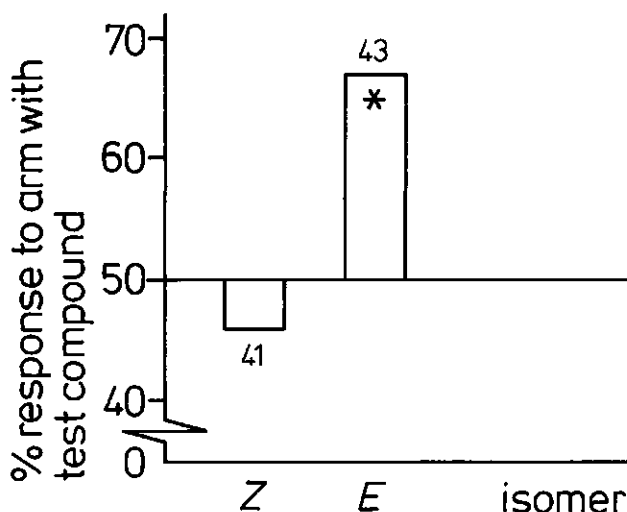Amblyseius potentillae responded to the volatile kairomone of

Figure 5.5: Final choice of P. persimilis females in Y-tube olfactometer to
4,8-dimethyl-1,3(E),7-nonatriene and 4,8-dimethyl-1,3(Z),7-nonatriene
isomers. Numbers under and above columns indicate the number of predators
tested. For explanation of asterisks, see Figure 5.3.

T. urticae when reared on a carotenoid-deficient diet such as V. faba pollen, but not when reared on the carotenoid-containing spider mite T. urticae (Dicke et al., 1986). Predators that differed in qualitative feeding history were offered the identified chemicals. The results (Figure 5.7) show that when amounts of 20 ug were offered, no chemical was attractive to A. potentillae (Tu). However, when reared on V. faba pollen, methyl salicylate and linalool were attractive, which resembles the data obtained for P. persimilis. However, in contrast to P. persimilis, A. potentillae (Vf) did not respond to 90:10 (E)/(Z) β -ocimene. Either (E)- β-ocimene is not attractive or the presence of 10% of the (Z) isomer or the other impurities disturbed the response of A. potentillae to the (E) isomer.

The response of A. potentillae to linalool was studied in more detail (Figure 5.8). Three different concentrations were tested: 20, 2 and 0.2 ug. When reared on V. faba pollen all three concentrations attracted predators that were starved during 20 h. Also when satiated these predators responded to 2 ug linalool, the only concentration tested for satiated A. potentillae (Vf). A response towards the volatile T. urticae kairomone in well-fed A. potentillae has only been observed when the predators were reared on V. faba pollen (Dicke, unpublished data) In contrast with the data for A. potentillae (Vf), amounts of 20 and 0.2 ug linalool were not attractive to A. potentillae (Tu) that were starved for 20 h, but when 2 ug linalool was offered a significant response was observed in these predators.

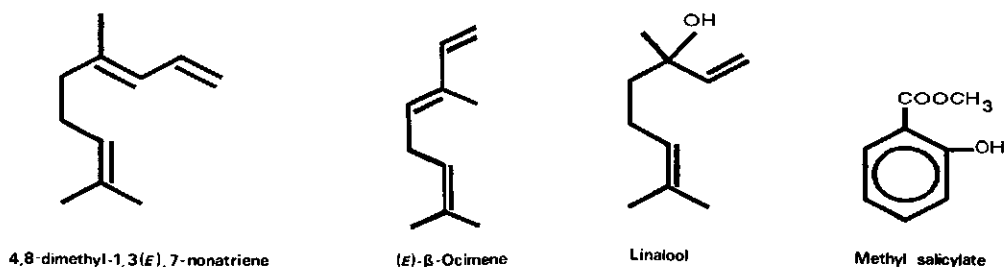The data for the responses of both predator species are summarized in Table 5.1.

4,8-dimethyl-1,3(E),7-nonatriene   (E)-β-Ocimene   Linalool   Methyl salicylate

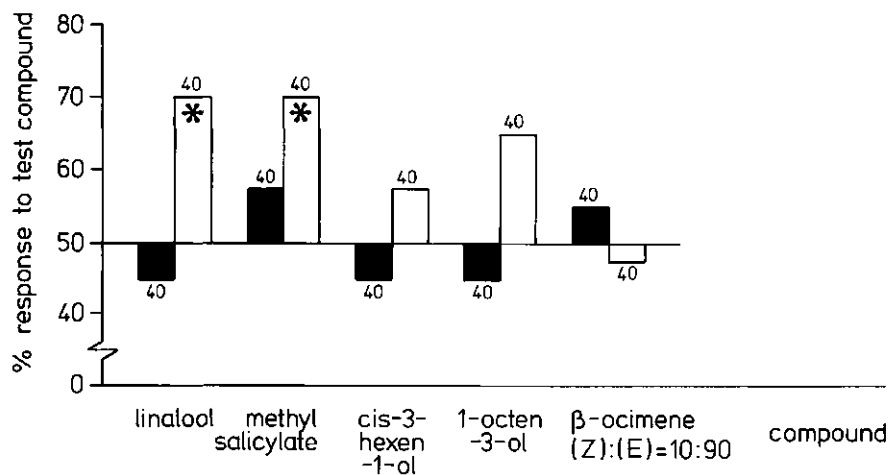Figure 5.6: Structures of active kairomone components for P. persimilis.

Figure 5.7: Final choice of starved female A. potentillae (Tu) (black bars) and A. potentillae (Vf) (white bars) in Y-tube olfactometer to synthetic chemicals (dose: 20 ug), identified after collection of volatiles from T. urticae-infested Lima bean plants. For explanation of asterisks, see Figure 5.3. Numbers under and above columns indicate the number of predators tested.

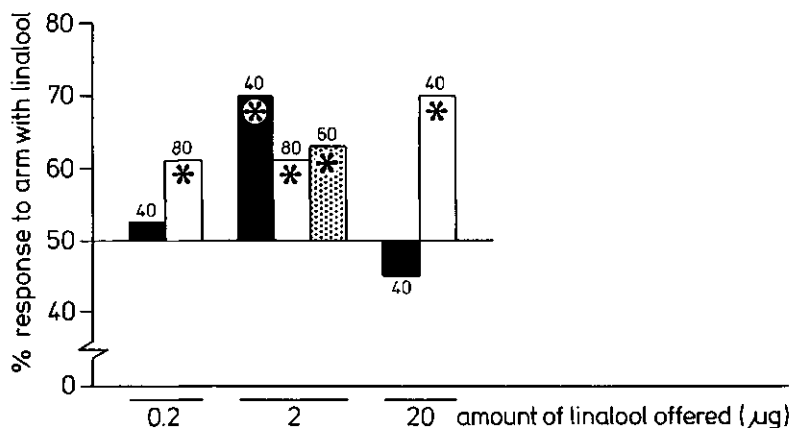

Figure 5.8: Final choice of starved female A.potentillae (Tu) (black bars) and starved and satiated A. potentillae (Vf) (white bars: starved; shaded bar: satiated) in Y-tube olfactometer to linalool at various doses. For explanation of asterisks, see Figure 5.3. Numbers under and above columns indicate the number of predators tested.

DISCUSSION

Damage inflicted on Lima bean plants by rubbing the leaves with carborundum or by infestation with spider mites caused emission of many volatiles. However, a clear difference was observed between the volatile mixture emitted from plants damaged in either way. Most of the major volatiles emitted from clean Lima bean plants, plants damaged mechanically or plants infested with T. urticae were identified. The small amount of volatiles collected from intact bean plants is remarkable when compared to reports on volatiles collected from other plants (e.g. Buttery et al., 1982, 1985; Buttery and Ling, 1984). However, these authors sampled for 24 h at a rate of 500 ml/min and analysed the total combination of chemicals adsorbed on several traps. The amounts of volatiles emitted from spider-mite infested plants were large enough to allow short sampling times in our investigation. However, minor compounds may have been missed.

These differences, observed between plants that are in different condition, will allow many organisms to distinguish between them.

Do different phytoseiid species use similar chemicals as volatile T. urticae kairomone?

Several species of predatory mites are able to distinguish between clean and T. urticae infested plants on the basis of volatile kairomones, but the conditions needed to demonstrate such a response vary. Phytoseiulus persimilis females respond to a volatile T. urticae kairomone when satiated or starved for 24 h (Sabelis and Van de Baan, 1983). This has also been established for carotenoid deficient A. potentillae (i.e. reared on V. faba pollen) (Dicke et al., 1986; Dicke, unpublished data).

Four of the synthetic potential kairomone components tested in the olfactometer elicited a significantly attractive response of at least one of the predator populations tested: these compounds are the terpenes linalool and (E)- β-ocimene, the methyl terpene 4,8-dimethyl-1,3(E),7-nonatriene and methyl salicylate.

All four compounds attracted P. persimilis females, although methyl salicylate only elicited this response when offered at a dose of 20 ug. Possibly the information conveyed by this latter compound is of more

importance when the chemical is presented together with linalool, (E)-β-ocimene, 4,8-dimethyl-1,3(E),7-nonatriene and/or other kairomone components. The effect of linalool and methyl salicylate on A. potentillae (Vf) is similar to that on P. persimilis. With respect to (E)-β-ocimene no definitive conclusion can yet be made, because it is not known whether the presence of 10% (Z)-isomer affected the behaviour of A. potentillae (Vf) towards (E)-β-ocimene.

With regard to the kairomone components linalool and methyl salicylate, the data obtained for A. potentillae reared on either of the two diets are in agreement with those of Dicke et al. (1986) and Dicke (unpublished data) on the response of A. potentillae to the volatiles emitted by T. urticae-infested plants. The only exception is the response of A. potentillae (Tu) to 2 ug linalool. This may indicate that A. potentillae (Tu) are sensitive to this component of the volatile T. urticae kairomone, though much less than A. potentillae (Vf) that respond much more consistently to linalool.

Our observations provide no reason to suppose that different predator species use different volatiles emitted from T. urticae-infested plants as kairomone. Investigations with other phytoseiid species, such as T. occidentalis or T. pyri are needed to investigate this further.

Do kairomone components function as dispersing pheromone for spider mites?

The spider mite T. urticae also distinguishes between undamaged and T. urticae-damaged Lima bean plants. Clean leaves emit a volatile kairomone that attracts spider-mite females. After infestation with T. urticae, the leaves also emit a dispersing pheromone. At a high ratio of dispersing pheromone : plant kairomone, the spider mites are repelled (Dicke, 1986). Moreover, Harrison and Karban (1986) reported that after 14 days since removal of T. urticae from a cotton plant on which these mites were feeding, conspecifics could distinguish these plants from previously uninfested clean plants. It will be interesting to study whether similar compounds are involved in the long-term and short-term effects of spider-mite infestation on the behaviour of conspecifics.

It is interesting to investigate whether the chemicals that act as a kairomone to predatory mites act as a spider-mite dispersing pheromone. Some indications for this can be found in a study on host-plant - spider-

mite interaction. Dabrowski and Rodriguez (1971) studied the response of
T. urticae females to essential oil components of strawberry foliage. In a
static air olfactometer, linalool repelled the mites at several
concentrations. Methyl salicylate was indifferent, whereas (E)-β-ocimene
or 4,8-dimethyl-1,3(E),7-nonatriene were not included in their study. In a
later study by Rodriguez et al. (1976), mixtures of compounds were used. A
mixture resembling strawberry essential oil attracted T. urticae females.
An increase in the proportion of nonanal, one of the components, reduced
the attractive effect. However, an increase in the proportion of methyl
salicylate had no effect whereas a decrease reduced the attractivity of the
mixture. Unfortunately, the effect of a change in the proportion of
linalool was not studied.

In the context of the present study and the one by Dicke (1986), the
data of Dabrowski and Rodriguez (1971) indicate that linalool is not only a
component of the T. urticae kairomone that attracts predatory mites, but
also of the T. urticae dispersing pheromone. This supports the hypothesis,
that the dispersing pheromone and the spider-mite kairomone have components
in common (Dicke, 1986). Rodriguez et al. (1976) showed that a change in
the blend affects the behaviour of T. urticae females. This is similar to
the data of Dicke (1986), who demonstrated that the ratio of clean and
infested leaves offered, affects the behavioural response of T. urticae to
the emitted blend of infochemicals.

Comparison of the data of Dabrowski and Rodriguez (1971) and Rodriguez
et al. (1976) show that the effect of methyl salicylate on T. urticae
behaviour can only be seen when changing the composition of the mixture. No
changes in composition of the mixture with respect to methyl salicylate
have been observed to cause a repellent effect on T. urticae. Thus, there
is no evidence for the kairomone component methyl salicylate to be a
component of the dispersing pheromone. These data on the identity of
components of the T. urticae dispersing pheromone are incorporated into
Table 5.1.

It will be interesting to investigate the effect of (E)-β-ocimene and
4,8-dimethyl-1,3(E),7-nonatriene on T. urticae females, to know whether
this kairomone component is also a component of the dispersing pheromone.

Origin of volatile kairomone.

The above considerations raise the question of the origin of the infochemicals that function in plant-spider mite-predatory mite interactions. The chemicals presumably are of plant origin as can be inferred from the classes of compounds identified (e.g. Karrer, 1976; Maurer et al., 1986). However, it cannot be concluded as yet, whether the plant has active control over the production or emission of the volatile, or whether the plant offers a substrate for spider-mite enzymes that subsequently produce the kairomone components. Investigation of e.g. site and moment of production, and possible storage of precursors are needed for that. Chemical identification of kairomone components was an essential first step for this.
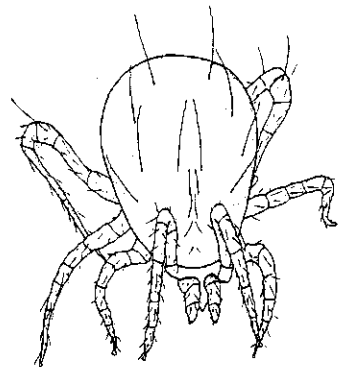
PART III. INFOCHEMICALS AND PREY PREFERENCE OF PHYTOSEIID MITES IN AN ORCHARD SYSTEM WITH TWO SPECIES OF PHYTOPHAGOUS MITES.

6. Vitamin A deficiency modifies response of predatory mite *Amblyseius potentillae* to volatile kairomone of two-spotted spider mite.

Marcel Dicke, Maurice W. Sabelis and Adrie Groeneveld

ABSTRACT

    A volatile kairomone of the two-spotted spider mite, Tetranychus
urticae, elicits a searching response of the phytoseiid predator Amblyseius
potentillae, only when reared on a carotenoid-free diet. However, after
addition of crystalline β-carotene or vitamin A acetate to the predator's
rearing diet this searching response was absent. Because vitamin A and
β-carotene are indispensable nutrients for diapause induction, the
carotenoid-deficient predators increase their fitness by searching for two-
spotted spider mites, when other spider mites are unavailable. Two-spotted
spider mites, among others, contain the carotenoids required for diapause
induction, but are an inferior prey due to the dense webbing they produce.
When the predators have carotenoids at their disposal, they do better by
searching for other spider mites, that are more profitable in terms of
reproductive success. Such a prey is the European red spider mite,
Panonychus ulmi. The volatile kairomone of this prey elicits a searching
response of the predator whether it has a carotenoid deficiency or not.

INTRODUCTION

    Amblyseius potentillae (Garman) (Acarina: Phytoseiidae) is a

polyphagous predatory mite that can feed and reproduce on phytophagous

spider mites (Acarina: Tetranychidae) (Kropczynska-Linkiewicz, 1971;

Overmeer, 1981) and pollen of several plant species (Overmeer, 1981). The

predator is known to respond to volatile kairomones: in Y-tube olfactometer

experiments it was shown that starved A. potentillae, when reared on two-

spotted spider mites (Tetranychus urticae Koch), respond to a volatile

kairomone of the European red spider mite (Panonychus ulmi (Koch)) but not

to a kairomone emitted from leaves infested by the two-spotted spider mite,

to which other predatory mites respond (Sabelis and Van de Baan, 1983).

Tetranychus urticae is an unprofitable prey species for A. potentillae as

the predator is hindered by the dense webbing produced by this spider mite (Sabelis, 1981). The European red spider mite that also produces silk but not a dense webbing, is a more profitable prey. These differences in values of these prey species for A. potentillae also are reflected in the ability of the predator to control populations of these mite species. The predator is able to control populations of P. ulmi (Van de Vrie, 1973; McMurtry and Van de Vrie, 1973; Rabbinge, 1976) but it is unable to control those of T. urticae (Sabelis, 1981).

When animals search for food they have to make decisions about where to search, how long to search at a specific site and which type of food to eat. In optimal foraging theory it is assumed that predators make decisions so as to maximize their genetic contribution to future generations. It is therefore assumed that animals maximize their net rate of food intake (Pyke et al., 1977, Krebs, 1978). Although it has received little emphasis in the literature, nutrient composition also is important and should be optimized. Examples are available for spiders (Greenstone, 1979) and moose (Belovsky, 1978). General dietary components like nitrogen, amino acids and proteins are usually thought to be important (Greenstone, 1979; Slansky and Feeny, 1977; Moss et al., 1972). In this paper a specific nutrient is taken into account: vitamin A, that is an indispensable nutrient for diapause induction in the eyeless mite Amblyseius potentillae (Veerman et al., 1983). Our work shows that a deficiency of vitamin A or its precursor β-carotene in the diet of A. potentillae affects its response to a kairomone of one of its prey species, the two-spotted spider mite. To our knowledge this is the first time that any single nutient has been shown to be of crucial importance in prey selection.

MATERIALS AND METHODS

Predators.

Amblyseius potentillae was reared on four different diets on plastic plates (McMurtry and Scriven, 1965) in a climate room at $25 \pm 1°C$, $60 \pm 10\%$ r.h. under continuous fluorescent light. (1) Two-spotted spider mites have been used as prey for many years. The mites were brushed off the host-plant leaves onto the plastic plates to circumvent the adverse effects of the

dense webbing of this prey. For about five years predators from this culture have been reared on (2) broad bean (Vicia faba L.) pollen, a carotenoid-free diet for A. potentillae (Overmeer and Van Zon, 1983a). For some experiments we added crystalline (3) β-carotene or (4) vitamin A acetate (Sigma Co., St. Louis, Missouri, U.S.A.) to the pollen (1 mg β-carotene or vitamin A acetate per 5 mg pollen). Overmeer and Van Zon (1983a) and Veerman et al. (1983) showed that A. potentillae take up these crystalline carotenoids when mixed with pollen.

Prey.

The two-spotted spider mite was reared in our laboratory for many years on Lima bean (Phaseolus lunatus L.) at 20-30°C under continuous fluorescent light that was added to the natural daylight regime.

The European red spider mite was collected from an apple orchard. The leaves, on which 20-40 active P. ulmi stages were present, were used in the bioassay on the day collected.

Bioassay.

We used an olfactometer that consisted of a glass tube with an iron wire in the centre, both of which are Y-shaped. Odour-emitting objects may be placed in PVC cages at the ends of both arms of the Y-tube olfactometer. Air was sucked out at the base of the tube and led to the outside of the climate room in which the olfactometer was positioned. The airstream speeds in both arms of the olfactometer were measured with a hot wire anemometer and standardized at 0.7 ± 0.1 m/s by inserting dry cotton wool in the inlet of the airstream and/or changing the total air speed with a valve. Female predators that had been deprived of food and water for 20 h at 25°C in a plastic tube were placed individually on the wire at the base of the basal tube and observed. Starved predators were used as these showed a response to the volatile kairomone of P. ulmi, unlike well-fed predators (Sabelis and Van de Baan, 1983). When the predator walked upwind and reached the far end of one of the arms the experiment was terminated. The maximum experimental time was 10 min. Predators that did not walk to the far end of one of the arms were left out of the statistical analysis.

The results have been analysed using a sign test. The null hypothesis is that the probabilities of the predators to walk to the far end of either

arm are equal to 50%. For a more detailed description of the olfactometer, the experimental procedure and statistical analysis, see Sabelis and Van de Baan (1983). The experiments were performed at $26 \pm 1°C$ and $60 \pm 10\%$ r.h.

RESULTS AND DISCUSSION

Results of experiments using the Y-tube olfactometer show that when A. potentillae were reared on broad bean pollen and then starved for 20 h, they responded to the volatile kairomones of T. urticae and P. ulmi. However, when they were reared on T. urticae they did not respond to the kairomone of T. urticae, but only to that of P. ulmi (Table 6.1). The

Table 6.1: Response of 20 h-starved female A. potentillae to kairomones of different spider mite species in a Y-tube olfactometer.

Predator reared on	Content of arm 1	Content of arm 2	n[a]	n(1)	n(2)	n(0)	Critical level
T. urticae	9 bean leaves infested with T. urticae	9 clean bean leaves	40	21	19	0	ns[b]
	18 apple leaves infested with P. ulmi	18 clean apple leaves	40	31	9	0	$P < 0.001$[b]
V. faba pollen	9 bean leaves infested with T. urticae	9 clean bean leaves	60	44	15	1	$P < 0.001$
	18 apple leaves infested with P. ulmi	18 clean apple leaves	53	38	14	1	$P < 0.001$

[a] n   = number of predators tested.
    n(1) = number of predators that walked to far end of arm 1.
    n(2) = number of predators that walked to far end of arm 2.
    n(0) = number of predators that did not walk to far end of one of the arms.

[b] Results of Sabelis and Van de Baan (1983).

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difference in response between predators reared on the two different diets could be accounted for either by a genetic difference due to different selection pressures in the two cultures or by a physiological difference due to different diets. No differences in fecundity, developmental time and mortality have been observed between A. potentillae reared on T. urticae or V. faba pollen (Overmeer, 1981). The predators can be reared equally well on both diets. However, it has been reported that predators reared on broad bean pollen cannot enter reproductive diapause while those reared on T. urticae can (Overmeer and Van Zon, 1983a). Because β-carotene is known to affect diapause in the two-spotted spider mite (Veerman and Helle, 1978), this compound was added to the broad bean pollen or to carotenoid-free eggs of albino T. urticae that A. potentillae were reared upon. This addition restored the predator's ability to enter diapause (Van Zon et al., 1981; Overmeer and Van Zon, 1983a). Adding vitamin A, a derivative of β-carotene, to the diet of A. potentillae also has the same effect (Veerman et al., 1983). Thus, a physiological difference between predators reared on the two different diets might cause the difference in response to the T. urticae kairomone.

When A. potentillae were reared for several generations on broad bean pollen mixed with β-carotene or vitamin A or just on broad bean pollen to which the carotenoid was only added five days prior to the experiment, the predators showed no response to the kairomone of T. urticae (Table 6.2). However, the response to the kairomone of P. ulmi was still present when the predators were reared for several generations on broad bean pollen to which crystalline β-carotene was added. Thus, the response of A. potentillae to the kairomones of T. urticae and P. ulmi is identical for predators reared on either T. urticae or pollen plus crystalline β-carotene as carotenoid source. These results show that β-carotene and vitamin A affect the response to the T. urticae kairomone. Predators respond to this kairomone when they lack these carotenoids. Because vitamin A is an essential nutrient for inducing diapause and hence for hibernation, it is thus worth foraging for since the ability to enter diapause is of selective advantage. Predators being under stress conditions (in this case carotenoid-deficiency) would do better by broadening their diet and the response to the T. urticae kairomone by carotenoid-deficient predators can be interpreted in this context.

Table 6.2: Response in a Y-tube olfactometer of 20 h-starved female
A. potentillae reared on a diet of V. faba pollen to which crystalline
carotenoids had been added.

Predators reared on	Content of arm 1	Content of arm 2	n[a]	n(1)	n(2)	Critical level
V. faba pollen + β-carotene	9 bean leaves infested with T. urticae	9 clean bean leaves	40	20	20	n.s.
V. faba pollen (β -carotene was added 5 days prior to the experiment)	9 bean leaves infested with T. urticae	9 clean bean leaves	41	17	24	n.s.
V. faba pollen + vitamin A-acetate	9 bean leaves infested with T. urticae	9 clean bean leaves	42	19	23	n.s.
V. faba pollen + β-carotene	18 apple leaves infested with P. ulmi	18 clean apple leaves	40	30	10	P< 0.001

[a]
n   = number of predators tested.
n(1) = number of predators that walked to far end of arm 1.
n(2) = number of predators that walked to far end of arm 2.

Amblyseius potentillae is found in orchards, where it is unlikely that
the pollen of V. faba would be an important food source. Therefore one
wonders under what circumstances carotenoid shortage could occur in the
field? This question cannot be answered yet but is of importance in
concluding how frequently carotenoids are a limiting factor, and thus how
decisive their role is in natural selection.

There are several ways in which A. potentillae can obtain carotenoids
and it would be interesting to study how they are affected by depriving A.
potentillae of carotenoids:
1. Predation on any seizable phytophagous prey. When deprived of
    carotenoids A. potentillae responds to the kairomones of more prey
    species than after consumption of these compounds (Dicke and

Groeneveld, 1986; this paper).

2. Cannibalism, a phenomenon often observed in cultures of A. potentillae on broad bean pollen, but not in those on T. urticae (Overmeer and Van Zon, 1983a; own observation). This only helps the carotenoid lack when the conspecific contains carotenoids.

3. Consumption of the types of pollen that have carotenoids available to the predator.

4. Consumption of red yeasts that are present on the host plant leaf. The phytoseiid mite Amblyseius finlandicus (Oudemans) has been found to feed and reproduce on fungal spores (Kropczynska, 1970), but so far A. potentillae has not been found to feed on fungi.

5. Phytophagy, a phenomenon reported for the predatory mite A. hibisci (Chant) (Porres et al., 1976) that has recently been confirmed for A. potentillae as well (Dicke, unpublished data).

Thus, it might seem that in the field A. potentillae usually should have no problem in obtaining carotenoids. Whether this is true, should be investigated by sampling predators in the field and testing their ability to enter diapause. However, if obtaining carotenoids is indeed not a problem for A. potentillae this might be the result of the development in evolutionary time of one or more of the five above-mentioned strategies to obtain carotenoids. As carotenoids are unstable under light conditions this might mean that the transparent A. potentillae has to replenish its supply regularly. Still, the amount of carotenoids needed for diapause induction seems to be small: after switching from a diet with carotenoids to one without, the offspring do not loose the ability to enter diapause until in the second generation (Overmeer and Van Zon, 1983a). However, a low amount of carotenoids may be insufficient to detect the photoperiodic signal under dim light conditions as has been reported for larvae of Bombyx mori (Shimizu and Kato, 1984).

Whether other functions of carotenoids in A. potentillae exist, is not known. In other organisms to date only photofunctions have been demonstrated for carotenoids (Krinsky, 1971), although many hypotheses about other functions have been put forward (Krinksy, 1971; Karnaukhov et al., 1977). Based on their isolation from bovine olfactory epithelium and the brownish-yellow colour of olfactory mucosa, it has also been suggested that carotenoids function as receptors for the energy from odorous

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molecules (Briggs and Duncan, 1961; Kurihara, 1967). It was thus postulated that smell is impaired by vitamin A deficiency (Briggs and Duncan, 1961). In A. potentillae vitamin A deficiency results in response to the kairomones of an increased number of prey species. In Y-tube olfactometer experiments in which different prey species were offered in each olfactometer arm carotenoid-deficient A. potentillae distinguished between the kairomones of these prey species (Dicke and Groeneveld, 1986). This indicates that olfactory discrimination in A. potentillae is rather improved. The hypothesis of Briggs and Duncan (1961) would predict however an impaired olfactory function and thus this hypothesis cannot hold for olfaction in A. potentillae.

A response to kairomones of a larger number of prey species can be thought to occur under stress situations other than carotenoid deficiency as well. Severe starvation can be such a stress situation. A response to the volatile kairomone of T. urticae may therefore be present in T. urticae-reared A. potentillae after starvation for a longer time than the 20 h of food deprivation used in this study.

Conditioning due to experience with a host species has been observed to affect host recognition by hymenopterous parasitoids (Arthur, 1971; Vinson et al., 1977; Vet, 1983). In the present case, predators that were reared on two-spotted spider mites for many years do not respond to the volatile kairomone of this prey species (Sabelis and Van de Baan, 1983). Only predators that were reared on broad bean pollen and never had any contact with two-spotted spider mites respond to the volatile kairomone of this prey. The response of A. potentillae to the volatile kairomone of T. urticae is not dependent on previous contact with this prey species but on whether the predator has available nutrients that are indispensable for diapause induction. The eyeless predatory mite A. potentillae can identify a deficiency in β-carotene or vitamin A in its body. As a result, the predator responds to a volatile kairomone of an unprofitable prey species when the alternative is no prey at all. The result of this response is that after finding and consuming this prey species the predator will have carotenoids available and thus will be able to enter diapause.

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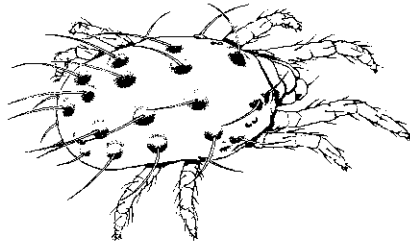
7. Hierarchical structure in kairomone preference of the predatory mite _Amblyseius potentillae_: dietary component indispensable for diapause induction affects prey location behaviour.

Marcel Dicke and Adrie Groeneveld

ABSTRACT

1.   The phytoseiid predator Amblyseius potentillae (Garman) responded to volatile kairomones emitted from leaves infested by the two-spotted spider mite (Tetranychus urticae Koch), the apple rust mite (Aculus schlechtendali (Nalepa)) or the thrips Frankliniella occidentalis (Pergande), only when the predators had been reared on a carotenoid-free diet. In contrast A. potentillae responded to the European red spider mite (Panonychus ulmi (Koch)) both when the predators had been reared on a carotenoid-containing and a carotenoid-free diet.
2.   Carotenoid-deficient predators did not respond to odour emitted from a host plant that was infested by larvae of the fruit-tree leaf roller (Adoxophyes orana (F.v.R.)), a carotenoid-containing phytophage, that cannot be preyed upon by A. potentillae.
3.   Carotenoids are indispensable for diapause induction in A. potentillae. Hence, carotenoid-deficient predators can increase their fitness by feeding from a carotenoid source. This may explain the response of carotenoid-deficient predators to the kairomones of the two-spotted spider mite, F. occidentalis and the apple rust mite (all containing ingestible carotenoids). As the fruit-tree leaf roller cannot serve as prey and thus as a carotenoid source, it makes sense that the predators lacking carotenoids do not respond to the odour emitted from plants infested by this phytophagous insect.
4.   Two-choice experiments in a Y-tube olfactometer showed that the kairomone preference of A. potentillae has a hierarchical structure: the kairomone of the European red spider mite is the most preferred one, followed by that of apple rust mite, whereas the kairomone of the two-spotted spider mite is the least preferred of these three.

INTRODUCTION

Amblyseius potentillae (Garman) (Acarina:Phytoseiidae) is a polyphagous predatory mite that can feed and reproduce on phytophagous mites, such as the European red spider mite (Panonychus ulmi (Koch)), the two-spotted spider mite (Tetranychus urticae Koch), and the apple rust mite (Aculus

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schlechtendali (Nalepa)) (Kropczynska-Linkiewicz, 1971), and on pollen of broad bean (Vicia faba L.) (Overmeer,1981). The predator occurs in orchards in the Netherlands. Panonychus ulmi and A. schlechtendali, and to a lesser extent T. urticae are the most abundant prey species of A. potentillae in Dutch apple orchards. Much research has been done on the predator-prey relationships of A. potentillae-P. ulmi (Van de Vrie & Boersma, 1970; Van de Vrie, 1973; Rabbinge, 1976) and A. potentillae-T. urticae (Sabelis, 1981). However, little knowledge exists on the relationship between A. potentillae and A. schlechtendali. As the apple rust mite has reached pest status more and more frequently in recent years (Van Epenhuijsen, 1981) it is necessary to elucidate the position of this mite in the diet of predatory mites, such as A. potentillae, that are or might be used in integrated pest management in apple orchards. To study the importance of a prey species in the diet of a predator one can study prey selection by the predator, which can occur at the level of distant prey location, close-range prey location or after prey contact (Greany and Hagen,1981). In this paper we will deal with distant prey location and present data on the response of A. potentillae to volatile kairomones.

In optimal foraging theory it is postulated that predators forage efficiently for prey, in order to maximize their contribution to future generations (for a review see Pyke et al., 1977; Krebs, 1978; Krebs and McCleery, 1984). The net rate of food intake is often assumed to be maximized by foraging predators. In predatory mites this results in maximization of reproductive success: the rate of digestion (conversion of food into eggs) and thus the rate of oviposition increase exponentially with increasing food content of the gut (Rabbinge, 1976; Sabelis, 1981). However, prey quality will also influence the predator's reproductive success. Therefore it can be assumed that predators search efficiently for those prey species that offer the best prospects for reproduction. Thus, we hypothesize that the predator shows a kairomone preference that corresponds with prey preference and that its prey-location behaviour results in maximization of reproductive success.

Sabelis and Van de Baan (1983) showed that A. potentillae, even though reared on T. urticae, did not respond to a volatile kairomone of T. urticae (to which other phytoseiid predators respond) or to a possible kairomone of A. schlechtendali, but that it did use a volatile kairomone of P. ulmi to

locate distant prey of this species. These authors argue that phytoseiid predators select particular spider-mite species with the aid of specific volatile kairomones, so as to maximize reproductive success. The two-spotted spider mite is a good food source for A. potentillae: fecundity, mortality and developmental time of the predator are the same on T. urticae and P. ulmi (Overmeer,1981). However, T. urticae produces a dense webbing, unlike P. ulmi, that produces sparse silk, but not a web. Amblyseius potentillae is hindered by the dense webbing of T. urticae: the predator's searching success is lower on a webbed substrate than on a substrate without webbing (Sabelis, 1981). Therefore, as was concluded by Sabelis and Van de Baan (1983), it was not surprising that this predator responds to the kairomone of P. ulmi and not to that of T. urticae.

In their research Sabelis and Van de Baan (1983) used predators that had been reared on a carotenoid-containing diet of T. urticae (Overmeer and Van Zon, 1983a). But recently, Dicke et al. (1986) showed that A. potentillae reared on broad bean pollen, a carotenoid-free diet for this predator (Overmeer and Van Zon, 1983a) did respond to a volatile kairomone of T. urticae. Carotenoids are indispensable for induction of reproductive diapause (Van Zon et al., 1981; Overmeer and Van Zon, 1983a; Veerman et al., 1983). The results obtained by Dicke et al. (1986) show that prey selection is not only related to short term reproductive success but also to the ability to survive during the winter, which is decisive to long-term reproductive success. When carotenoid-deficient predators have a choice in a Y-tube olfactometer between no prey and an 'inferior' prey (T. urticae), on which reproduction is possible and from which carotenoids can be obtained, they prefer the prey-containing arm (Dicke et al., 1986). In the present paper we will deal with the following aspects of distant prey location by A. potentillae:

1. Kairomone of A. schlechtendali. Does A. potentillae respond to a volatile kairomone of A. schlechtendali? Sabelis and Van de Baan (1983) who did not find a response of T. urticae-fed predators to leaves infested by low densities of A. schlechtendali, suggest that higher rust-mite densities might elicit a response of A. potentillae. If this is not the case for T. urticae-fed predators it should be investigated whether carotenoid deficiency elicits a response in A. potentillae to the kairomone of A. schlechtendali.

2.   Kairomones of other phytophages. Does A. potentillae respond to
volatile kairomones of other phytophages that occur in orchards, and if so,
is this response dependent on carotenoids being present in the diet of the
predators? Do the carotenoid-deficient predators respond to kairomones of
phytophages (which probably contain carotenoids derived from the plant) in
general or only to those phytophages on which they can feed and from which
they thus obtain carotenoids? In this context we studied the response of A.
potentillae to plants infested by the thrips Frankliniella occidentalis
(Pergande) or larvae of the lepidopteran fruit-tree leaf roller, Adoxophyes
orana (F.v.R.). These two phytophages differ in their value for A.
potentillae as phytoseiid predators can feed on thrips (Ramakers, 1978) but
not on larvae of A. orana as these move vigorously, when attacked.
3.   Kairomone preference in two-choice experiments. So far, in all
olfactometer experiments with predatory mites the predators could choose
between no prey at all or a certain prey species (Sabelis and Van de Baan,
1983; Dicke et al., 1986). Sabelis and Van de Baan (1983) found that T.
urticae-fed A. potentillae showed no response to the T. urticae kairomone
but did show a positive response to the P. ulmi kairomone. Therefore it can
be concluded that P. ulmi is preferred to T. urticae. The results obtained
by Dicke et al. (1986) raise the question of which prey is preferred by the
carotenoid-deficient predators, as these respond to kairomones of both prey
species. To study the preference of carotenoid-deficient A. potentillae we
observed the predators when they were offered different kairomones in each
arm of the Y-tube olfactometer.

By studying these three aspects of prey location we will try to
elucidate the structure of kairomone preference of A. potentillae as an
indication of prey preference.

MATERIALS AND METHODS

Predators.

Amblyseius potentillae was reared on three different diets on plastic
plates (McMurtry and Scriven, 1965) in a climate room at $25 \pm 1°C$, $60 \pm 10\%$
r.h. and LD 18:6. (1) Two-spotted spider mites have been used as prey for
A. potentillae for many years. The spider mites were brushed off the host

plant leaves onto the plastic plate. This rearing method diminished the adverse effects of the dense webbing of the spider mites on the predators. (2) For the last 6 years, predators from this culture have been reared on broad bean pollen, a carotenoid-free diet for A. potentillae (Overmeer and Van Zon, 1983a). (3) Finally, predators were used that had been reared for at least half a year on a diet of broad bean pollen to which crystalline β-carotene (Sigma Co., St. Louis, Missouri, U.S.A.) had been added (1 mg β-carotene per 5 mg pollen).

Prey.

Both the European red spider mite and the apple rust mite were collected from orchards. Only leaves containing more than 10 spider mites or 3000 rust mites were selected and used in an olfactometer on the same day. Only one of these prey species was present per leaf.

The thrips F. occidentalis was sampled in glasshouses from tomato and cucumber plants and was reared in the laboratory on Lima bean (Phaseolus lunatus L.) at 20–30°C, 60 $\pm$ 10% r.h. and continuous fluorescent light.

The two-spotted spider mite had been reared in our laboratory for many years on Lima bean under the same conditions as F. occidentalis.

The fruit-tree leaf roller A. orana was sampled from an apple orchard and had been reared in our laboratory for about a year on an artificial diet (Ankersmit et al., 1977) to which ground apple leaves were added. Rearing conditions were 18 $\pm$ 2°C, 60 $\pm$ 10% r.h. and continuous fluorescent light. Lima bean leaves infested by 4th instar larvae for 24 h were used in an olfactometer.

Methods.

A Y-tube olfactometer was used that consisted of a glass tube with an iron wire in the centre, both of which are Y-shaped. At the end of both arms of the olfactometer odour-emitting objects may be placed in PVC cages. Air was sucked out at the base of the tube and led to the outside of the climate room in which the olfactometer was positioned. The air speed in both arms of the olfactometer was measured with a hot wire anemometer and standardized at 0.7 $\pm$ 0.1 m/s by adding dry cotton wool at the inlet of the airstream and/or changing the total airflow speed with a valve. Female predators that had been deprived of food and water for 20 h in a plastic

tube were placed on the wire at the base of the basal tube and observed
individually. Starved predators were used because, unlike well-fed
predators they are known to respond to the volatile kairomone of P. ulmi
(Sabelis and Van de Baan,1983). When the predator walked upwind and reached
the far end of one of the arms, the experiment was terminated. The maximum
duration of an experiment was 10 min. A sign test was used to analyse the
results. The null hypothesis was that the probabilities of the predators
walking to the far end of either arm are equal. Predators that did not walk
to the far end of one of the arms were left out of the statistical
analysis. For a more detailed description of the olfactometer, the
experimental procedure and statistical analysis, see Sabelis and Van de
Baan (1983). The experiments were performed at $25 \pm 1°C$ and $60 \pm 10\%$ r.h.
Leaves were used for a maximum of 3 h except for the experiments using 7
apple leaves infested by 4,000 apple rust mites each: these leaves were
used for 1 h only.

RESULTS AND DISCUSSION

Response to prey kairomones when odour of uninfested leaves is the
alternative.

     Predators reared on several diets were used to study their response to
host plants infested by different phytophagous prey species.
     As Sabelis and Van de Baan (1983) did not find a response of A.
potentillae, reared on T. urticae, to 9 apple leaves infested by ca. 400
rust mites each, we used a higher number of rust mites. When we offered the
predators reared in the same way a total number of 120,000 rust mites, no
response was observed either. However, as Table 7.1 shows, a response did
occur in this situation when the predators had been reared on a carotenoid-
free diet, i.e. broad-bean pollen. Predators reared in this way also
responded to a volatile kairomone when fewer apple rust mites (28,000) were
offered. In the latter case, however, leaves that had been used for more
than 1 h in the bioassay no longer elicited the response: after the leaves
had been used for more than one hour in the bioassay, six predators walked
into the arm that contained apple rust mites and seven into the control

Table 7.1. Response of starved A. potentillae females, reared on carotenoid-free and carotenoid-containing diets, to volatile kairomones of several prey species in a Y-tube olfactometer. Each kairomone was offered separately against a control of uninfested host-plant leaves.

Prey species and number	Number of leaves, and species of host plant in each olfactometer arm	Response of predators reared on									
		Carotenoid-free diet[b]					Carotenoid-containing diet				
		n^a	n (prey)[a]	n (control)[a]	n (nc)[a]	critical level	n	n (prey)	n (control)	n (nc)	critical level
Aculus schlechtendali 120,000	30 apple	49	38	11	0	< 0.001	40	21	19	0	n.s.[c]
							63	34	29	0	n.s.[d]
Aculus schlechtendali 28,000	7 apple	40	30	10	0	< 0.001					
Frankliniella occidentalis 250	9 bean	63	42	21	0	0.006	39	17	20	2	n.s.[c]
Adoxophyes orana 25	15 bean	35	19	16	0	n.s.					

a   n   = number of predators
    n(prey)   = number of predators walking to far end of arm containing infested host plant leaves
    n(control) = number of predators walking to far end of arm containing uninfested host plant leaves
    n(nc)   = number of predators that did not walk to the far end of either arm
b   Diet was broad bean pollen
c   Diet was broad bean pollen mixed with β-carotene
d   Diet was T. urticae
e   Given the null hypothesis and n (prey) and n (control) the critical level can be determined by use of the table of the binomial distribution

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arm. Predators reared on broad-bean pollen to which β -carotene had been added, did not respond to the kairomone of apple rust mites, just like predators that had been reared on T. urticae. Thus, apple leaves infested by A. schlechtendali emit a volatile kairomone to which carotenoid-deficient A. potentillae females respond. This resembles the response of A. potentillae to the volatile kairomone of T. urticae (Dicke et al., 1986). At the present time we have found that carotenoid-deficient predators respond to the kairomones of three phytophagous mites: the spider mites P. ulmi and T. urticae (Dicke et al., 1986) and the rust mite A. schlechtendali. In contrast, A. potentillae females that have carotenoids available respond to the kairomone of only one of the aforementioned three prey species: i.e. P. ulmi (Sabelis and Van de Baan, 1983; this paper).

The question arises whether the carotenoid-deficient predators respond: (a) to kairomones that indicate a host plant that is damaged by phytophagous mites, (b) to kairomones of potential, carotenoid-containing prey species in general or (c) to damaged host plants in general. To answer this question we offered the predators odours of host plants infested by different phytophages. Table 7.1 shows that the predators respond to a volatile kairomone of a non-mite species, the thrips F. occidentalis, but only when they are deficient in carotenoids. These results correspond to those for the kairomones of T. urticae (Dicke et al., 1986) and A. schlechtendali. Carotenoid-deficient predators do not respond, however, to leaves that are infested by A. orana larvae (Table 7.1). Thus, under carotenoid deficiency the predator responds to several prey species, both acarine and non-acarine, that can be preyed upon, but when the predators have carotenoids available they only respond to P. ulmi. When deficient in carotenoids, the predator does not respond to odour emitted from leaves infested by a phytophage that cannot serve as prey, in this case A. orana larvae. Such a response would increase neither long- nor short-term reproductive success as neither food nor carotenoids can be obtained from this phytophagous insect.

The response of carotenoid-deficient A. potentillae to volatile kairomones of A. schlechtendali and F. occidentalis would contribute to long-term reproductive success, if these prey species were a carotenoid source for A. potentillae. Carotenoids in the host plant are present in plastids in parenchymous and stomatal cells. Host plants that are damaged

by F. occidentalis show whitish spots where chloroplasts seem to be absent.
Lewis (1973) and Chisholm and Lewis (1984) state that thrips ingest whole
chloroplasts. This means that thrips are indeed a carotenoid-containing
prey. With respect to A. schlechtendali it is assumed that the stylets are
too short to penetrate into host-plant cell layers deeper than the
plastidless epidermis (Jeppson et al.,1975; Schliesske, 1977; M.A.
Easterbrook, pers. comm.). This would mean that only stomatal cells could
provide the rust mite with carotenoids. However, damage to the host plant
caused by A. schlechtendali (russeting of the underside of the leaves)
suggests that this phytophage not only affects the epidermis but also
deeper cell layers. After histological examination of plum leaves that were
infested by the rust mite A. fockeui (Nalepa et Trouessart), Schliesske
(1977) states that the most obvious damage to deeper cell layers is the
destruction of plastids. Dicke (unpublished data) studied diapause ability
of A. potentillae when reared on apple rust mites and found that A.
schlechtendali is indeed a carotenoid source for A. potentillae, and
therefore apple rust mites can probably feed from cell layers under the
epidermis.

Response to prey kairomones, when kairomones of other prey are the
alternative.

     Results obtained by Sabelis and Van de Baan (1983) and Dicke et al.
(1986) have shown that irrespective of its carotenoid content, A.
potentillae responds to a volatile kairomone of P. ulmi. As the responses
to kairomones of T. urticae (Dicke et al., 1986). A. schlechtendali and F.
occidentalis are dependent on carotenoid deficiency of the predator, it
seems that P. ulmi is not only preferred to T. urticae (Sabelis and Van de
Baan, 1983), but also to A. schlechtendali and F. occidentalis. Will
carotenoid-deficient predators respond selectively when they are offered a
choice between two prey species in the Y-tube olfactometer? When both of
these prey species can provide the predator with carotenoids, but differ in
prospects for other fitness characteristics, we hypothesize the predators
do choose the prey species that offers the best prospects. Whether this
occurs has been studied with respect to the three prey species that occur
most often in Dutch apple orchards: P. ulmi, T. urticae and A. schlechtendali.

Table 7.2. Response of starved <u>A. potentillae</u> females, reared on carotenoid-free diet, in a Y-tube olfactometer when offered kairomones of European red spider mite and two-spotted spider mite simultaneously.

Number of P. ulmi in arm 1[a]	Number of T. urticae in arm 2[b]	n[c]	n(1)	n(2)	Critical[d] level
200-300	ca. 2,700	44	29	15	0.024
200-300	ca. 5.400	64	42	22	0.008

[a] 10-15 active stages/pear leaf. Arm 1 also contained the same number of clean bean leaves as of infested bean leaves in arm 2

[b] ca. 300 active stages/bean leaf. Arm 2 also contained the same number of clean pear leaves as of infested pear leaves in arm 1

[c] n = number of predators
n(1) = number of predators walking to far end of arm 1
n(2) = number of predators walking to far end of arm 2

[d] Given the null hypothesis and n(1) and n(2) the critical level can be determined by use of the binomial distribution

Table 7.3. Response of starved <u>A. potentillae</u> females, reared on carotenoid-free diet, in a Y-tube olfactometer when offered kairomones of apple rust mite and European red spider mite simultaneously.

Number of P. ulmi in arm 1[a]	Number of A. schlechtendali in arm 2[b]	n[c]	n(1)	n(2)	Critical[d] level
200-300	ca. 120,000	28	14	13	n.s.
200-300	ca. 60,000	20	9	11	n.s.
200-300	ca. 28,000	40	29	11	0.003

[a] 10-15 active stages/pear leaf. Arm 1 also contained the same number of clean apple leaves in arm 2

[b] ca. 4,000 rust mites/apple leaf. Arm 2 also contained 20 clean pear leaves

[c,d] see legends of Table 7.2.

Table 7.2 indicates that carotenoid-deficient <u>A. potentillae</u> prefer the European red spider mite kairomone over that of the two-spotted spider mite, even although 10 - 20 times more individuals of the latter species are present. When offered a choice between the kairomones of European red spider mite and apple rust mite the response of the predators depended on the ratio of the prey numbers offered in both arms (Table 7.3). When ca. 200 or more rust mites per active <u>P. ulmi</u> stage are present, no preference can be established. However, when this ratio drops to ca. 100 rust mites

per active spider mite stage, the predators preferred the arm with P. ulmi kairomone. When two-spotted spider mites were offered in one arm and apple rust mites in the other, the predators preferred the arm with kairomone of apple rust mite irrespective of the prey number ratios, that ranged from ca. 44 to as low as ca. 5 rust mites per active spider mite stage (Table 7.4).

In these experiments we offered different ratios of prey numbers to the

Table 7.4. Response of starved A. potentillae females, reared on carotenoid-free diet, in a Y-tube olfactometer when offered kairomones of apple rust mite and two-spotted spider mite simultaneously.

Number of T. urticae in arm 1[a]	Number of A. schlechtendali in arm 2[b]	n[c]	n(1)	n(2)	Critical[d] level
ca. 2,700	ca. 120,000	51	18	33	0.024
ca. 2,700	ca. 28,000	40	14	26	0.040
ca. 5,400	ca. 28,000	40	11	29	0.003

[a] ca. 300 active stages/bean leaf. Arm 1 also contained the same number of clean apple leaves as of infested apple leaves in arm 2
[b] ca. 4,000 rust mites/apple leaf. Arm 2 also contained the same number of clean bean leaves as of infested bean leaves in arm 1
[c,d] see legends of Table 7.2.

predators. For the predator, however, not only prey numbers are important, but also predation success, prey quality, problems in finding these prey, etc. To understand the results of Tables 7.2, 7.3 and 7.4 we should compare the two prey species involved with respect to factors as mentioned above. For A. potentillae the prey quality of T. urticae is comparable to that of P. ulmi (Overmeer,1981), but due to its dense webbing T. urticae is an unprofitable prey for this predator (Sabelis, 1981). This is reflected in the results shown in Table 7.2: T. urticae is less attractive than P. ulmi.

Aculus schlechtendali numbers cannot simply be compared with spider mite numbers as the former prey is much smaller. One adult rust mite contains about four times less food for the predator than a spider mite larva (based on their weights, 0.2-0.25 ug and 0.8-1.0 ug respectively). Thus the biomass of the lowest number of rust mites used (28,000) was

equivalent to 3,000 - 7,000 spider mite larvae (depending on the proportion of adult rust mites present). Table 7.4 shows that even when this amount of rust mites was offered as an alternative to ca. 5400 T. urticae stages, a preference exists for the kairomone of apple rust mite. However, Table 7.3 shows that 200 - 300 P. ulmi stages were preferred over 28,000 rust mites. This rust-mite biomass is much larger than the P. ulmi biomass. Only after a 2- to 5-fold increase of this high rust mite number can no preference of the predators be established. Hence, if we base our conclusion on the biomass ratio of rust mite and European red spider mite, the kairomone of the latter is preferred.

What aspect of the relationship between A. potentillae and A. schlechtendali accounts for rust mites being ranked lower than P. ulmi as food source for A. potentillae? Differences in physical resistance probably do not account for this as the small rust mite is less resistant to attack and is suspected to have a thinner cuticle than spider mites. No information is available on the encounter probability of the two prey species for the predator. Rust mites are much smaller than spider mites and the predator may therefore have difficulties in finding them between the relatively large hairs on an apple leaf. A lower nutritional value of A. schlechtendali for the predator, resulting in a lower fitness may be involved but too few data exist to draw firm conclusions. Kropczynska-Linkiewicz (1971) reports the developmental time of A. potentillae to be 8.8 and 9.5 days and the total fecundity 12.1 and 17.7 eggs on apple rust mite and European red spider mite respectively. However, as she does not mention at what intervals she observed the predators, what prey stages she used and in what densities, it is difficult to draw conclusions on the significance of the differences that she reports. For analysis of reproductive success of A. potentillae when feeding on P. ulmi or A. schlechtendali see chapter 12. A study is currently in progress to elucidate prey preference when both prey species are encountered by the predator at the same location. If the data correspond with the data on kairomone preference, it will be interesting to determine whether prey quality (different nutrients) or encounter probability can explain the results.

At all prey ratios offered, A. potentillae preferred the rust mite kairomone to that of T. urticae (Table 7.4): probably the two-spotted

spider mite is less preferred than apple rust mite by A. potentillae due to the dense webbing.

From these data we conclude that carotenoid-deficient A. potentillae females show a hierarchy of preference for the kairomones of the three prey species studied: European red spider mite produces the most preferred kairomone, apple rust mite the second preferred one and two-spotted spider mite the least preferred. Tables 7.2, 7.3 and 7.4 show that the predators distinguish between the kairomones of different prey species: carotenoid-deficient predators do not respond to semiochemicals that indicate the presence of a phytophage in general but to prey-specific kairomones. Chemical analysis of the kairomones is needed to elucidate the differences in kairomones of the different prey species.

Our work shows that carotenoid-deficient A. potentillae respond to kairomones of more prey species than do predators that have carotenoids available. This may reflect a diversification of the diet under carotenoid-deficiency.

Future research on predation by A. potentillae is needed to ascertain whether kairomone preference does indeed correspond to the prey preference found in predation experiments. If so, it is worthwile to study whether olfactometer experiments offer a quick alternative to the more laborious predation experiments for elucidating prey preference and, hence, the usefulness of natural enemies for biological control (see also Sabelis and Dicke, 1985).

ACKNOWLEDGEMENTS
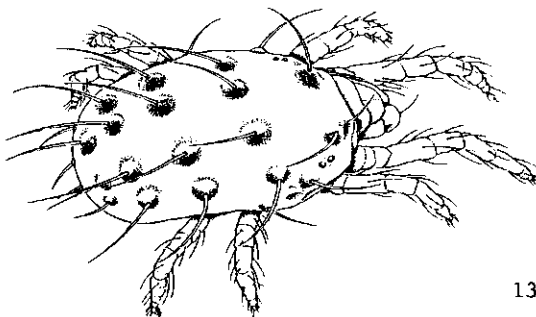
8. Prey preference of the phytoseiid mite *Typhlodromus pyri*: response to volatile kairomones.

Marcel Dicke

ABSTRACT

Using a Y-tube olfactometer, a study has been made of the response of females of the predatory mite Typhlodromus pyri Scheuten (Acarina: Phytoseiidae) to volatile kairomones of three prey species: the European red spider mite (Panonychus ulmi (Koch)), the two-spotted spider mite (Tetranychus urticae Koch) and the apple rust mite (Aculus schlechtendali (Nalepa)).

Predators that had been reared on T. urticae responded only to the volatile kairomone of P. ulmi. In contrast, when reared on Vicia faba L. pollen, they responded to the kairomones of all three prey species. Pollen-reared predators, offered a choice between kairomones of two different prey species, prefer the P. ulmi kairomone to those of A. schlechtendali or T. urticae.

The difference in response between predators reared on V. faba pollen and T. urticae seems to be caused by the low carotenoid content of V. faba pollen. Predators that had been reared on V. faba pollen mixed with crystalline β-carotene, behaved in a way similar to conspecifics that had been reared on the carotenoid-rich prey mite T. urticae. Obviously pollen-reared T. pyri females are in need of carotenoids, which can be obtained from e.g. P. ulmi, T. urticae or A. schlechtendali. This may explain why pollen-reared predators respond to more prey species than T. urticae-reared predators.

Why T. pyri females need carotenoids has not been established. The only function of carotenoids known in mites, is involvement in diapause induction (Veerman, 1985; Overmeer, 1985a). However, as pollen-reared T. pyri enter reproductive diapause under short-day conditions, they either extract sufficient amounts of carotenoids from V. faba pollen, or do not need carotenoids for diapause induction.

Apart from the effect of dietary requirements on prey selection, food deprivation also affects the predator's response to kairomones. All the data mentioned above have been obtained for predators that had been starved for 20 h. Predators that had been reared on T. urticae and starved for 48 h before the experiment, did respond to the volatile kairomone of T. urticae, in contrast to predators from the same culture that had been starved for 20 h. Thus foraging decisions by T. pyri are affected by both starvation time and specific hunger for carotenoids.

INTRODUCTION

Polyphagous animals may consume many different food types. When such animals would feed indiscriminately, the diet of an individual would depend on such factors as the relative abundance and vulnerability of food types. Genetic variation in food intake, both in terms of quantity and quality, may result in differences in reproductive success. Natural selection will then favour those individuals that select food so as to optimize their diet and thereby maximize reproductive success. Therefore, a correlation may be expected to exist between foraging decisions and the reproductive success that will result from them.

In this paper I will present data on prey selection of the polyphagous predatory mite Typhlodromus pyri Scheuten. This predator can feed and reproduce both on animal food (e.g. spider mites, rust mites) and plant food (e.g. pollen) (Chant, 1959; Overmeer, 1981). It occurs in Dutch orchards, where the European red spider mite (Panonychus ulmi (Koch)), the apple rust mite (Aculus schlechtendali (Nalepa)) and to a lesser extent the two-spotted spider mite (Tetranychus urticae Koch) may be available as prey. The predator is used in integrated pest management (IPM) in orchards to control P. ulmi (Van de Vrie, 1985b). For the selection of natural enemies for biological control, previous screening of biological characteristics of natural enemies has been advocated (Sabelis and Dicke, 1985; Van Lenteren, 1986b) to find the 'best' natural enemy species. However, despite application of T. pyri in IPM, the biology of this predatory mite has not been studied extensively (Gruys, 1982). For instance, nothing is known about the prey preference of this polyphagous predator. Detailed knowledge on prey preference is needed for a better understanding of the effect of a polyphagous biological control agent in an environment where more than one prey species is present in order to evaluate its role in regulation of pest numbers.

The first question to be asked in the study of prey selection is: at what stages of prey selection may the animals make decisions? Predatory mites are wingless creatures that disperse in air currents. They have probably little control of where to land, so that the first opportunity of selecting prey is after landing. At that stage, the predator may decide to stay and search for prey or to take off again and start another aerial

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voyage. Information upon which to base this decision may be provided by volatile kairomones (sensu Dicke and Sabelis, 1988), that are emitted from host plant leaves that are infested with prey (see Sabelis and Dicke, 1985 for review). Prey selection decisions may also be made at later stages. For instance, once the predator arrives in a prey patch it may decide which prey stage (or species, if more than one is present) to feed on. At some time, when prey numbers decline, the predators will be faced with the decision of leaving the prey patch or not.

In the study of prey selection by T. pyri, I have investigated prey preference with regard to the response to volatile kairomones. This will give a measure of the predator's prey preference in the first stage of prey selection. In this context, preference can be inferred from two types of experiments: (1) Response to the kairomone of some prey species but not to that of others, in a test situation where one prey species is offered at a time. Several species of predatory mites use volatile prey kairomones in prey selection (see Sabelis and Dicke, 1985 for review). The data show that volatile kairomones of some prey species are responded to, but that kairomones of others species are not (Sabelis and Van de Baan, 1983, Dicke and Groeneveld, 1986). However, no data are available for T. pyri. Whether T. pyri shows a response to the kairomones of P. ulmi, A. schlechtendali and/or T. urticae when only one of these species is offered at a time, was the first question to be answered. (2) Preference of the volatile kairomone of one prey species over that of another, in a situation where more than one prey species is offered simultaneously. If the previous experiment (see (1)) reveals that the predators respond to the volatile kairomones of more than one prey species, it may be questioned whether they prefer one kairomone to the other when both are presented in a choice situation. This was investigated consequently.

In a study on the response of predatory mites towards kairomones, it is essential to take account of the feeding state of the predators. Both quantitative and qualitative aspects of the feeding history have been found to affect the response of some species of phytoseiids. Satiated Metaseiulus occidentalis (Nesbitt) and Amblyseius potentillae (Garman) did not respond to a volatile kairomone of, respectively T. urticae or P. ulmi. However, after 24 h of starvation each of these predator species did respond to the volatile kairomone of the spider mite species indicated (Sabelis and Van de

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Baan, 1983). The response of A. potentillae towards a volatile kairomone of T. urticae is dependent on the carotenoid availability of the predators (Dicke et al., 1986). Carotenoids are indispensable nutrients to this predator: when deficient in carotenoids, the females cannot enter reproductive diapause (Overmeer and Van Zon, 1983a). These nutrients also affect prey selection behaviour. When deficient of carotenoids, the predators do respond to the carotenoid-containing T. urticae, but when they have carotenoids available they do not respond to this prey. Carotenoid-deficient A. potentillae can increase their fitness by finding a carotenoid source, which may explain the response of carotenoid-deficient predators towards the inferior prey T. urticae, that may overcome the carotenoid lack. However, carotenoid-deficient A. potentillae prefer the P. ulmi kairomone to that of T. urticae when given a choice between the two, because the predator's fitness is lower on T. urticae whose webbing severely hinders the predator (Sabelis, 1981). The effect of food quality and quantity on the response of T. pyri towards volatile kairomones has also been incorporated in the present study.

MATERIALS AND METHODS

Predators.

   Typhlodromus pyri was obtained from A. Q. van Zon and W.P.J. Overmeer (University of Amsterdam; cf. Overmeer, 1981) and reared on three different diets on plastic plates (McMurtry and Scriven, 1965) in a climate room at $25 \pm 1°C$, $60 \pm 10\%$ r.h. and continuous fluorescent light. (1) The predators have been reared on a diet of two-spotted spider mites for 6-8 years. The spider mites were brushed off the host plant leaves onto the plastic plate. This rearing method diminished the adverse effects of the dense webbing of the spider mites on the predators (McMurtry et al., 1970; Overmeer, 1981). (2) Another rearing from the same was fed broad-bean pollen (variety Suprifin, Jansen Zaad, Dinxperlo, The Netherlands). Both cultures of T. pyri have been in our laboratory since 1982. (3) To study whether lack of carotenoids affected the response of T. pyri to volatile kairomones the predators were reared on a diet of V. faba pollen to which crystalline β –carotene (Sigma Co., St. Louis, Missouri, USA) had been added (1 mg

β-carotene per 5 mg pollen). When predators from this culture were used in experiments, the culture had been maintained for at least 6 months.

Prey.

Both the European red spider mite and the apple rust mite were collected from orchards where only one of these phytophages was present. Only leaves containing more than 10 spider mites or 2000 rust mites were selected and used in an olfactometer on the same day.

The two-spotted spider mite had been reared in our laboratory for 5–7 years on Lima bean (Phaseolus lunatus L.) at 20–30°C, 60 ± 10% r.h. and continuous fluorescent light.

Methods.

Olfactometer.

A Y-tube olfactometer was used that consisted of a glass tube with an iron wire in the centre, both of which are Y-shaped. At the end of both arms of the olfactometer, leaves with or without prey mites may be placed in PVC cages. Air was sucked out at the base of the tube to the outside of the climate room in which the olfactometer was positioned. The air speed in both arms of the olfactometer was measured with a hot-wire anemometer and standardized at 0.7 ± 0.1 m/s by adding dry cotton wool in the PVC cages and/or changing the total airflow speed with a valve. Unless stated otherwise, female predators that had been deprived of food and water for 20 h at 25°C in a plastic tube (one mite per tube) were placed on the wire in the basal tube and observed individually. When the predator walked upwind and reached the far end of one of the arms, the experiment was terminated. Maximum duration of an experiment was 10 min. After every 10 replications the PVC cages of the two arms were interchanged to eliminate possible positional effects. Leaves were used for a maximum of 3 h.

A sign test was used to analyse the results. The null hypothesis was that the probabilities of the predators walking to the far end of either arm are equal. Predators that did not walk to the far end of one of the arms were left out of the statistical analysis because the 10-min criterion for ending an observation is arbitrary. Given the null hypothesis and the number of predators walking into either arm, the critical level can be

determined by use of the table of the binomial distribution. When the critical level was below 0.05 it was concluded that the predators distinguished between the odours offered in both arms. For a more detailed description of the olfactometer, the experimental procedure and statistical analysis, see Sabelis and Van de Baan (1983). The experiments were performed at $25 \pm 1°C$ and $60 \pm 10\%$ r.h.

Diapause.

To study the induction of reproductive diapause, predator eggs obtained from the regular cultures on T. urticae or V. faba pollen were reared to adulthood in groups, on plastic plates, at $17 \pm 1°C$, 70 ± 10 % r.h. and LD 8:16. When matings were observed, the plates were examined every two days during 18 days. When eggs were found in a group of predators, females were isolated individually on plastic discs four days later to determine the percentage of predators that reproduced (non-diapause females). When no eggs were observed during the time period indicated, although sufficient males were present to inseminate all females, the experiment was terminated.

RESULTS

Response to volatile kairomones.

When apple leaves infested with P. ulmi were provided in one arm of the olfactometer, the predators walked upwind into the arm with this prey significantly more often than into the control arm in which clean apple leaves were present. This response was not dependent on the diet the predators were reared on, which contrasts with the response towards volatile kairomones of T. urticae and A. schlechtendali (Figure 8.1). Female T. pyri did not respond to a volatile kairomone of T. urticae when the predators were reared on this prey or on V. faba pollen mixed with β-carotene. On the other hand, when reared on V. faba pollen only, the predators did respond to the kairomone of T. urticae. This result is similar to that obtained with A. schlechtendali as prey. When reared on V. faba pollen, the predators responded to a volatile kairomone of
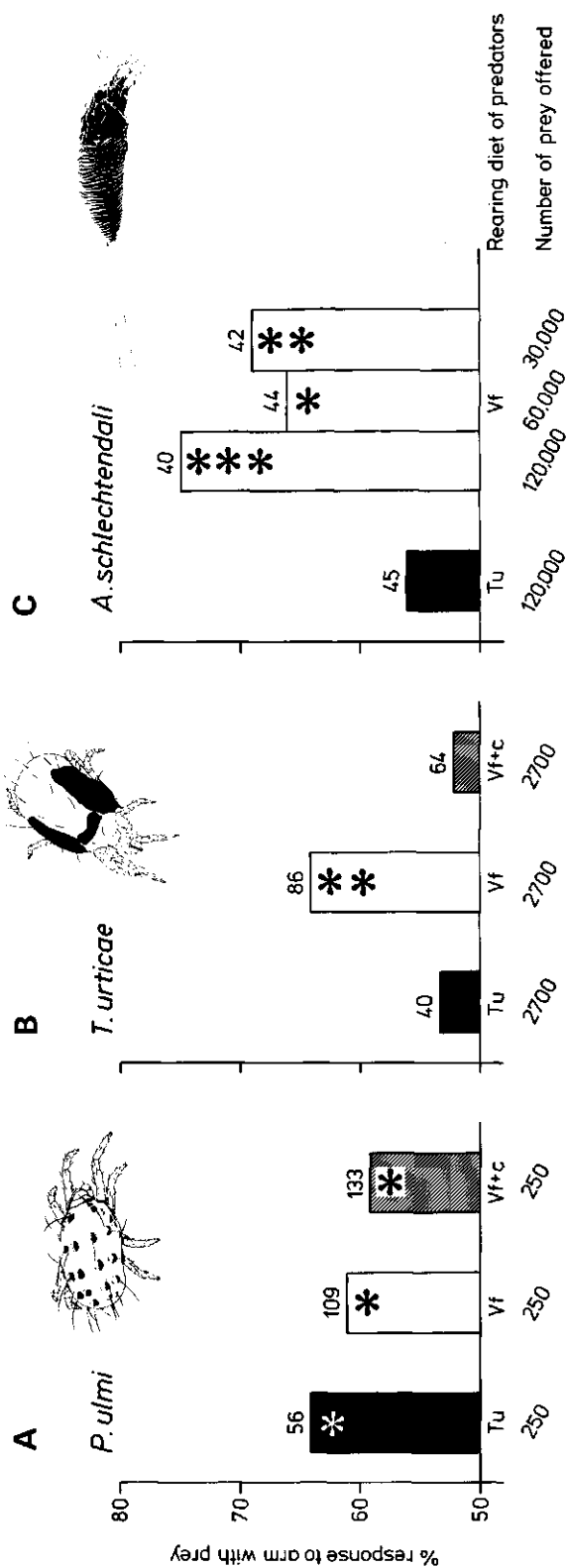
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Figure 8.1: Y-tube olfactometer experiments in which leaves infested with P. ulmi (A), T. urticae (B) or A. schlechtendali (C) were offered versus clean leaves to 20 h starved T. pyri females, reared on different diets. Each bar represents one experiment. Numbers above bars indicate number of prey offered. Numbers above bars indicate number of predators observed. Asterisks indicate level of significance:

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*   =   0.01 < P < 0.05
**  =   0.001 < P < 0.01
*** =           P < 0.001
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Tu = T. urticae; Vf = V. faba pollen; Vf+c = V. faba pollen mixed with β‑carotene.

A. schlechtendali when the rust mite number varied between 30 000 and 120 000. However, when reared on V. faba pollen mixed with -carotene no response was observed when 120 000 A. schlechtendali were offered in the olfactometer. Thus, it can be concluded that female T. pyri respond to volatile kairomones of P. ulmi, T. urticae and A. schlechtendali, but that the response to the kairomones of the latter two prey species is dependent on the diet on which the predators were reared.

Two-spotted spider mites and rust mites are suitable prey for T. pyri. The predators can feed and reproduce on these phytophages (Chant, 1959; Overmeer, 1981). Therefore it was unexpected that carotenoid-containing predators that had been deprived of food for 20 h did not respond to the kairomone of T. urticae when the alternative was odour of clean leaves. One reason may be that the starvation level at which the predators respond had not been reached after food deprivation for during 20 h. Therefore, predators were starved for 48 h on plastic plates where free water was available in the surrounding tissue barrier to prevent the predators from dying from water deficiency. When these predators were offered bean leaves with T. urticae vs. clean bean leaves, the predators walked significantly more often into the arm with T. urticae kairomone than into the control arm (Figure 8.2). Thus it can be concluded that, apart from the qualitative feeding history, the quantitative feeding history also affects the response of T. pyri females towards the volatile kairomone of T. urticae. The question then may be asked whether pollen-reared predators, starved for a period shorter than 20 h, respond to the volatile kairomone of T. urticae. To study this satiated predators from the culture on V. faba pollen were examined in the olfactometer with respect to their response towards the volatile kairomone of T. urticae. The result (Figure 8.2) shows that these predators do not respond to the volatile kairomone of T. urticae, so that the critical satiation level is attained after a starvation period that lies between 0 and 20 h.

Kairomone preference.

Female T. pyri reared on T. urticae, responded only to the volatile kairomone of P. ulmi (Figure 8.1). This implies that the predators prefer the P. ulmi kairomone when reared on T. urticae. However, since predators
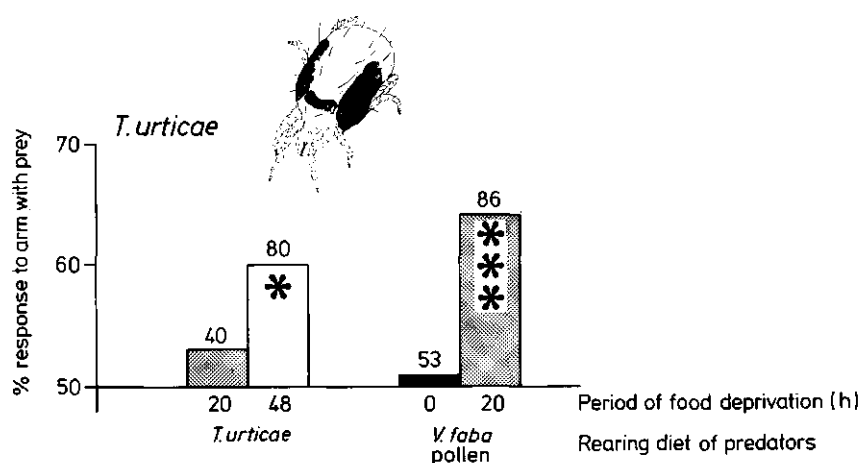
Figure 8.2: Effect of diet and starvation period on the response of T. pyri
to volatile kairomone of T. urticae. Each bar represents one experiment.
Numbers above bars indicate number of predators observed. For explanation
of asterisks, see Figure 8.1.

reared on V. faba pollen responded to the kairomones of all three prey
species, it may be questioned whether these predators distinguish between
these infochemicals and thereby have a relative kairomone preference.
Alternatively they may respond to a general chemical associated with host-
plant damage. To test this, the pollen-reared predators were offered one
prey species in one arm and another prey species in the other. When T.
urticae was offered in one arm and either P. ulmi or A. schlechtendali in
the other, the phytoseiids walked significantly more often to the end of
the arm that contained P. ulmi or A. schlechtendali (Figure 8.3a,b). When
the predators could choose between the kairomones of the two spider-mite
species, the number of two-spotted spider mites offered was 20 times higher
than the number of P. ulmi. However, despite the relative abundance of T.
urticae, the predators walked significantly more often towards P. ulmi.
Therefore it can be concluded that pollen-reared T. pyri prefers the P.
ulmi kairomone to that of T. urticae.

    To compare rust mite numbers and spider mite numbers, one should bear
in mind that a rust mite is much smaller than a spider mite. An adult rust
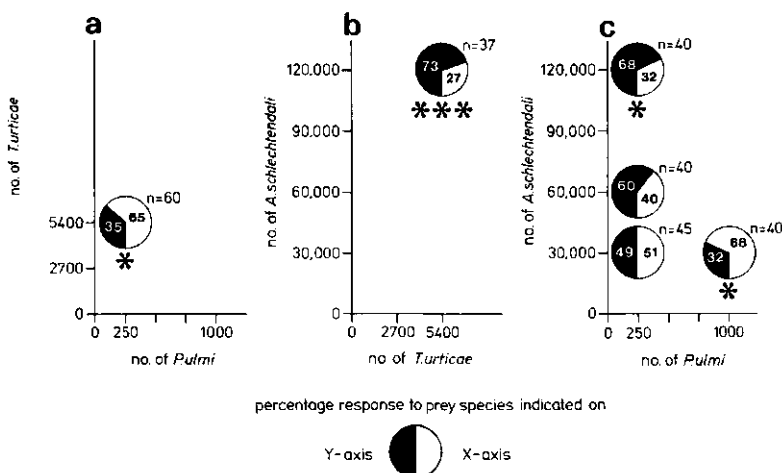mite weighs about 4 times less than a spider mite larva and 60-80 times

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Figure 8.3: Olfactometer experiments in which two prey species were offered to pollen-reared T. pyri, starved for 20 h. One prey species was offered per arm. For explanation of asterisks, see Figure 8.1.

less than an adult spider mite. It is obvious that in the choice situation with rust mite kairomone and two-spotted spider mite kairomone, the rust mites (120 000) not only outnumber but also outweigh the spider mites (5400) (the proportion of adult female spider mites is low). Therefore, it is difficult to reach a firm conclusion on prey kairomone preference in this situation. The data only suggest that T. pyri prefers the A. schlechtendali kairomone to the T. urticae kairomone at the ratio tested, but the effect of other ratios on the response is unknown, as yet.

The effect of varying the ratio of prey numbers on the response of the predators has been studied in more detail with respect to the prey species P. ulmi and A. schlechtendali (Figure 8.3c). When 120 000 rust mites were present in one arm and 250 P. ulmi in the other, the predators preferred the A. schlechtendali kairomone. In this situation the rust mites outnumbered and outweighed the spider mites. When the rust mite number was reduced to 60 000 or 30 000 without a change in the spider mite number, no kairomone preference could be observed. Furthermore, when the spider-mite number was raised to 1000 and the rust mite number in the other arm was 30 000, the predators preferred the P. ulmi kairomone to that of

A. schlechtendali. In the latter situation, the rust mites offered still
outnumbered and outweighed the spider mites. Therefore, these data show
that T. pyri prefers the P. ulmi kairomone to that of A. schlechtendali.

Diapause.

The results in Table 8.1 show that all T. pyri females enter
reproductive diapause irrespective of their diet. These results agree with
those obtained by W.P.J. Overmeer and A.Q. Van Zon (personal communication,
1986) for this predator. However, they contrast to data on A. potentillae,
another phytoseiid species occurring in Dutch orchards, that is unable to
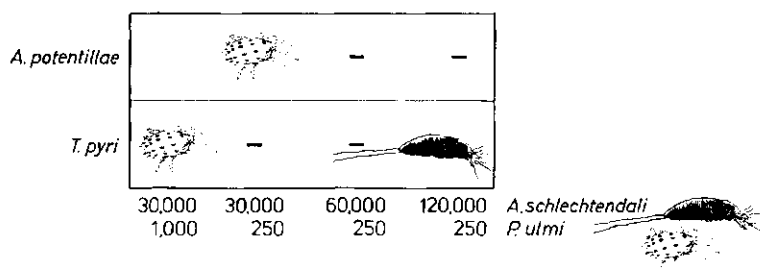enter diapause when reared on V. faba pollen (Overmeer and Van Zon, 1983a).

Figure 8.4: Comparison of the effect of A. schlechtendali : P. ulmi prey-
number ratio on the response of the predator species T. pyri and
A. potentillae in the Y-tube olfactometer. Both predator species were
reared on V. faba pollen and subsequently starved for 20 h. At each prey-
number ratio the preferred prey species is indicated; "-" indicates no
preference. Data on A. potentillae from Dicke and Groeneveld (1986).

DISCUSSION

Diet-dependent response to kairomones.

Female T. pyri that were reared on T. urticae for many years did not
respond to the volatile kairomone of this prey species. However, when
reared on V. faba pollen, this predator species distinguished between clean
and T. urticae infested leaves. With respect to the volatile kairomone of
A. schlechtendali the same phenomenon was observed. These results
correspond to those obtained for another phytoseiid mite, A. potentillae
(Sabelis and Van de Baan, 1983; Dicke et al., 1986; Dicke and Groeneveld,

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1986). In both predator species the differences in response to volatile
kairomones is dependent on carotenoid intake. Compared to other pollen
species, e.g. pollen of the ice plant, <u>Dorotheantus bellidiformis</u> (Burman),
<u>V. faba</u> pollen contain carotenoids in minute amounts: 8.5 vs. 0.26 ug
β -carotene / g pollen (H.C.J.F. Nelis, W.P.J. Overmeer and A. Veerman,
personal communication, 1986). When reared on <u>V. faba</u> pollen mixed with
crystalline β-carotene, the predators responded in a way similar to
conspecifics that had been reared on <u>T. urticae</u>, which is rich in
carotenoids, obtained from the host plant (Veerman, 1974).

   At present, only one function of carotenoids is known in mites:
involvement in diapause induction (Veerman 1985; Overmeer, 1985a). In
<u>A. potentillae</u>, this function of carotenoids has been corroborated. This
species is unable to enter diapause when reared on the carotenoid-poor
<u>V. faba</u> pollen. Therefore, the response of pollen-reared <u>A. potentillae</u> to
kairomones of a larger number of prey can be seen as a way of increasing
chances of finding carotenoid sources (Dicke et al., 1986; Dicke and
Groeneveld, 1986). In <u>T. pyri</u> however, no function for carotenoids is
known. Females entered reproductive diapause either when they were reared
on <u>T. urticae</u> or when they were reared on broad bean pollen, under short
day conditions. This would mean that either <u>T. pyri</u> females do not need
carotenoids for diapause induction, or that, if they do, they can extract
sufficient amounts of carotenoids from this pollen species, in contrast to
<u>A. potentillae</u>. Anyway, the data suggest that β -carotene is an important
nutrient for the predators and that, when they are reared on <u>V. faba</u>
pollen, the predators are in need of carotenoids: addition of a single
chemical to the diet of <u>T. pyri</u>, when reared on <u>V. faba</u> pollen changes the
predator's response to the volatile kairomone of the carotenoid-containing
food source <u>T. urticae</u>. Maybe other functions for β-carotene (see
discussion in Dicke et al., 1986) exist in <u>T. pyri</u>, for which the
predators, when reared on <u>V. faba</u> pollen have insufficient amounts of this
nutrient. Unfortunately, one cannot infer a function for β -carotene from
the present knowledge of the predator's physiology. The solution to this
intriguing problem is likely to be a matter of serendipity.

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Kairomone preference.

The data of the olfactometer experiments in which one prey species was present in each arm, show that T. pyri preferred the P. ulmi kairomone to that of T. urticae and A. schlechtendali. No definite conclusion can be drawn on the kairomone preference of this predator with regard to the kairomones of A. schlechtendali and T. urticae. To conclude on that, it is necessary to study the response when offering a lower number of rust mites and the same high number of T. urticae as was offered in the present experiment.

Comparison of the data in Figure 8.3c with the data of Dicke and Groeneveld (1986) on A. potentillae, shows a similarity: both predator species prefer the P. ulmi kairomone to the A. schlechtendali kairomone. However, some differences exist (Figure 8.4). Dicke and Groeneveld (1986) did not find a situation in which A. potentillae walked significantly more into the arm with rust mite kairomone, whereas in one of the present experiments using the same prey ratios as for A. potentillae (120 000:250) T. pyri did prefer the arm with rust mites. Compared to A. potentillae, T. pyri needs a higher P. ulmi : A. schlechtendali ratio to show a response that favours the P. ulmi kairomone. It may be expected that apart from varying the prey number ratio, also the overall prey number offered affects the predator's response.

Table 8.1: Diapause induction of T. pyri when reared on two different diets.

Predators reared on	% diapause	n
T. urticae	100	35
V. faba pollen	100	33

Relative prey preference.

The observations show that T. pyri prefers P. ulmi to A. schlechten-dali. This conclusion refers to a choice made at a distance, when kairomones of both prey species were offered simultaneously but in different arms of the olfactometer. In the field, the two prey species can occur together on the same leaf. Therefore, after finding a prey patch where both prey species occur, the predators may selectively feed on one of the two prey species. An interesting question is not only whether they do so, but also whether they show the same preference as was observed in the olfactometer study. This can be inferred from the work by Dicke and De Jong (1988) with respect to the prey species P. ulmi and A. schlechtendali.

Dicke and De Jong (1988) made an electrophoretic analysis of the diet of T. pyri females collected in an orchard where only P. ulmi and A. schlechtendali were present as prey. With this method they could identify both prey species in the gut of individual predators. Their data show that at various ratios of P. ulmi to A. schlechtendali, P. ulmi was identified as the main prey species in the gut of T. pyri. Dicke and De Jong (1988) concluded that T. pyri prefers P. ulmi to A. schlechtendali.

These independently obtained data from field collected predators agree with the present data on prey kairomone preference of laboratory reared predators, which is conclusive evidence that T. pyri prefers P. ulmi to A. schlechtendali at different stages of prey selection. No field data on prey selection of T. pyri with respect to T. urticae and P. ulmi are available.

Preference and reproductive success.

Typhlodromus pyri prefers the P. ulmi kairomone to that of T. urticae. The reproductive success obtained by the predators on these two prey species seems to be similar in terms of mortality, developmental time and rate of oviposition. Any differences that have been reported would rather suggest that T. urticae is a more profitable prey than P. ulmi (Overmeer, 1981). However, the data with respect to T. urticae have been obtained under conditions without webbing. As webbing hinders the predators (McMurtry et al., 1970; Overmeer, 1981), their reproductive success under

natural conditions will be lower on T. urticae than on P. ulmi. This
explains T. pyri's preference of P. ulmi to T. urticae. Comparison of the
reproductive succes of T. pyri on A. schlechtendali and P. ulmi as prey
will be the topic of a future paper.
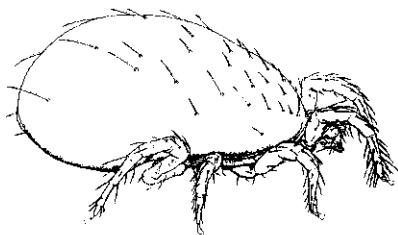
9. Does prey preference change as a result of prey species being presented together? Analysis of prey selection by the predatory mite *Typhlodromus pyri* (Acarina: Phytoseiidae).

Marcel Dicke, Maurice W. Sabelis, Henk van den Berg.

ABSTRACT

1. Prey-selection behaviour of the phytoseiid mite Typhlodromus pyri
Scheuten was studied with a Markov-type model of feeding-state dynamics and
feeding-state dependent searching behaviour (Sabelis, 1981, 1986; Metz and
Van Batenburg, 1985a,b).
2. All behavioural characteristics of the predator which are independent on
the feeding state were represented by one parameter. The remaining feeding-
state dependent characteristics were represented by a function of the
feeding state, with one parameter.
3. The best parameter values to describe a predator – prey interaction were
determined by fitting the model to the predation rates in monocultures.
Assuming that the parameter values are not dependent on the composition of
prey species supply, the diet of the predators in mixed cultures can be
predicted from parameters estimated in monoculture experiments.
4. Apple rust mite (Aculus schlechtendali (Nalepa)) adults and European red
spider mite (Panonychus ulmi (Koch)) larvae were the two prey types
studied. A large discrepancy was observed between calculated and
experimentally determined predation rates of T. pyri in mixed cultures: the
predators actually killed 3-7 times more P. ulmi larvae than was predicted
by the model.
5. Observed changes in the behaviour of the prey, as a result of being
presented together are too small to account for the large difference
between observed and predicted predation rates in mixed cultures. Moreover,
a hypothetical change in walking speed or activity of the predator cannot
explain this difference either. Therefore, it was concluded that the prey
selection behaviour of the predators had changed: the predatory mite T.
pyri prefers P. ulmi to A. schlechtendali.

INTRODUCTION

When foraging on a leaf inhabited by a mixture of prey types, predatory

mites have to decide which prey to accept and which to reject. These

decisions may be based on two types of information. Firstly, there is time-

invariant information resulting from (locally) static properties of the

predator-prey system. Examples are food quality, food quantity per prey
individual and defensive capacity of each prey type. Secondly, there is
information that changes at a short-term time scale. Examples are food
content of the gut, density of each prey type and prey type composition.
The outcome of the acceptance/rejection decisions reflects prey preference
of the predatory mite. Sabelis (1981, 1986) showed (1) that the feeding
state of predatory mites determines the probability of a successful attack
after contact with prey (success ratio) and (2) that, given a particular
feeding state, these probabilities may differ substantially with respect to
developmental stages of the prey. He argued that to detect changes in prey
stage preference as a result of prey stages being presented together, one
should take the feeding state of the predators into account. Ideally this
could be accomplished by standardizing the feeding state of the predator
and investigating prey acceptance when offering a series of prey stages.
This method, however, is not appropriate because prey consumption changes
the feeding state considerably. Moreover, feeding is likely to be an
integral part of the sampling process required to adapt the
acceptance/rejection decision to changed conditions of prey density and
prey composition. To account for a continuously changing feeding state
Sabelis (1981, 1986, in prep.) and Metz and Van Batenburg (1985a,b)
developed a Markov-type model. The parameters of the model were estimated
in monocultures of each prey stage. These estimated parameter values were
used to predict the composition of the predator's diet in mixed cultures
under the assumption that the feeding-state dependent success ratios would
not change as a result of the prey stages being presented together. Sabelis
(1986, in prep.) found that the difference between predicted and measured
diet composition in a mixed prey-stage supply was absent or small, and
concluded that prey stage composition does not affect prey stage
preference. The prey-predator system consisted of <u>Tetranychus</u> <u>urticae</u> Koch
and <u>Phytoseiulus</u> <u>persimilis</u> Athias-Henriot. In this paper we will use the
same method to analyse whether prey preference changes as a result of prey
species being presented together.

   Our method, however, differs in one important respect. We are
interested in prey selection behaviour of another predatory-mite species
than studied by Sabelis (1981, 1986, in prep.), i.e. <u>Typhlodromus</u> <u>pyri</u>
Scheuten. The feeding-state dependent behaviour of this predator species

has not yet been studied in detail and it is quite laborious to obtain
sufficient data on this aspect. Therefore, we simplified the model by
reducing the number of parameters to three: (1) a gut emptying constant,
(2) a constant that represents the feeding-state independent part of the
rate of successful attack and (3) a constant that governs the shape of the
feeding-state dependent part of the rate of successful attack. Each of
these parameters has been estimated by fitting model calculations to the
experimentally determined functional response to each prey species. The
parameters estimated in this way were then used to predict the predator's
diet in a mixture of these prey species, again assuming no change in the
parameters as a result of the prey species being presented together. To
check whether our changes in the method affect the conclusions, our
analysis method has also been applied to the data of Sabelis (1986) to
compare both model calculations.

The phytoseiid mite T. pyri can feed and reproduce on several
phytophagous prey species. The two most abundant prey species in Dutch
apple orchards are the European red spider mite, Panonychus ulmi (Koch) and
the apple rust mite, Aculus schlechtendali (Nalepa), two economically
important acarine pests. The predators frequently encounter both prey
species because these phytophages often occur on the same leaves. Therefore
this is an appropriate system to investigate whether composition of prey-
species supply affects prey preference.

METHODS AND MATERIALS

The methodology is essentially the same as that used by Sabelis (1986).
He used a predator species of which all behavioural data required were
available. No such data were available on the foraging behaviour of
T. pyri. Instead of performing these experiments to determine the
parameters needed, we simplified the procedure to obtain estimates of the
attack rate, σ. Sabelis (1981, 1986) calculated the attack rate from
walking speed and activity of prey and predator, width of searching path,
success ratio (s) of the predator and density of the prey. All these
components of σ were determined in relation to the level of gut fullness
(g) for four phytoseiid species and several stages of the two-spotted

spider mite. These parameters can be divided into components dependent on g and components independent on g, so that the attack rate (σ) may be represented as follows:

$$\sigma = c * f(g) * D, \qquad (1)$$

where c represents the components of the above-mentioned parameters that are not affected by g, f(g) represents the components that are dependent on g and D is the prey density. The data of Sabelis (1981) demonstrate that the success ratio is the parameter that is most dependent on g. The shape of the function f(g) may therefore be assumed to resemble the success ratio curves as measured for several species of predatory mites by Sabelis (1981). These success ratio curves as a function of g can roughly be classified as convex or concave negative exponentials. We chose a one-parameter function for f(g) that describes a set of convex and concave curves:

$$f(g) = \frac{1 - g}{1 + z*g} \qquad z \in <-1, \infty> \qquad (2)$$

where z is a constant.

Thus, the relationship between σ and D, the functional response, is dependent on two unknown constants: c and z, that represent the predator's behaviour. If the functional response is determined experimentally, values for c and z can be found by fitting the model to the experimental data. These estimates of c and z (c^* and z^*) with respect to each of the two prey species are used in the model to predict predation rates in the mixed culture. To investigate to what extent the conclusions depend on the shape of f(g), we also made an analysis with a sigmoid function of f(g) instead of only convex and concave curves. We used the Weibull function for that purpose:

$$f(g) = 1 - \exp^{(a*g^b)}, \qquad (3)$$

where a and b are unknown parameters, determining the scale and shape of f(g) respectively. The use of this function results in a three-parameter function for σ with three unknown constants a, b and c.

Mites.

The prey mites, P. ulmi and A. schlechtendali, have been collected in commercial Dutch apple orchards. Winter eggs of P. ulmi on apple twigs were collected in March and stored at 5°C. The emerging larvae (after 2-4 days at 26°C) were used immediately in predation experiments. Panonychus ulmi larvae that had been collected in an orchard during summer were used for some of the experiments. Apple rust mites were collected on apple leaves in an orchard during July / August and were stored in the laboratory at 5°C in plastic bags. Thirty min - 2 h after transferring the leaves to 26°C, we collected the largest active mites for immediate use in the predation experiment. Apple rust mites can be stored at 5°C for up to 2 - 3 months, but the mites that were used in the experiments have been stored for a maximum of 3 - 4 weeks.

The predatory mites were obtained from W.P.J. Overmeer and A.Q. van Zon (Amsterdam) (for details see Overmeer, 1981) and were reared on Vicia faba L. pollen on plastic plates for c. 5 years. During the last 2 years pollen of V. faba variety Suprifin (Janssen Zaad B.V., Dinxperlo, The Netherlands) was applied. Young females in the oviposition phase (3 days since the final moult) were used in the experiments.

Predation experiments.

Predation was measured on plastic discs on wet cotton wool. These discs (1385 mm^2) were transparant and provided with a mm grid on the underside. The grid facilitated counting of the tiny and numerous rust mites. Prey densities were 1, 2, 4, 8 or 15 P. ulmi larvae or 4, 8, or 40 A. schlechtendali adults per disc for the monocultures and 2 or 6 P. ulmi larvae in combination with 30 adult A. schlechtendali for the mixed cultures. One female predator was introduced per disc immediately after placing the prey on the disc. At regular intervals, varying from 30 min (density 1 P. ulmi or 2 A. schlechtendali per disc) to 2 h (15 P. ulmi or 40 A. schlechtendali per disc) the prey consumed were recorded and replaced by fresh ones. Furthermore, drowned prey were replaced and predator eggs

removed. This procedure was executed during 8 h before the experiment started and served as an adaptation period, to reach a steady state of the food content of the gut (Rabbinge, 1976; Sabelis, 1981, 1986). After the adaptation period, predation was determined during 6 h by treating the discs as had been done during the adaptation period. The experiments were performed in a climate room at $26\pm1°C$ and 60 ± 10 % r.h.

Weighing.

To quantify feeding and food conversion the weights of the prey types, predator females and eggs should be determined. Both prey species were weighed using a quartz fiber fish pole balance (Lowry and Passonneau, 1971), whereas predator females and their eggs were weighed using a Cahn electrobalance.

Behaviour of prey.

A difference between measured and predicted predation rates in mixed culture does not necessarily indicate a change in the predator's prey preference. For that, it is necessary to exclude that the behaviour of one or both prey species is affected by the other species in such a way that its rate of encounter with the predator changed as a result of the two species being presented together. Therefore, two aspects of prey behaviour were studied in mono and mixed cultures: percentage activity and walking speed. Prey densities were 2, 4 or 8 P. ulmi larvae or 4, 15 or 50 A. schlechtendali adults per disc (500 mm^2) for monocultures and 2 P. ulmi larvae and 50 A.schlechtendali adults per disc in mixed cultures. To measure the percentage activity instantaneous observations of the mites were made at 30 min intervals: each prey individual was classified as walking or standing still. Drowned mites were replaced by fresh ones.

With a 'drawing tube' connected to a stereo microscope one can draw walking paths on graph paper with a millimeter grid. A trail was drawn during 3 min, except when the mite stopped before this time limit was reached. Only trails of more than 1 min were analysed. Walking speed was established by counting the number of mm squares traversed, by transforming this to the pathlength (Reddingius et al., 1983) and by dividing by the time elapsed.

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RESULTS

Food conversion parameters.

The relative rate of gut emptying.

The ingested food leaves the gut through resorption and egestion, processes that are dependent on the temperature:

$$r_g = r_{rs} + r_{eg},$$

where r_g is the relative rate of gut emptying, r_{rs} is the relative rate of resorption and r_{eg} is the relative rate of egestion. r_{rs} can be estimated with a 'mass balance equation' (Sabelis, 1986):

$$r_{rs}*g_{max} = r_{rt}*b + R_{ov}*E,$$

where g_{max} is the maximum food content of the gut, r_{rt} the relative rate of respiration and transpiration, b the somatic body weight (total weight minus gut content, weight of integument and terminal oocyte), R_{ov} the rate of oviposition and E the weight of an egg at deposition. The values for these parameters are given in Table 9.1. Some assumptions made during calculation were: (1) The somatic body weight and g_{max} of T. pyri represent similar proportions of the weight of a female in the oviposition phase as has been established for four other phytoseiid species (Sabelis, 1981). (2) r_{rt} and r_{eg}, whose contribution to r_g is small compared to that of R_{ov} were assumed to have the same value as those of other phytoseiid species: 0.04 * (temperature - 11) and 0.01 * (temperature - 11) respectively (Sabelis, 1981). From the parameter values as given in Table 9.1 r_g can be calculated to be 1.72/day at 26°C.

The ingestible weights of the two prey types.

A P. ulmi larva weighed 0.77 ± 0.07 ug (n=28) and an A. schlechtendali adult 0.20 ± 0.06 ug (n=27). We used values of 0.8 and 0.2 ug for the ingestible weights of these prey types respectively. Especially for P. ulmi larvae this is an overestimation, because a significant part of its biomass, consisting of integument and legs, is not ingestible. However, the model is not very sensitive to the weight of P. ulmi, as a result of partial ingestion at the upper range of g values. In the model we divided the gut volume (3.5 ug) into 35 classes of 0.1 ug each. Thus a P. ulmi larva is equivalent to 8 classes and an adult A. schlechtendali to 2

Table 9.1: Parameters needed to calculate the relative rate of gut emptying (r_g).

Parameter	Symbol	Value	n^a	Remark
Weight of young oviposition female just before egg deposition	F	10.5 ± 1.3 ug[b]	10	
Somatic body weight	b	2.3	–	Calculated from F on the assumption that b takes up the same proportion of F (0.22) as in other phytoseiid mites (Sabelis, 1981)
Maximum food content of the gut	g_{max}	3.5^c	–	Calculated from F on the assumption that b takes up the same proportion of F (0.33) as in other phytoseiid mites (Sabelis, 1981)
Weight of freshly laid egg	E	1.9 ± 0.5 ug[b]	9	
Rate of oviposition	R_{ov}	2.0 ± 0.3/day[b]	44	Temperature $26 \pm 1°C$
Relative rate of resorption and transpiration	r_{rt}	0.60	–	Assumed to have the same value as for other phytoseiid mites (Sabelis, 1981), temperature 26°C.
Relative rate of egestion	r_{eg}	0.15	–	Assumed to have the same value as for other phytoseiid mites (Sabelis, 1981), temperature 26°C.

a Number of predators
b mean $\pm$ standard deviation
c Value of g_{max} used in balance equation was set to a slightly lower value ($=g_{max} - 0.2$) to approximate the dynamics of g_{max} during the oviposition rate determination.

classes. This grid of gut content classes is fine enough to account for partial ingestion of both prey types at high levels of gut fullness.

Predicted and measured diet in mixed cultures.

(a) Phytoseiulus persimilis. Sabelis (1986) used empirically determined behavioural data in the model. To investigate whether our indirect and simplified method of estimating the attack rate affects the conclusions, we first applied this method to reanalyse predation of P. persimilis in a
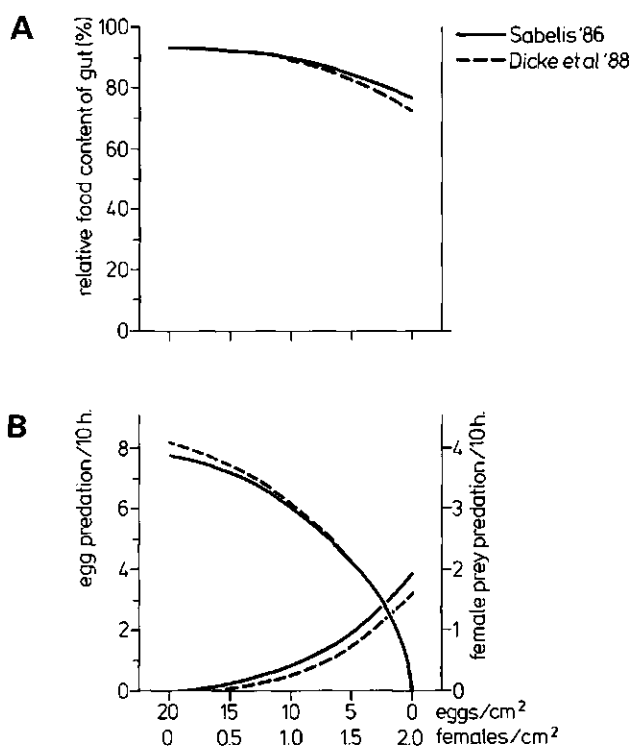

Figure 9.1: Comparison of model steady state calculations for Phytoseiulus persimilis in mixed cultures of T. urticae eggs and females. Continuous lines represent calculations by Sabelis (1986) who included experimentally determined behavioural characteristics into the model. Dashed lines represent calculations made with indirectly estimated behavioural parameters.

A Relative food content of the gut.
B Predation rates.

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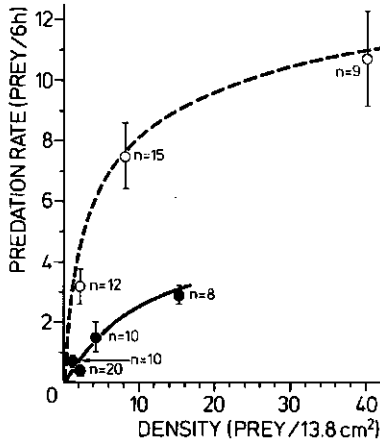
Figure 9.2: Functional response of Typhlodromus pyri to the density of Panonychus ulmi larvae (———) or Aculus schlechtendali adults (----). The lines represent steady-state calculations made with the model, where f(g) is represented by the one-parameter equation (equation 2). The mean measured predation is indicated by a dot, the bar represents the standard error.

Table 9.2: Values of attack rate parameters that result in best fit of model to measurements of predation rates in monocultures

A: Predator: Phytoseiulus persimilis

Prey	c	z
T. urticae eggs	550	−0.72
T. urticae preoviposition females	800	0.30

B: Predator: Typhlodromus pyri

Prey	a	b	c	z
one-parameter function for f(g)				
P. ulmi larvae	−	−	2500	−0.94
A. schlechtendali adults	−	−	13000	−0.95
Weibull function for f(g)				
P. ulmi larvae	1.08	10	3000	−
A. schlechtendali adults	1.06	10	10000	−

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mixture of eggs and preoviposition females of <u>T. urticae</u>. Estimations of c
and z are given in Table 9.2a. Comparison of model predictions by Sabelis
(1986) and by our method (Figure 9.1) do not show a clear difference. Model
calculations of relative gut fullness are also similar (Figure 9.1). Thus,
our simplifications have not affected the conclusions on prey preference of
<u>P. persimilis</u> and therefore our method may be used to analyse prey species
preference by <u>T. pyri</u>.

(b) <u>Typhlodromus pyri</u>. Predation rates in monocultures of each prey species
are presented in Figure 9.2. The best fit of the model to each functional
response is shown for the simulation where $f(g)$ was represented by the one
parameter (z) function (equation 2). Estimations of c and z are presented
in Table 9.2b. For both prey species the value of z^* indicates that $f(g)$ is
a convex curve, that steeply declines at high values of g.

The observed and predicted predation rates in the mixed cultures are
given in Figure 9.3. The 95% confidence intervals of the observations
indicate that the predicted values for the predation rates on apple rust
mites do not differ from the observations. However, the experimental values
for the predation rate on European red spider-mite larvae differ largely
from the prediction: the observed predation rate on <u>P. ulmi</u> is 3 — 7 times
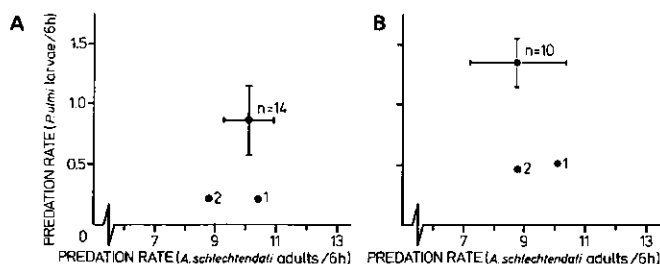

Figure 9.3: Comparison of measured and predicted predation rates of
<u>Typhlodromus pyri</u> in mixed cultures of <u>Panonychus ulmi</u> larvae and <u>Aculus
schlechtendali</u> adults. Dot with bars (representing standard errors)
indicates measured predation rates. Numbered dots represent predicted
predation rates when using (1) the one parameter or (2) the two parameter
function for $f(g)$.

A: 2 <u>P. ulmi</u> larvae and 30 <u>A. schlechtendali</u> adults per disc.
B: 6 <u>P. ulmi</u> larvae and 30 <u>A. schlechtendali</u> adults per disc.

higher than predicted.

Also when the Weibull function is used to represent $f(g)$, the prediction of the predation rates in the mixed cultures differs largely from the experimental values (Figure 9.3, Table 9.2 for estimated values of a, b and c). The conclusion is thus not dependent on the function used for $f(g)$.

Are the deviating predation rates due to changes in predator or prey behaviour?

The difference between observed and predicted predation rates of T. pyri in mixed cultures is too large to be explained by differences with respect to walking activity or speed of prey or predator. In fact changes in prey behaviour as a result of being in a mixed culture are negligible. Some differences in prey activity or walking speed occur between monocultures (Table 9.3), but these characteristics did not differ significantly between the mixed culture and the corresponding monocultures.

The analysis leads to the conclusion that foraging decisions of T. pyri are not solely affected by the food content of the gut but also by the composition of prey species supply. Typhlodromus pyri preferentially feeds on P. ulmi.

We studied whether variation in either food conversion parameters or attack-rate parameters affected the conclusion.

(1) Food conversion parameters. We varied r_g from 1.0 to 3.0 (26°C) and determined c^* and z^* values for each value of r_g. When the value of r_g was as low as 1.0 no fully satisfactory fit for the functional response to the A. schlechtendali density could be obtained. In that case, c and z values that resulted in the least bad fit were used for prediction of the predation rates in mixed cultures. Figure 9.4 shows that the conclusion is not affected by the drastic changes in r_g that we investigated.

(2) Attack rate parameters. Figure 9.5a shows the product of $c^*f(g)$ (which describes the relationship between attack rate (σ) and prey density (D)), against g for several values of a, b and c (Weibull function) with respect to P. ulmi as prey (note that always $f(1.0)=0.0$). The corresponding predicted predation rates in mixed culture are shown in Figure 9.5b. In these calculations, values of a^*, b^* and c^* with respect to A. schlechten-

Table 9.3: Walking speed and activity of prey in monocultures and mixed culture (2 _Panonychus ulmi_ and 50 _Aculus schlechtendali_ per 500 mm^2)

A: _P. ulmi_

Density	Activity(%)	n	Walking speed (mm/s)[1]	n
Mono culture				
2	60-70	30	0.19 + 0.06 a	6
4	60-70	40	0.20 + 0.06 a	10
8	60-70	64	0.22 + 0.03 b	13
Mixed culture				
2	70-80	12	0.15 + 0.05 b	24

[1] mean + standard deviation
* Walking speed data were analysed by ANOVA: F=5.09, df=3, 49, P=0.004. Subsequently, significant differences were detected with the Tukey test (Zijp, 1974). Walking speed values followed by the same letter are not significantly different (5% level).

B: _A. schlechtendali_

Density	Activity(%)	n	Walking speed (mm/s)[1]	n
Mono culture				
4	50-60	28	0.07 + 0.02 b	9
15	60-70	75	0.07 + 0.01 b	9
50	50-60	150	0.06 + 0.02 a	9
Mixed culture				
50	50-60	300	0.05 + 0.02 a	30

[1] See Table 9.3A. ANOVA: F=5.94, df= 3, 53, P=0.002

dali are as determined from the best fit of the functional response. Figure 9.5 shows that only when f(g)=1 (independent of g for all gut-content classes except the highest class, line 1 in Figure 9.5a), the predicted predation rates in the mixed cultures are similar to the measured rates. Similar manipulations of f(g) with respect to _A. schlechtendali_, while using values of a*, b* and c* with respect to _P. ulmi_ as determined from the monoculture, revealed that changes that result in increased _P. ulmi_ predation cause a strong decrease in _A. schlechtendali_ predation. Thus, a severe change in the success ratio with respect to _P. ulmi_ is the only factor capable of making the model calculations correspond with the experimental predation rates.
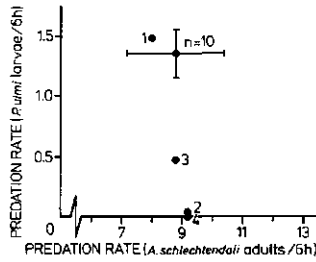
Figure 9.4: Comparison of measured and predicted predation rates of
<u>Typhlodromus</u> <u>pyri</u> in a mixed culture of 2 <u>Panonychus</u> <u>ulmi</u> larvae and 30
<u>Aculus</u> <u>schlechtendali</u> adults. Dot with bars (representing standard errors)
indicates measured predation rates. Predictions were made with different
values of r_g (indicated in the figure).
$c*$ and $z*$ values at different r_g values:

r_g= 1.0: <u>P.ulmi</u>: $c*$=3000, $z*$=-.97; <u>A.schlechtendali</u>: $c*$=30,000, $z*$=-1.0
r_g= 1.4: <u>P.ulmi</u>: $c*$=2900, $z*$=-.93; <u>A.schlechtendali</u>: $c*$=15,000, $z*$=-.97
r_g= 2.0: <u>P.ulmi</u>: $c*$=2900, $z*$=-.90; <u>A.schlechtendali</u>: $c*$=15,000, $z*$=-.85
r_g= 3.0: <u>P.ulmi</u>: $c*$=2700, $z*$=-.85; <u>A.schlechtendali</u>: $c*$=10,000; $z*$=-.3
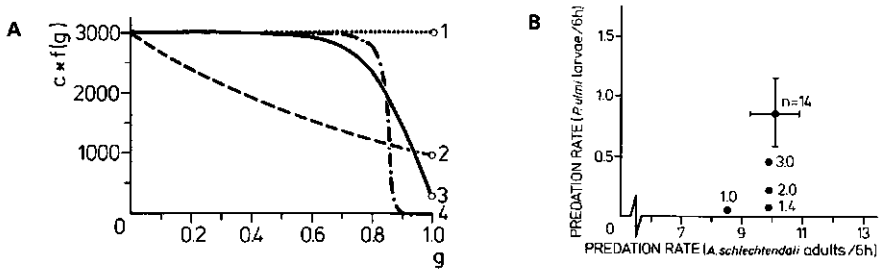
Figure 9.5: Effect of varying model parameters a and b in $f(g)$ (equation 3)
for the interaction of <u>Typhlodromus</u> <u>pyri</u> and <u>Panonychus</u> <u>ulmi</u>.

<u>T.</u> <u>pyri</u> - <u>A.</u> <u>schlechtendali</u> interaction:
$a*$=1.06; $b*$=10; $c*$=10,000

<u>T.</u> <u>pyri</u> - <u>P.</u> <u>ulmi</u> interaction:
1 : $a*$=0.10; $b*$=10; $c*$=3000
2 : $a*$=1.18; $b*$=15; $c*$=3000
3 : $a*$=1.08; $b*$=10; $c*$=3000
4 : $a*$=1.18; $b*$=50; $c*$=3000

A: Effect on the value of $c*f(g)$ at several levels of gut fullness (g).
B: Comparison of the predicted predation rates in a mixed culture of 6
   <u>Panonychus</u> <u>ulmi</u> larvae and 30 <u>Aculus</u> <u>schlechtendali</u> adults with the
   measured predation rates.

DISCUSSION

Causal analysis.

Sabelis (1986) developed a method for causal analysis of prey preference in phytoseiid species, for which many data on behavioural and food conversion parameters were available. If such data are not available, our simplified procedure that only needs quantification of food conversion parameters and functional responses to both prey species is a suitable first step in prey preference analysis. After comparison of predicted and measured predation rates in mixed cultures one may decide whether detailed behavioural observations are needed for the causal analysis or for a functional analysis of prey preference (see below).

The conclusion on prey preference as obtained in this study can be compared with other independent preference analyses to investigate the consistency of the conclusions. Dicke (1988a) investigated prey preference of T. pyri by studying the response of a laboratory population towards prey kairomones. Dicke and De Jong (1988) analysed the diet of a field population of T. pyri by electrophoresis of the gut content. The conclusions of these independent analyses of prey preference agree with the current one and thereby strengthen it: T. pyri prefers P. ulmi to A. schlechtendali.

Functional analysis.

The causal analysis of prey preference asks for a functional analysis of why T. pyri prefers European red spider mites. Two functional arguments can be given why T. pyri would not prefer European red spider mites to apple rust mites: (1) In terms of the predator's intrinsic rate of increase, apple rust mite is a better prey species for T. pyri than European red spider mite (Dicke et al., 1988c). (2) Under selective feeding the time needed to find and catch a prey is longer than when both prey species are accepted. Thus, selective feeding results in a lower level of gut fullness and thereby in a lower rate of resorption of food from the gut (Sabelis, 1981).

Because of the discrepancy between these suggestions and the

observations, it seems that T. pyri sacrifices a high intrinsic rate of increase in order to increase the proportion of P. ulmi in the diet. Why would the predators do this? A prey type may be more preferred e.g. because it (1) is easier attacked and caught, (2) has a more profitable composition of nutritious components, (3) contains less toxic components that need to be detoxified or (4) is a more prolific organism, that provides more prey and for a longer period. Do one or more of these arguments provide an indication why T. pyri prefers P. ulmi?

Rust mites are smaller and are expected to have a thinner integument than spider mites. Therefore it is inconceivable that rust mites are more difficult to get hold of than European red spider mites. Behavioural observations support this view.

A difference in the intrinsic rates of population increase (r_m) of P. ulmi and A. schlechtendali may be hypothesized to affect foraging decisions when the predators have a choice between two spatially separated prey patches. However, this does not hold for prey selection in a patch where both the most prolific and the least prolific prey species occur together. If no difference exists in nutritious or toxic compounds, the predator would do better by feeding non-selectively.

The effect of food quality on food selection behaviour, termed 'specific hunger' by Dethier (1976), is known for many organisms, among which phytoseiid mites. The behavioural response of females of T. pyri and Amblyseius potentillae (Garman) to volatile kairomones is affected by the rearing diet of the predators: when reared on broad bean pollen these predators have a 'specific hunger' for carotenoids (Dicke et al., 1986; Dicke and Groeneveld, 1986; Dicke, 1988a). This is the only qualitative factor known to affect the behaviour of predatory mites. Amblyseius potentillae needs carotenoids for diapause induction (Overmeer, 1985a; Van Houten et al., 1987). The amount needed is small: females that feed on a carotenoid source can supply their offspring with enough carotenoids for diapause induction (Van Zon et al., 1981). Both P. ulmi and A. schlechten-dali may provide the predators with carotenoids (Van der Geest, 1985; Dicke et al., 1988c). However, Dicke et al. (1988c) recorded that these two prey species are not identical in this respect. After transfer of A. potentillae females to a carotenoid-deficient diet, the effect that previous feeding on P. ulmi had on diapause induction in the offspring lasted longer than the

effect of previous feeding on A. schlechtendali. Whether this is caused by
a (quantitative or qualitative) difference in carotenoid content cannot be
concluded from their data. These nutrients are also important for T. pyri,
but their function for this predator species is unknown yet (Dicke, 1988a).
Therefore, it is difficult to conclude on the effect of a difference in
carotenoid-content of the two prey species on prey preference.

Current knowledge is insufficient to explain why T. pyri prefers
P. ulmi to A. schlechtendali. More investigations are needed to understand
which selection pressures may have moulded prey preference of T. pyri. For
discussion see Dicke et al. (1988c).
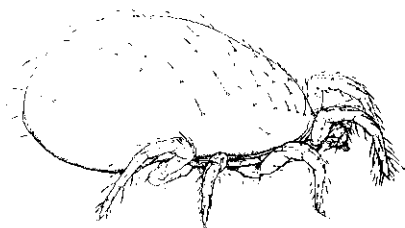
10. Prey preference of the phytoseiid mite *Typhlodromus pyri*: An electrophoretic diet analysis.

Marcel Dicke and Marijke de Jong

ABSTRACT

  The prey selection of the phytoseiid mite Typhlodromus pyri Scheuten
was studied by using polyacrylamide gel electrophoresis to analyse the diet
of field collected predators. The predators were obtained from an orchard
where the phytophagous prey mites Panonychus ulmi (Koch) and Aculus
schlechtendali (Nalepa) were present in various density ratios.
  Esterases of both prey species were identified in the predators, but on
all sampling dates, P. ulmi esterases were discovered much more frequently
than those of A. schlechtendali. The data show that T. pyri hardly fed on
A. schlechtendali over a range of prey density ratios. It is therefore
concluded that T. pyri prefers P. ulmi to A. schlechtendali.

INTRODUCTION

  The phytoseiid mite Typhlodromus pyri Scheuten (Acarina: Phytoseiidae)
is a polyphagous predator. It may feed and reproduce on many food types,
e.g. the European red spider mite, Panonychus ulmi (Koch) and the apple
rust mite, Aculus schlechtendali (Nalepa) (Chant, 1959; Overmeer, 1981).
These mites are the most abundant prey species available to this predator
in Dutch orchards. However, polyphagy does not imply that the predators
feed indiscriminately.

  In a laboratory study on prey selection, Dicke (1988a) concluded that
T. pyri prefers P. ulmi to A. schlechtendali. This conclusion was based on
the predator's response to volatile kairomones of these phytophages.
However, one needs to ascertain whether the data obtained under laboratory
conditions, using animals that have been reared on one food source for many
years, correspond to the field situation. A field population may undergo
physiological and genetic changes when introduced into the laboratory (Van
Lenteren, 1986b). In recent reviews of the present status of the knowledge

of interactions between phytoseiid mites and spider mites, attention has
been drawn to the lack of direct evidence of the diet composition of these
mites in the field (Overmeer, 1985b; Sabelis and Dicke, 1985). Analysing
the prey preference of phytoseiid mites under field conditions is difficult
because the animals are small, have a low feeding rate and are inactive for
a large part of the time (Sabelis, 1981). Therefore, researchers have been
reluctant to make direct observations of feeding behaviour under field
conditions. Nevertheless, it is possible to measure prey preference by
qualitatively determining the gut content of individual predators with
polyacrylamide gel electrophoresis (Murray and Solomon, 1978). This method
allows unravelling of the predation history over a specific time period,
dependent on food digestion rate of the predator and sensitivity of the
detection technique. Since its introduction, the method has been used in
only one study on the diet composition of a predatory mite under field
conditions, namely, the Antarctic Gamasellus racovitzai (Trouessart) by
Lister (1984). The present study on electrophoretic analysis of the diet of
T. pyri, collected from a Dutch apple orchard was set up to validate
laboratory-determined data on prey preference (Dicke, 1988a).

MATERIAL AND METHODS

Mites.

Samples of predators were obtained weekly from an unsprayed apple
orchard near Biddinghuizen (Flevoland, The Netherlands) during August and
September 1983. Branches having leaves on which P. ulmi, A. schlechtendali
and T. pyri were present, were selected. Ten successive leaves were
collected from these branches and stored individually in marked petri
dishes. In the laboratory, c. 2 - 3 h after sampling of the leaves, female
predators were removed from the leaves and stored individually in plastic
tubes at - 20°C. Their gut contents were analysed the next day.

The predators were selected on the basis of their behaviour: T. pyri
moves much more slowly than other phytoseiid species that occur in Dutch
orchards such as Amblyseius finlandicus, the other abundant phytoseiid.
Verification of this selection criterion was made in two ways. (1) A sample
of selected predators (n=20) was mounted in Berlese's medium for

identification. All these predators were identified as T. pyri. (2) The
esterase pattern of all predators subjected to electrophoresis was known.
The esterase pattern of A. finlandicus differs distinctly from that of
T. pyri (Figures 10.1 and 10.2). The esterase patterns of the predators
selected on the basis of their behaviour, did not show marked differences.
No esterase patterns were found that resembled that of A. finlandicus.
Thus, based on these tests, we conclude that all analysed predators were T.
pyri.

The leaves were stored at 4°C for 1-2 days, after which the numbers of
prey were counted under a stereo microscope (magnification 25 x). The
numbers of P. ulmi were counted per stage, whereas all A. schlechtendali
were counted as one category. At rust mite densities exceeding ca. 600 per
leaf, we counted these mites on one quarter of a leaf and extrapolated this
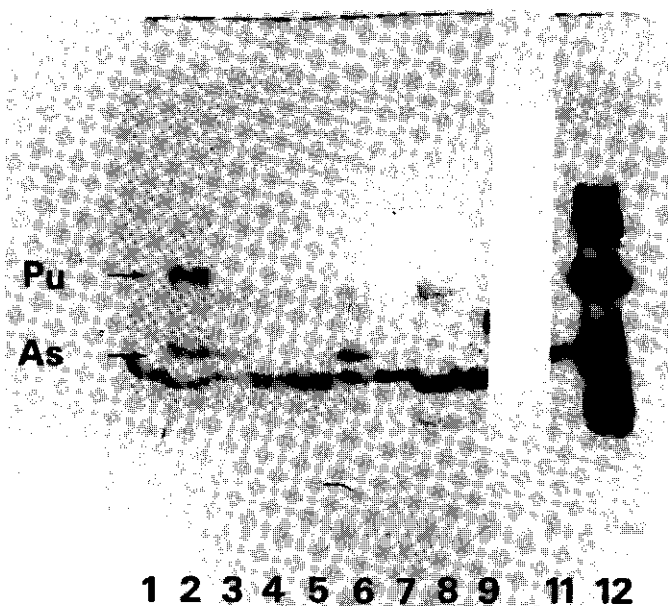
Figure 10.1: Predation of field-collected T. pyri on P. ulmi and/or
A. schlechtendali
     lanes 1-9: field collected T. pyri; predators 2,3 and 6 had fed on
                A. schlechtendali and predator 2 had also fed on P. ulmi;
     lane   11: 20-30 A. schlechtendali;
     lane   12: one female P. ulmi;

As: A. schlechtendali esterase that is detected in T. pyri;
Pu: P. ulmi esterase that is detected in T. pyri.

number to the density per leaf, on the basis that at high densities rust
mites are evenly distributed over a leaf (M. Dicke and M. De Jong, personal
observation). Other prey species were rarely observed.

To determine the detection retention time we used female predators from
cultures that had been maintained in the laboratory on broad bean (Vicia
faba L.) pollen or two-spotted spider mites (T. urticae Koch) for ca. 5-6
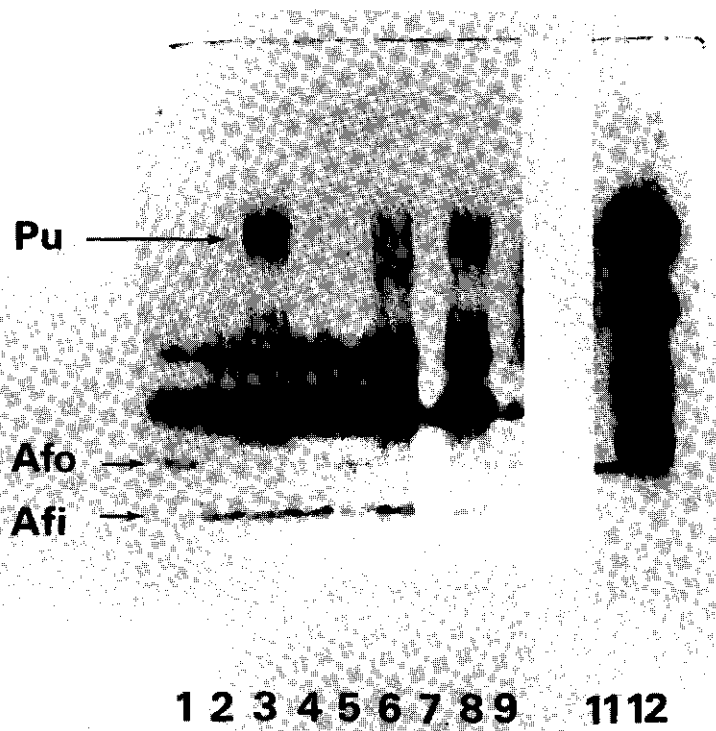years. For rearing conditions, see Dicke (1988a).

Figure 10.2: Electrophoresis of A. finlandicus collected in a Morello
cherry orchard in Wessem, The Netherlands, where P. ulmi and the rust mite
Aculus fockeui (Nalepa et Trouessart) were present.
    lanes 1-9: field-collected A. finlandicus; predators 3, 6 and 8 had
                  fed on P. ulmi and predator 1 had fed on A. fockeui;
    lane 11: 20-30 A. fockeui;
    lane 12: one female P. ulmi;

Afo: A. fockeui esterase that is detected in A. finlandicus.
Pu : P. ulmi esterase that is detected in A. finlandicus.
Afi: A. finlandicus esterase that clearly distinguishes this predator
    species from T. pyri.

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Electrophoresis.

To identify prey in the gut of the predators, we applied polyacrylamide
gel electrophoresis on vertical slab gels prepared with a total gel
gradient of 5-28 %, a cross-linker gradient of 3.5-6.2 % and with 0.2% w/v
Triton X-100. Thus, the pore size in the gel decreases from the top to the
bottom and the mite proteins will migrate until restricted by the pore
size. After electrophoresis, the esterases in the gel were stained with a
0.2 M phosphate buffer (pH 6.9) in which 1- and 2-naphthylacetate (both
1.34 mM) were dissolved and to which 1 ml of a solution of Fast Blue RR in
acetone (15 mg/ml) was added. Esterases hydrolyse the naphthylacetates to
form acetic acid and 1- or 2-naphthol. The naphthols react with Fast Blue
RR and thereby stain the gel. Staining was performed overnight (18 h) at
ca. 20°C in darkness.

Female predators and spider mites were squashed individually in a drop
of maceration buffer in a shallow pit in a perspex plate, by means of a
glass rod. The rust mite sample that was subjected to electrophoresis as a
control consisted of 20 - 30 mites, squashed together with the aid of a
needle. The samples were applied to the gel with a microsyringe. After all
samples had been applied to the gel (this took c. 1 - 2 h in all),
electrophoresis was carried out at 20 - 25°C at 200-300 V for 3 h. For more
details on the method and composition of the buffers, see Murray and
Solomon (1978).

Analysis of detection retention time.

To ascertain how long A. schlechtendali enzymes can be detected in the
predators, female T. pyri were starved individually for 24 h in a plastic
tube to remove the pollen from their gut. Then they were offered an ample
supply of A. schlechtendali on apple leaf discs for 24 h. Subsequently, the
predators were deprived of food but not of water, for a period of 0 - 40 h
with 5 h intervals, on plastic discs. After these treatments at 25°C the
predators were stored at -20°C and their gut content was analysed within
two days.

Several esterases can be seen in a sample of one P. ulmi female (Figure 10.1). This contrasts with the data obtained by Murray and Solomon (1978) who detected only one P. ulmi esterase. However, they applied a sample representing 10 % of a P. ulmi female to the gel and stained for much shorter periods (1-3 h). Fitzgerald et al. (1986) detected four esterase bands in P. ulmi after short staining periods (up to 4 h). They showed that both the duration of staining and the quantity of P. ulmi greatly affected the staining intensity. One or two of the P. ulmi esterases can be seen in T. pyri that fed on P. ulmi (Figure 10.1). Murray and Solomon (1978) demonstrated that a P. ulmi esterase could be identified in the gut of T. pyri for at least 31 h (temperature not mentioned) after the predators had stopped feeding on this prey. In A. schlechtendali we regularly discovered only one esterase with our staining method (Figure 10.3). Occasionally a second esterase was detected at the bottom of the gel, but this esterase has never been detected in T. pyri that were fed A. schlechtendali. The detection retention time of the A. schlechtendali enzyme in the gut of T. pyri was more than 40 h of starvation at 25°C (Figure 10.3). In the field, average temperatures were below 25°C. Esterases of both prey species were discovered in the field-collected predators. No interference was observed between the A. schlechtendali esterase and one of the P. ulmi esterases. This means that electrophoretic identification of prey esterases may be used to study the diet of T. pyri. Personal observations indicated that in the field, T. pyri remains on one leaf for a long time (if prey is available, for more than 24 h). On the basis of these observations and the data on the detection retention time, we assume that the electrophoretic analysis of the predator's gut content conveys information on its prey selection on the leaf the predator was collected from.

Figure 10.4 shows that the frequency of occurrence of the esterases of the two phytophagous prey species differed greatly in field-collected T. pyri. In the majority, esterases of P. ulmi were recovered (65+4 %), whereas A. schlechtendali esterase was identified in only 6 (4+2) % of the predators.

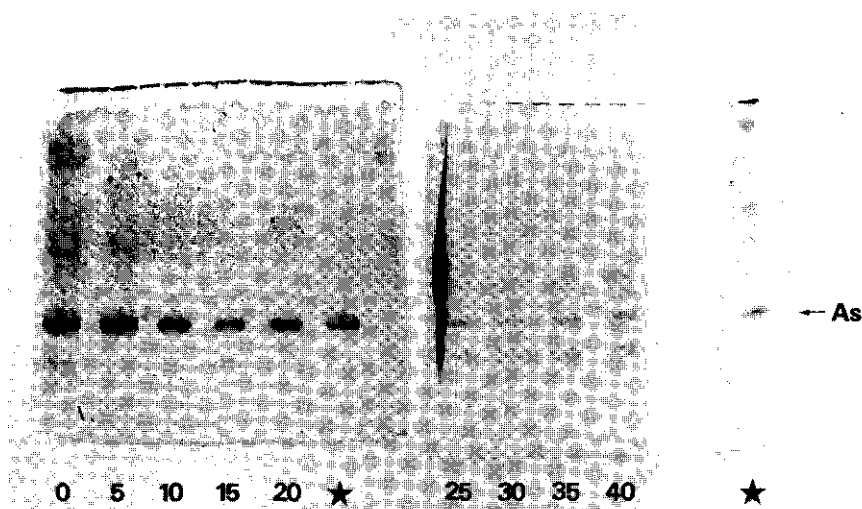All predators were collected from leaves on which both prey species

Figure 10.3: Detection retention time analysis of A. schlechtendali esterase in T. pyri. Every lane represents a predator with a different starvation time. Numbers indicate period of food deprivation in hours. The right-hand lane on each gel (indicated with an asterisk) represents 20-30 A. schlechtendali as reference.

As: esterase of A. schlechtendali.

were present. Yet the densities of these prey species on the sampled leaves varied from 1-220 spider mites (eggs excluded) and 1- >2000 rust mites per leaf. Thus, the predators were present at very different prey density combinations. To see whether quantitative differences in prey availability affected the data, the results were analysed per prey density combination (Figure 10.5). From this figure, it is obvious that at most prey density combinations, either P. ulmi esterases or no prey esterases at all were detected in the predators. Only at the lowest prey density combination (1-5 P. ulmi, 1-50 A. schlechtendali) were predators found with rust mite esterase as the only prey esterase. Predators with esterases of both prey species were found occasionally at several prey density combinations.

Predators in which only rust mite esterase was identified were collected on the last sampling date (15 September). But on this date too, the majority of T. pyri had fed on P. ulmi. Predators with esterases of both prey species were collected on several sampling dates.

On all sampling dates and at most prey density combinations, a proportion of predators existed in which neither P. ulmi esterases nor A. schlechtendali esterase were identified.
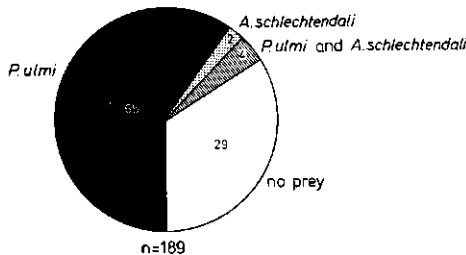

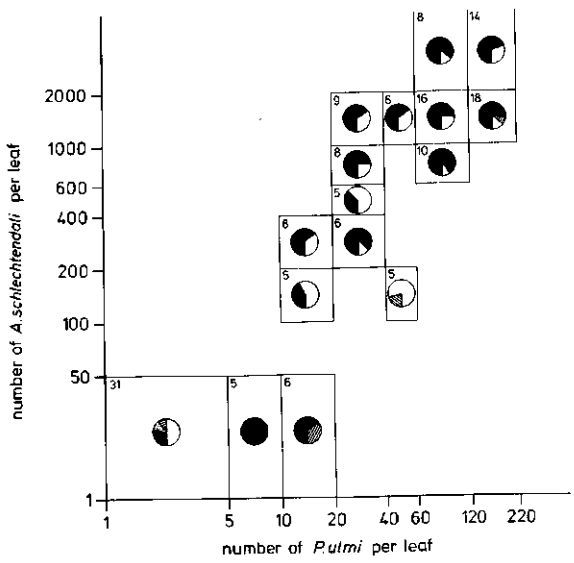Figure 10.4: Percentages of prey esterases in field-collected T. pyri.

Figure 10.5: Percentages of prey esterases in field-collected T. pyri at several prey density (all stages except eggs) combinations. Per class of prey density combinations (represented as a rectangle), a pie diagram indicates the esterase percentages detected; the number in the top left corner of the rectangle indicates the number of T. pyri analysed. For explanation of the pie diagram sectors, see figure 10.4. Axes on log scale.

DISCUSSION

The main prey esterases that were identified in T. pyri were those of P. ulmi, even when the predators had been collected from leaves where in addition to this prey, very high densities of A. schlechtendali were present. This may be explained in two ways: (1) rust mite esterase is more difficult to detect than spider mite esterases or (2) rust mites form a minor component of the diet of T. pyri, except when the density of rust mites is low to high and that of spider mites very low.

Our laboratory results show that rust mite predation can be detected in the predators long after feeding actually took place. After starvation during 40 h at 25°C the gut of a T. pyri female is almost empty: only 6 % of the gut volume contains food (relative rate of gut emptying, r_g, at 25°C is 1.72/day, Dicke et al., 1988a; fraction emptied is $1 - e^{(-r_g * time)}$, Sabelis, 1981). This indicates that very small amounts of rust mite food can be detected in the predators, which is corroborated by the observation that rust mite esterase can be detected in predators that just before fed on only one adult rust mite (data not shown). Consumption of one adult rust mite (0.20 ug) accounts for 6% of the maximum food content of the gut (c. 3.5 ug, Dicke et al., 1988a). These data contrast with the conclusion by Van der Geest and Overmeer (1985), who stated that demonstration of the rust mite esterase is only possible when the sample contains 30-40 rust mites. Their conclusion was based on electrophoresis of a sample of squashed rust mites and a staining procedure that usually lasted for 2 h (L.P.S. van der Geest, pers. comm. 1987). Squashing of the tiny rust mites is difficult with the current procedure: after extensive squashing of a sample of 20-30 rust mites in a drop of buffer, we did not squash all individuals. It is possible that Van der Geest and Overmeer were less successful in squashing the rust mites. Recently Murray and Solomon (1985) developed a technique that is more suitable for squashing rust mites. Also, the short duration of staining may have affected the conclusion by Van der Geest and Overmeer (1985): Fitzgerald et al. (1986) showed that the intensity of the least intensive esterase band of P. ulmi increased linearly with the duration of staining. Our data show that the conclusion of Van der Geest and Overmeer (1985) was premature. The low detection frequency of rust mite esterase cannot be explained by a difficulty in detecting this esterase. As our

procedure can detect small amounts of rust mites in T. pyri, it is unlikely that rust mite predation by the field-collected predators has occurred frequently. We therefore conclude that P. ulmi is a much more important dietary factor of T. pyri than A. schlechtendali. Because the fraction of predators with only spider mite esterases is much larger than the fraction with only rust mite esterase and the fraction of predators with both prey species in the gut is small, the use of a quantitative analysis of prey esterases (cf. Fitzgerald et al., 1986) is not needed to reach this conclusion.

What did those predators, in which neither P. ulmi nor A. schlechten-dali esterases were identified, feed on? We could not discover other prey in the orchard. Moreover, the esterase patterns of the predators without esterases of P. ulmi or A. schlechtendali did not suggest predation on other prey species. Therefore, predation on other arthropods cannot explain the high percentage of predators in which no prey esterases were identified. On the other hand, the chance that a predator had not encountered a prey item in the 24 h before sampling is negligible at the high prey densities observed. Movement of the predators inside a prey colony is random. As a result, the inter-encounter periods are random too and meet the conditions of a Poisson process (Fransz, 1974). The probability of no encounter is then equal to $e^{(- \text{encounter rate} * \text{time})}$. The rate of encounter is dependent on the size of prey and predator, the walking speeds of prey and predator, the fraction of time they are active and the prey density (Sabelis, 1981). If we assume that the predator has a diameter of 0.6 mm, a speed of 0.1 - 1 mm/s, and is active for 10 % of the time (values based on Sabelis, 1981), whereas the prey are points (without diameter), do not move and have a density of 2000 rust mites and 200 spider mites per leaf of 50 cm^2, then the average rate of encounter varies from 9.5 - 95 per h (for equation used to calculate the rate of encounter, see Sabelis, 1981). Then, the probability that the predator did not encounter a prey item in 24 h is 10E-991 to 10E-99. So, other causes must be responsible for the phenomenon of a high percentage of predators without detectable prey esterases.

Lister (1984) reports proportions of c. 10-30 % of G. racovitzai without prey esterases during the feeding season. Also from his data no correlation can be seen between prey availability and the percentage of

predators without prey esterases. In addition to qualitatively analysing the diet of field collected predators, Lister (1984) also made a quantitative analysis of the prey esterase content of laboratory-fed G. racovitzai, to estimate the detection time of a prey species in this predator. From his data it is obvious that the variation is large: even without starvation or after a relatively short starvation period, the amounts of prey esterase measured in some (6-18%) predators are close to zero. Typhlodromus pyri females in which no prey esterases were identified might have fed on either P. ulmi or A. schlechtendali or both. We assume that these predators consumed the prey species in the same proportions as found for conspecifics in which prey esterases were detected. Alternatively they may have fed on non-prey food sources such as pollen, fungi or plant juices, which we have not been able to detect with electrophoresis.

Predators with A. schlechtendali esterase as the only prey esterase were found exclusively at the lowest densities. So T. pyri preferentially feeds on P. ulmi and only includes A. schlechtendali in its diet when spider mites are scarce. In our situation this only happened at the end of the season. Overmeer (1985b) suggests that Aculus species are important as an alternative food source for predatory mites early in the season, before P. ulmi winter eggs hatch. Although we did not collect T. pyri in spring, our data, obtained in August and September support this view.

Dicke (1988a) analysed prey preference of T. pyri by offering prey kairomones to starved predators. His data relate to a situation where the predators were outside the prey patch. In choice situations the predators preferred the P. ulmi kairomone to the A. schlechtendali kairomone. The data from the present study, where the predators were in a prey patch with both P. ulmi and A. schlechtendali demonstrate that the predators show the same preference. This indicates that P. ulmi may not only be a more favourable prey species when long-distance prey finding is the first hurdle to be cleared, but also when close-distance prey finding, prey handling, etc. are involved.

ACKNOWLEDGEMENTS
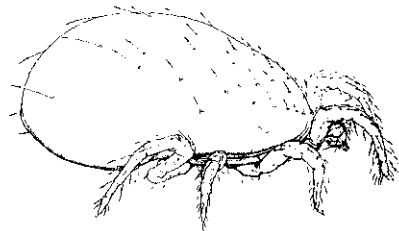
11. Analysis of prey preference in phytoseiid mites by using an olfactometer, predation models and electrophoresis.

Marcel Dicke, Maurice W. Sabelis and Marijke de Jong

ABSTRACT

Prey preference of three phytoseiid species, Typhlodromus pyri Scheuten, Amblyseius potentillae (Garman) and A. finlandicus (Oudemans), that occur in Dutch orchards was analysed with respect to two economically important phytophagous mites: Panonychus ulmi (Koch) (European red spider mite) and Aculus schlechtendali (Nalepa) (apple rust mite). Two types of laboratory experiments were carried out:
(1) Olfactometer tests to study the response when volatile kairomones of both prey species were offered simultaneously.
(2) Predation tests in mixtures of the two prey species and comparison with calculated predation rates using a model provided with parameters estimated from experiments with each of both prey species alone.
In addition, the diet of field-collected predators was analysed using electrophoresis.
For each predator species the results of the different tests were consistent in that T. pyri and A. potentillae preferred P. ulmi to A. schlechtendali, whereas A. finlandicus preferred A. schlechtendali to P. ulmi.

INTRODUCTION

Phytoseiid mites may feed on a variety of food sources. Some species such as Phytoseiulus persimilis Athias-Henriot only feed on spider mites, others such as Amblyseius potentillae (Garman) may feed on a wide range of unrelated prey species, not restricted to the Acari. Also plant tissue may be included in the diet of some phytoseiid species (Porres et al., 1977). Variation in prey selection behaviour, resulting in quantitative and/or qualitative differences in food intake, may result in differences in reproductive success. If this variation is genetically determined natural selection is expected to favour those genotypes that maximize their contribution to future generations. It is therefore expected that phytoseiid mites do not feed indiscriminately but prefer certain food types

over others.

From an applied point of view prey specificity has been regarded as one of the criteria for pre-introductory evaluation of natural enemies (Beddington et al., 1978; Hassell, 1978; Van Lenteren, 1986a) and was regarded to be an indispensable characteristic for biocontrol agents in (seasonal) inoculative release programs (Van Lenteren, 1986a). In a comparison of characteristics of predatory mites that are successful in biological control, McMurtry (1982) discussed prey specificity in terms of the range of prey/food types that could be fed on and that supported survival and reproduction of the phytoseiid species. Moreover, not only the range of possible prey/food items is important in assessment of prey specificity, but also the preference of the predator (Sabelis and Dicke, 1985).

For assessment of prey preference it is essential to regard what foraging decisions are made during different stages of prey searching. (1) A phytoseiid mite that lands after aerial dispersal may decide to stay and search for prey or take off and start a new aerial voyage. If it decides to stay, it faces the decision of where to search and how long to search on specific locations. (2) After location of a prey colony, it may decide how long to stay, which prey stages, or prey species to accept and which to reject. (3) Finally, if it decides to leave the (exterminated) prey colony, it may choose whether to take off for an aerial voyage, or to disperse by walking (see Sabelis and Dicke, 1985, for review of prey searching behaviour). Analysis of a single foraging decision may provide information on preference at that particular stage of prey selection. To obtain a more comprehensive view of prey preference it is indispensable to study foraging behaviour at different stages of prey selection.

To analyse prey preference of phytoseiid mites, we used two independent preference analysis methods to be carried out under laboratory conditions: (1) Investigation of the response to volatile kairomones. This investigation regards decisions of the predators, when prey individuals are not contacted, as is the situation after termination of an aerial voyage, or after eradication of a prey colony. A Y-tube olfactometer was used in this analysis.
(2) Investigation of predation rates. This relates to acceptance/rejection decisions during contacts with prey items. For this analysis of prey

preference we used a Markov-type predation model that accounts for feeding-state dependent behaviour (Sabelis, 1981, 1986; Dicke et al., 1988a).

The above two analyses are performed under artificial conditions, where only one aspect of prey selection behaviour is investigated. Moreover, field populations may undergo physiological and/or genetic changes when introduced into the laboratory (Van Lenteren, 1986a). Preference analyses under field conditions constitute a significant complement to the conclusions obtained in the laboratory. For this purpose we made an electrophoretic analysis of gut contents of predators collected in orchards.

Prey selection of three phytoseiid species that occur in Dutch apple orchards was investigated: Typhlodromus pyri Scheuten, Amblyseius potentillae and A. finlandicus (Oudemans). All three phytoseiid species can feed and reproduce on the two most abundant pest mite species that occur in Dutch orchards: the European red spider mite (Panonychus ulmi (Koch)) and the apple rust mite (Aculus schlechtendali (Nalepa)) (Chant, 1959; Overmeer, 1981; Kropczynska-Linkiewicz, 1971; Dicke et al., 1988c). These two prey species can occur on different but also on the same leaves. Thus predators have to make selection decisions in all stages of the prey selection process outlined above.

METHODS AND MATERIALS

Preference analyses.

Olfactometer.

To investigate prey preference on the basis of volatile kairomones, a Y-tube olfactometer was used. Predators were starved in plastic tubes at 26°C for 20 h. For a detailed description of the olfactometer, its use and the environmental conditions, we refer to Sabelis and Van de Baan (1983), Dicke and Groeneveld (1986) and Dicke (1988a).

In the olfactometer the predators were offered a choice between two substrates. These were either clean and prey-infested host plant leaves (prey vs. no prey choice), or host plant leaves that were infested with each of two different prey mites (simultaneous prey choice). Simultaneous

prey choice was studied in a series of experiments differing in the ratio of prey numbers offered. For a comparative approach in the latter situation prey (kairomone) quantities should be compared. This may be done on the basis of prey numbers. However, because kairomone production seems to be related to feeding on the host plant (Sabelis et al., 1984a) and because prey biomass of spider mites and rust mites differs largely, we prefer a comparison on the basis of prey biomass.

Analysis of predation rates.

The value of predation models that include feeding-state dependent behaviour for analysing prey preference has been established for several predator-prey interactions (Sabelis, 1986; Dicke et al., 1988a). For details of the methods used here, we refer to Dicke et al. (1988a). The basic idea is as follows. At first, the functional response of the predators to the density of each prey type is determined. Two parameters of a Markov-type model are estimated for each functional response curve by trial and error. These parameters represent the feeding-state independent behaviour (c) and the feeding-state dependent behaviour ($z \in$ <-1 ;∞>). The parameter values thus obtained are used in an extended version of the model to predict predation rates in mixed cultures under the assumption that the parameters are not affected by the prey species being presented together. If this model prediction differs from experimentally determined predation rates in mixed cultures, and if neither a change in behaviour of the prey nor a change in walking speed of the predator can explain this difference, the conclusion is that the predator has a preference in terms of changes in success ratio or width of the searching path in response to the prey types being presented together (Sabelis, 1986). Thus, this analysis of preference uses prey numbers as a comparison index, on the basis of a model that accounts for prey biomass differences (Sabelis 1981, 1986).

In the predation model food conversion is determined by the relative rate of gut emptying, which is the combined process of resorption, respiration, transpiration and egestion. The relative rate of respiration and transpiration (r_{rs}) and the relative rate of egestion were assumed to be similar to those of other phytoseiid species (cf. Sabelis, 1981). The parameters needed to calculate the relative rate of resorption are: maximum food content of the gut (g_{max}; ug), somatic body weight (b; ug), rate of

oviposition (R_{ov}; day^{-1}) and weight of an egg (E; ug). These parameters were estimated by weighing procedures using a Cahn electrobalance (g_{max}; b and E), or by experimental determination (R_{ov}; Dicke et al., 1988a).

The experiments were performed at 26 $\pm$ 1°C and 70 $\pm$ 10 % r.h.

Electrophoresis.

Prey preference under field conditions was analysed by tracing prey esterases in predators by means of polyacrylamide gel electrophoresis. For procedures see Murray and Solomon (1978) and Dicke and De Jong (1988). Recently Fitzgerald et al. (1986) developed a technique to use this method quantitatively. Since we analysed gut contents qualitatively, conclusions on preference are based on presence/absence of each prey species' esterases. The predators were collected from leaves in an orchard where only two prey species were available. Leaves constituted the experimental unit. Prey numbers on the leaves from which predators had been collected, were counted and predators were analysed individually. The data on gut contents were analysed in the context of prey densities experienced by individual predators.

Mites.

Prey.

The prey mites P. ulmi and A. schlechtendali were collected from commercial Dutch apple orchards. For predation experiments rust mites and spider mite eggs were collected and stored at 5°C for up to some weeks (rust mites) or two months (P. ulmi winter eggs). Prior to the experiments spider mite eggs were kept at 26°C for 2-4 days and rust mites for 0.5-2 h. Rust mite adults and recently emerged spider-mite larvae were offered to the phytoseiids in the predation experiments. Leaves that were used in the olfactometer had been collected on the day of the experiment.

The spider mite Tetranychus urticae Koch was reared on Lima bean plants (for rearing conditions see Dicke (1986)). This spider mite was used to rear A. potentillae and T. pyri.

Predators.

The phytoseiid mites T. pyri and A. potentillae were reared on
T. urticae or Vicia faba L. pollen on plastic plates. For details on
rearing methods see Dicke (1988a) and Dicke and Groeneveld (1986)
respectively. Typhlodromus pyri had been reared on these food sources for
6-8 years, one strain of A. potentillae had been reared on T. urticae for
more than 12 years, and another strain, that originated from the first one,
had been reared on V. faba pollen for c. 6 years.

Amblyseius finlandicus was collected in Wageningen in 1984 from leaves
of Prunus sp. This predator species was reared in the laboratory on V. faba
pollen on Lima bean leaves at $26\pm1°C$, $60\pm10\%$ r.h. and continuous
fluorescent light. The leaves were placed upside down on wet cotton wool.

RESULTS

Typhlodromus pyri

Olfactometer.

The response of 20 h-starved T. pyri females towards volatile
kairomones is dependent on nutritional quality of the diet used for
predator rearing (Dicke, 1988a). Predators reared on T. urticae responded
to a volatile kairomone of P. ulmi, but not to a volatile kairomone of
A. schlechtendali (Dicke, 1988a). These data indicate that T. pyri, when
reared on T. urticae preferred European red spider mites to apple rust
mites.

When reared on V. faba pollen, 20 h-starved T. pyri females responded
to the volatile kairomones of both P. ulmi and A. schlechtendali in prey
vs. no-prey choice-experiments (Dicke, 1988a). When these two prey species
were offered simultaneously, the response of pollen-reared predators was
dependent on the ratio of prey numbers used (Figure 11.1). They walked
significantly more to the end of the arm with A. schlechtendali when the
ratio of A. schlechtendali : P. ulmi numbers was 120,000 : 250 but they
preferred the arm with P. ulmi when the ratio of prey numbers was 30,000 :
1000. In both choice situations the rust mites outweighed the spider mites
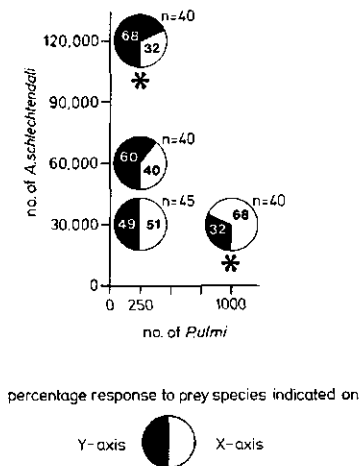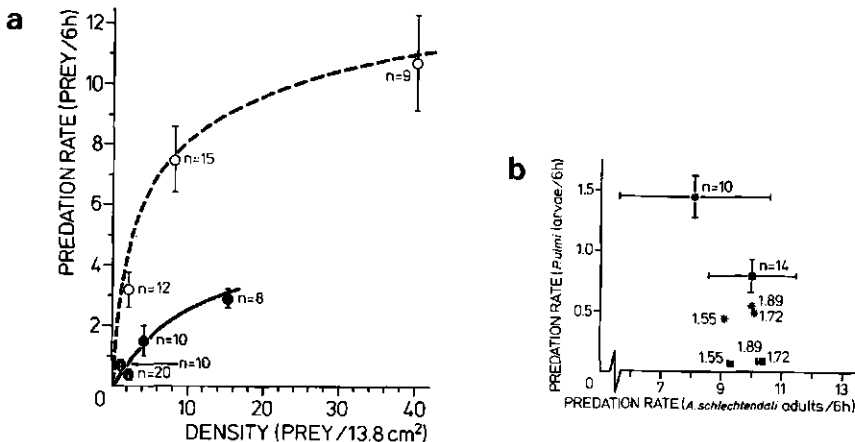(the weight of the smallest active spider mite stage is four times that of

Figure 11.1: Response of T. pyri females reared on V. faba pollen and starved for 20 h to volatile kairomones in a Y-tube olfactometer. Choice between leaves infested with P. ulmi or A. schlechtendali (after Dicke, 1988a). Numbers in sectors indicate percentage response to each prey species. Numbers above pie diagrams represent quantity of predators tested. Asterisks indicate level of significance:
* = 0.01 < P < 0.05
** = 0.001 < P < 0.01

Figure 11.2: Predation rates of T. pyri females reared on V. faba pollen. (a) Functional response to prey density of P. ulmi larvae (———) or A. schlechtendali adults (-----). Lines, dots and bars indicate best fit of model, mean predation rate, and standard error respectively. (b) Predation rates in mixed cultures of prey supply; squares and asterisks denote model predictions for the three values of r_g indicated, at prey densities of 2 P. ulmi larvae and 30 A. schlechtendali adults per 5 cm^2 (■) or 6 P. ulmi and 30 A. schlechtendali per 5 cm^2 (*); square and asterisk with bars indicate observed predation rates with 95% confidence interval (after Dicke et al., 1988a).

the largest rust mite stage). Thus, the observations of the choice between 120,000 rust mites and 250 spider mites do not permit a conclusion on prey preference on the basis of a prey biomass criterion. However, the result of the experiment where 30,000 rust mites were offered vs. 1000 spider mites demonstrates that the predators preferred P. ulmi to A. schlechtendali. Thus, the conclusions on prey preference for T. pyri reared on each of the two diets are similar.

Comparison of predation rates.

The predators used in these experiments had been reared on V. faba pollen. The relative rate of gut emptying was calculated to be 1.72/day (Table 11.1). Estimated values for c and z values that best described the predation rates in monocultures (Figure 11.2) are shown in Table 11.2. The values for z indicate that the predator's success ratio sharply declines at high levels of gut fullness (Dicke et al., 1988a). The predicted predation rate on A. schlechtendali in one of the mixed cultures (2 P. ulmi larvae and 30 A. schlechtendali adults) was similar to the experimental value, but in the other mixed culture it was higher than the observed predation rate. Observed predation rates on P. ulmi in both mixed cultures were significantly higher than those predicted. Observations on walking speed and walking activity of the prey in mono and mixed cultures indicated that a change in these behavioural characteristics as a result of being together cannot explain the difference between predicted and measured predation rates in mixed cultures (Dicke et al., 1988a). Simulations showed that the differences between model calculations and observations are too high to be explained by an increase in walking speed of the predators. Varying the estimated value of r_g did not affect the conclusion on prey preference (Figure 11.2; Dicke et al., 1988a). Only a drastic increase in the success ratio with respect to P. ulmi resulted in a predicted predation rate on P. ulmi that was similar to the observed value (Dicke et al., 1988a). It is therefore concluded that T. pyri reared on V. faba pollen preferred P. ulmi larvae to A. schlechtendali adults.

Electrophoresis.

Esterases of both prey species could be detected in the gut of field collected T. pyri. Esterases of P. ulmi were detected in 69% and esterases

Table 11.1: Values of parameters needed to calculate relative rate of gut emptying (r_g) for three phytoseiid species.

	T.pyri	A.potentillae[1]	A.finlandicus
Weight of female carrying a full grown egg (ug)	10.5+1.3 (n=10)	17.9	15.4+0.6 (n=13)
Somatic body weight (ug)	2.3[2]	5.1	4.4[2]
Maximum gut volume (ug)	3.5[2]	5.2	4.5[2]
Weight of egg at deposition (ug)	1.9+0.5 (n=9)	3.1	2.8+0.3 (n=28)
Rate of oviposition at 26°C (eggs/day)	2.0+0.2[3]	2.8+0.5[3]	2.4+0.4[3]
Calculated relative rate of gut emptying[4] at 26°C (day^{-1})	1.72	2.50	2.33

[1] Data of Sabelis (1981), except for rate of oviposition
[2] Calculated from weight of female carrying fullgrown egg, under the assumption that the fraction that is made up by the somatic body weight and the maximum food content of the gut is similar for A. potentillae and A. finlandicus and for Typhlodromus occidentalis and T. pyri.
[3] Data of Dicke et al. (1988c).
[4] Calculation with balance equation (Sabelis, 1986) where g_{max} was set to a slightly lower value ($=g_{max}$ – 0.2) to approximate the dynamics of g_{max} during the oviposition rate determination.

Table 11.2: Values of model parameters c and z that best describe predation rates of females of three phytoseiid species in monocultures in which P. ulmi larvae or A. schlechtendali adults were offered.

Predator species	Prey species			
	Panonychus ulmi		Aculus schlechtendali	
	c^*	z^*	c^*	z^*
Typhlodromus pyri	2500	–.94	13000	–.95
Amblyseius potentillae	11000	–.60	6000	–.70
Amblyseius finlandicus	6200	–.97	7200	–.95

of A. schlechtendali in 6% of the predators (n=189). These predators were collected from leaves with known ratios of prey numbers. When the predators were classified according to these prey to prey ratios it was clear that at
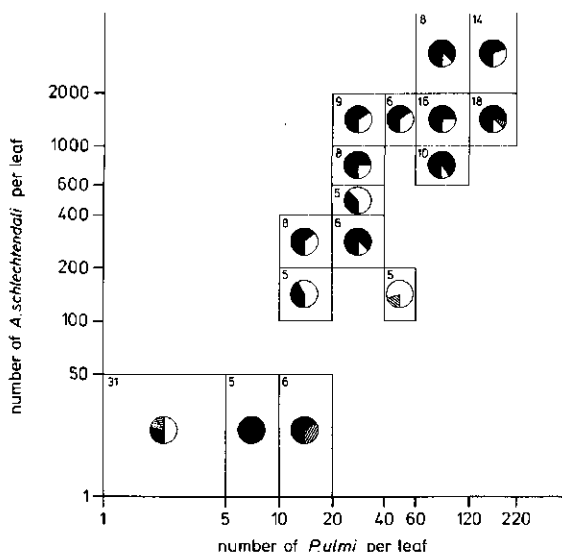
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Figure 11.3: Percentages of field–collected T. pyri with different prey esterases at several prey density combinations. Per class of prey density combinations (represented as a rectangle), a pie diagram indicates the esterase percentages detected; the number in the top left corner of the rectangle indicates the number of T. pyri analysed. Axes on log scale. (Dicke and De Jong, 1988).

all ratios most predators contained P. ulmi esterases (Figure 11.3), whereas at the lowest prey densities only 10 % of the predators were found with rust mite esterase but no spider mite esterases, which is low because 32% of the predators contained only P. ulmi esterases. Because the differences in detection frequencies were not the result of a lower chance of detection of rust mite esterases in T. pyri (see Dicke and De Jong (1988) for discussion), these data show that T. pyri collected in a Dutch apple orchard preferred P. ulmi to A. schlechtendali.

Amblyseius potentillae

Olfactometer.

The data on the response of 20 h–starved A. potentillae females towards the kairomones of P. ulmi and A. schlechtendali in the prey–no prey choice experiment (Dicke and Groeneveld, 1986) are similar to those for T. pyri. A

nutritional difference between the diets of T. urticae and V. faba pollen
also caused the difference in the response between A. potentillae reared on
either of the two diets (Dicke and Groeneveld, 1986). Simultaneous prey
choice experiments with predators reared on V. faba pollen led to the
conclusion that A. potentillae preferred P. ulmi to A. schlechtendali
(Figure 11.4). In contrast to the data for T. pyri none of the prey number
ratios used, resulted in a preference of the arm with rust mites. Thus,
A. potentillae reared on either of the two diets preferred P. ulmi to
A. schlechtendali.
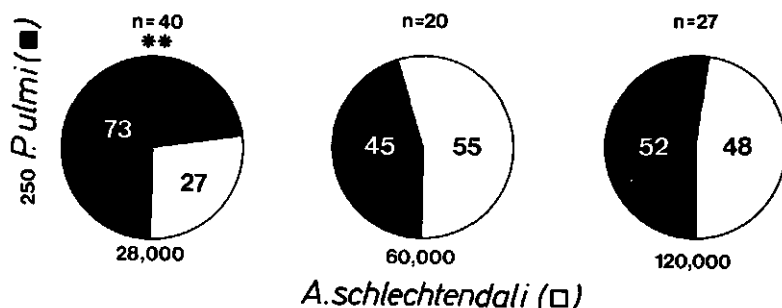
Figure 11.4: Response of A. potentillae females reared on V. faba pollen
and starved for 20 h to volatile kairomones in a Y-tube olfactometer.
Choice between leaves infested with P. ulmi or A. schlechtendali (after
Dicke and Groeneveld, 1986).
Numbers above pie diagrams represent quantity of predators tested.
For explanation of asterisks, see Figure 11.1.

Comparison of predation rates.

Amblyseius potentillae females used in predation experiments had been
reared on T. urticae. Estimated values for r_g, c and z are given in
Tables 11.1 and 11.2. The predation rates on P. ulmi in monocultures
(Figure 11.5a) are similar to those reported by Rabbinge (1976). The
predicted predation rate on A. schlechtendali in the mixed culture (2 P.
ulmi larvae and 50 A. schlechtendali adults) was similar to the observed
value, but the calculated predation rate on P. ulmi was lower than the one
observed. Differences in walking speed and activity between mono and mixed
cultures are too small to explain the difference between predicted and
measured predation rates (Dicke et al., 1988a). Moreover, simulations
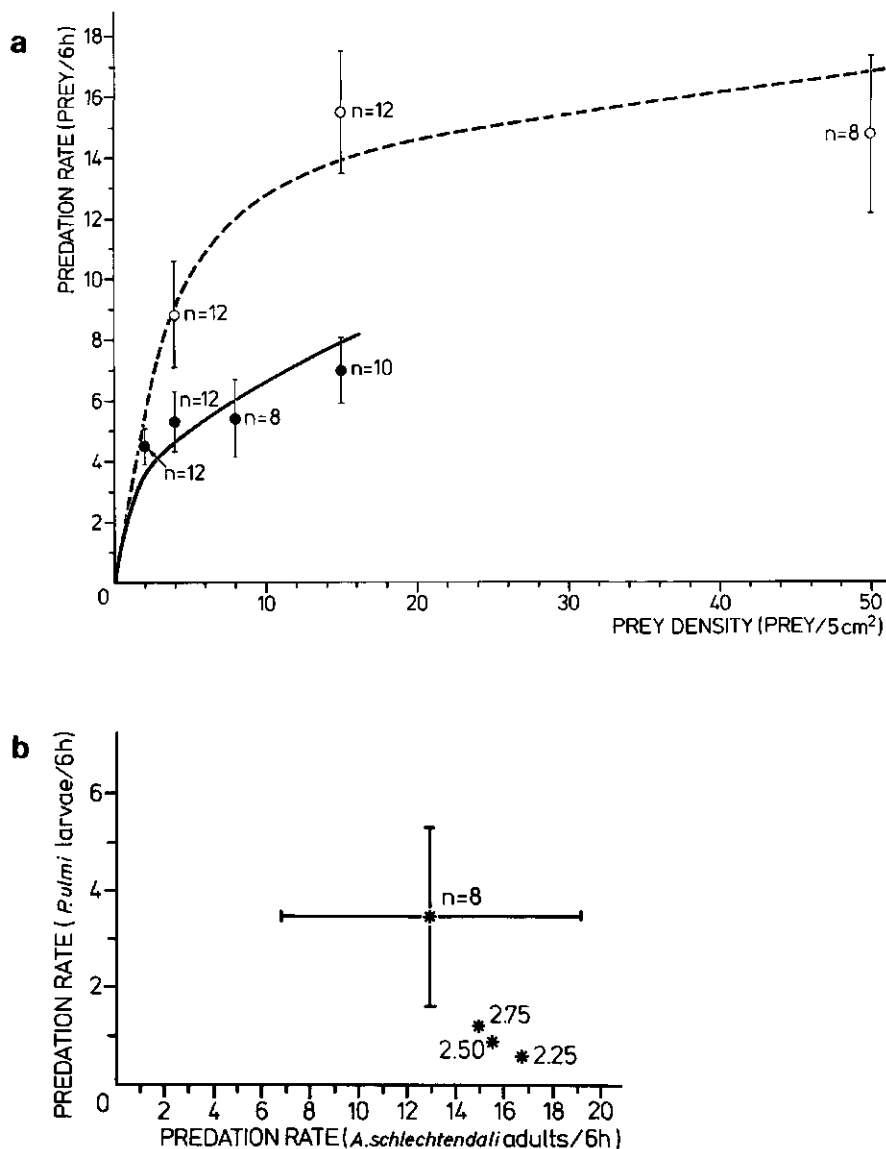showed that a change in walking speed of the predators also cannot account

Figure 11.5: Predation rates of A. potentillae females reared on
T. urticae. (a) Functional response to prey density of P. ulmi larvae
(————) or A. schlechtendali adults (-----). Lines, dots and bars indicate
best fit of model, mean predation rate, and standard error respectively.
(b) Predation rates in mixed culture of prey supply; asterisks denote model
predictions for three values of r_g indicated, at prey densities of 2
P. ulmi larvae and 50 A. schlechtendali adults per 5 cm^2; asterisk with
bars indicates observed predation rates with 95% confidence interval.

for that difference. Thus, the data show that A. potentillae prefers P. ulmi larvae to A. schlechtendali adults. A 10% increase or decrease in the value of r_g does not affect this conclusion (Figure 11.5b).

Electrophoresis.

No data on gut content of A. potentillae have been obtained because we were unable to find a Dutch orchard where European red spider mites, apple rust mites and A. potentillae were present.

Amblyseius finlandicus

Olfactometer.

Amblyseius finlandicus females that were starved during 20 h responded to the kairomones of P. ulmi and A. schlechtendali (Figure 11.6a). The response to the kairomone of P. ulmi was rather weak (Figure 11.6a). In a simultaneous prey choice experiment in the olfactometer the predators preferred the arm with rust mites when 35,000 rust mites were offered versus 1000 P. ulmi (Figure 11.6b). Because the rust mites outweigh the spider mites in this choice situation it cannot be concluded whether the
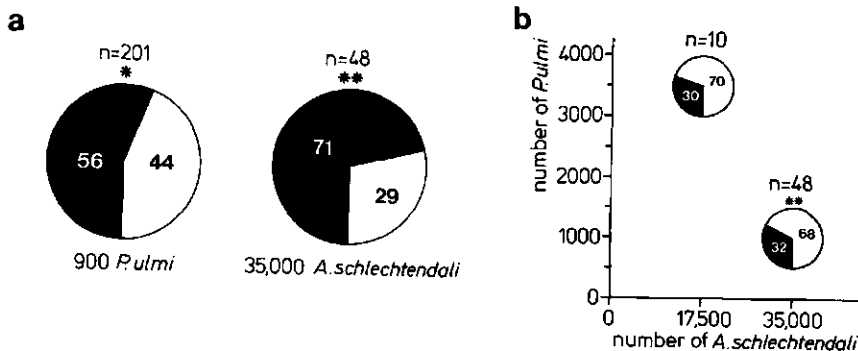

Figure 11.6: Response of A. finlandicus females, reared on V. faba pollen on Lima bean leaves and starved for 20 h, to volatile kairomones in a Y-tube olfactometer. (a) Predators were offered prey infested leaves (■) vs. clean leaves (□). (b) Predators were offered a choice between leaves infested with P. ulmi or A. schlechtendali.
Numbers in sectors indicate percentage response to each prey species.
Numbers above pie diagrams represent quantity of predators tested.
For explanation of asterisks, see Figure 11.1.

predators prefer A. schlechtendali to P. ulmi. The response in the simultaneous prey choice experiment between 3500 P. ulmi and 17,500 A. schlechtendali, which represents a 1:1 biomass ratio, suggests that the predators prefer the arm with rust mites (Figure 11.6b).

Comparison of predation rates.

Estimated values for r_g, c and z are given in Tables 11.1 and 11.2. Predation rates of A. finlandicus (Figure 11.7a) were much higher than those of A. potentillae (Figure 11.5a). Partial prey consumption clearly occurred in most of the successful attacks. Two mixed cultures have been offered to the predators. When 2 P. ulmi larvae and 50 A. schlechtendali adults were offered, the predicted predation rate on P. ulmi was similar to the observed value (Figure 11.7b). However, the measured predation rate on A. schlechtendali was significantly higher than predicted (Figure 11.7b). This indicates that A. finlandicus preferred apple rust mites to European red spider mites, which is even more obvious from the other mixed culture, where 15 P. ulmi larvae and 4 A. schlechtendali adults were offered to the predators. The predicted predation rate on P. ulmi is significantly higher than the observed value, whereas the predicted predation rate on A. schlechtendali is significantly lower than predicted (Figure 11.7b). Simulations showed that these differences cannot be explained by a change in the walking speed of the predator and observations on walking speed and activity of prey in monocultures and mixed cultures revealed that differences were negligible (Dicke et al., 1988a). The data clearly show that A. finlandicus prefers A. schlechtendali adults to P. ulmi larvae.

Electrophoresis.

Predators were collected in a Morello cherry orchard where only P. ulmi and the plum rust mite Aculus fockeui (Nalepa et Trouessart), which is closely related to A. schlechtendali, have been observed as prey. Spider mite and rust mite esterases were detected in 35% and 37% resp. of the predators (n=107) (Dicke and De Jong, 1986). The range of ratios of prey numbers that were present on the leaves was small (Figure 11.8). Comparison of Figures 11.3 and 11.8 shows that A. finlandicus feeds much more consistently on rust mites than T. pyri. In only one of the six classes (10-20 P. ulmi and 1-50 A. fockeui) no predator was found with rust mite
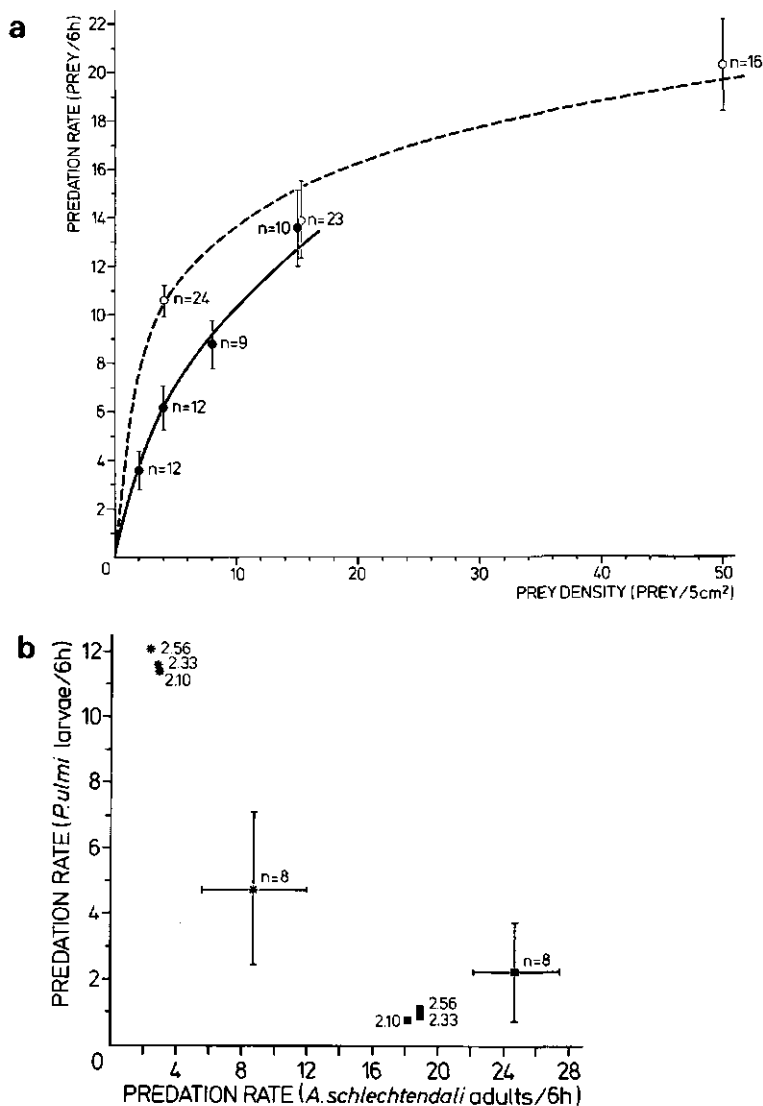
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Figure 11.7: Predation rates of A. finlandicus females reared on V. faba pollen on Lima bean leaves. (a) Functional response to density of P. ulmi larvae (———) or A. schlechtendali adults (-----). Lines, dots and bars indicate best fit of model, mean predation rate, and standard error respectively. (b) Predation rates in mixed cultures of prey supply; squares and asterisks denote model predictions for three values of r_g indicated, at prey densities of 2 P. ulmi larvae and 50 A. schlechtendali adults per 5 cm^2 (■) or 15 P. ulmi and 4 A. schlechtendali adults per 5 cm^2 (*); square and asterisk with bars indicate observed predation rates with 95% confidence interval.

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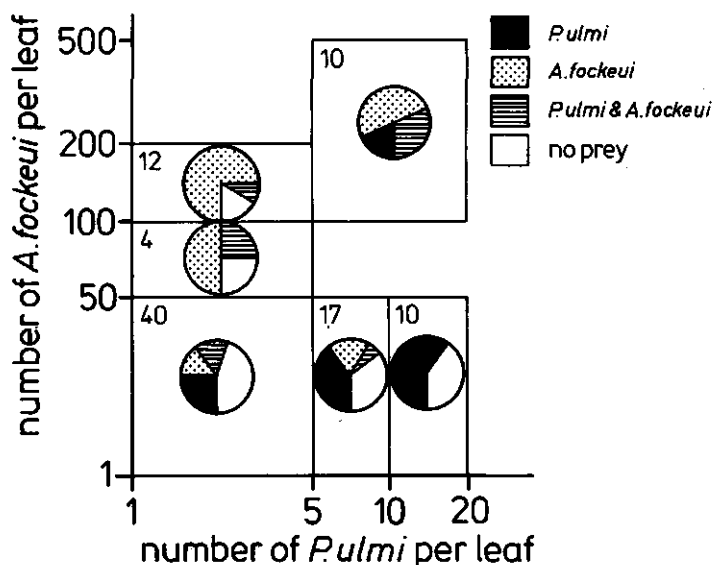
Figure 11.8: Percentages of field-collected A. finlandicus with different
prey esterases at several prey density combinations. Per class of prey
density combinations (represented as a rectangle), a pie diagram indicates
the esterase percentages detected; the number in the top left corner of the
rectangle indicates the number of A. finlandicus analysed. Axes on log
scale (after Dicke and De Jong, 1986).

esterase. The data do not allow for a definite conclusion on prey
preference of A. finlandicus under field conditions. Data over a larger
range of prey densities are needed.

DISCUSSION

   Sabelis and Dicke (1985) reviewed knowledge of prey selection by
phytoseiid mites. At that time only data were available on the response of
some predator species to prey cues. The current paper is the first to give
a comprehensive analysis of prey selection by several phytoseiid species
based on several independent bioassays (Figure 11.9).
   The prey selection analyses carried out for T. pyri and A. potentillae
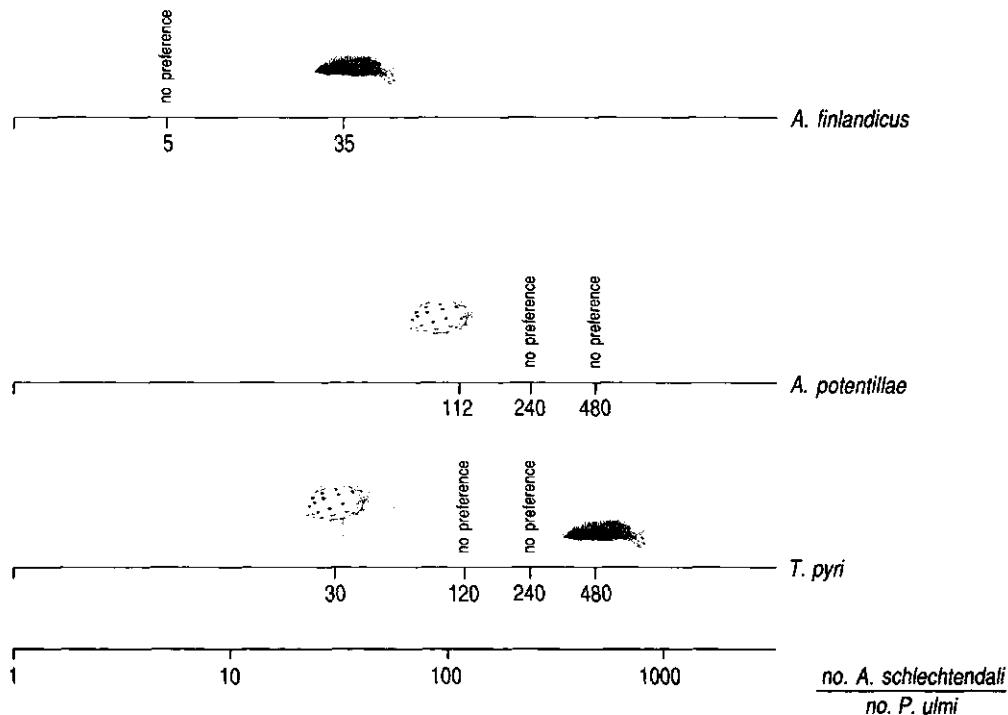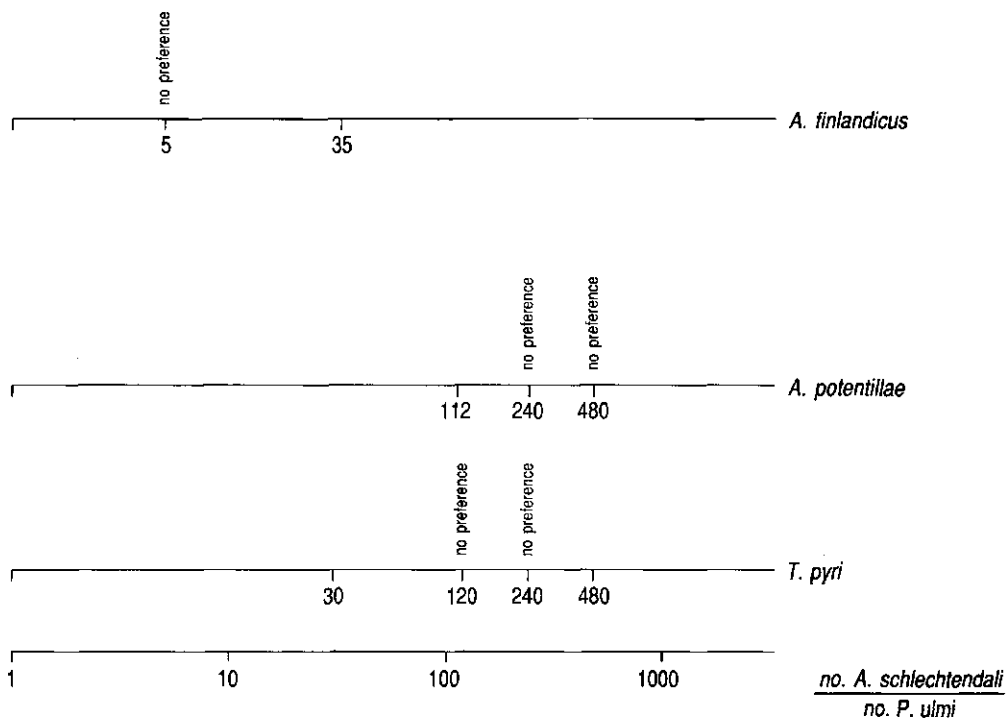invariably indicated that these predator species prefer P. ulmi to A.

Figure 11.9: Summary of data of simultaneous prey choice experiments in Y-tube olfactometer at several prey number ratios.
At each prey-number ratio the preferred prey species is shown.

Figure 11.9: Summary of data of simultaneous prey choice experiments in Y-tube olfactometer at several prey number ratios.
At each prey-number ratio the preferred prey species is shown.

schlechtendali.

For A. finlandicus not all analyses resulted in a firm conclusion. The predation experiments demonstrated a preference for A. schlechtendali over P. ulmi, whereas no definite conclusion could be drawn from the olfactometer experiments. However, a comparison of the decisions of all three predator species in comparative choice experiments (Figure 11.10) shows that of all three predator species A. finlandicus prefers the arm with rust mite kairomone at the lowest rust mite to spider mite biomass ratio. No conclusion could be drawn from the analysis of field predation of A. finlandicus, but comparison with data on field predation by T. pyri indicates that A. finlandicus feeds much more on rust mites than T. pyri (Figures 11.3 and 11.8). Thus, the conclusion on A. finlandicus' preference of A. schlechtendali to P. ulmi, obtained from the comparison of observed and predicted predation rates, is not rejected.

The establishment of prey preference of these three phytoseiid species

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Figure 11.10: Summary of data on prey preference obtained from three independent analysis methods. For each analysis the preferred prey species is shown.

raises several questions, such as why do they exert prey preference and why do T. pyri and A. potentillae prefer P. ulmi whereas A. finlandicus prefers A. schlechtendali?

Different prey types may differ in profitability for the predator. Dicke et al. (1988c) determined life-history components of all three predator species, when feeding on eiter P. ulmi or A. schlechtendali. In addition they investigated the effect of prey species on diapause induction in A. potentillae. From their data it is obvious that apple rust mites are a better prey species for A. finlandicus than European red spider mites. To a lesser extent this was also true for A. potentillae and T. pyri. Thus, on the basis of current data prey preference of A. finlandicus can be understood in terms of reproductive success, whereas this is not true for A. potentillae and T. pyri.

The functional response of predatory mites to the density of phytophagous mites has often been found to be of type II. Evidence for type III responses is weak (Sabelis, 1985e), but this applies to experiments with one or more developmental stages of one prey species at a time. The results presented in this paper show that other (e.g. type III) responses may occur when more than one prey species is involved. When two prey species were presented as alternatives in olfactometer experiments, prey choice appeared to depend on the biomass ratio of the two prey species. Moreover, predation experiments in a mixture of two prey species cannot be predicted from parameters measured in monoculture predation experiments. More experiments on prey choice are needed to elucidate how the functional response to the density of one prey species is modified by the presence of alternative prey species. This may reveal type III or even more complex responses that could be crucial in understanding coexistence in and stability of acarine predator–prey systems (Roughgarden & Feldman, 1975; Murdoch & Oaten, 1975; Comins & Hassell, 1976; Murdoch, 1977; Hassell & Commins, 1978; Nunney, 1980).

PART IV. INFOCHEMICALS, PREY PREFERENCE AND REPRODUCTIVE SUCCESS. A SYNTHESIS.

A. potentillae, Overmeer (1981) for T. pyri, and Sabelis (1985b) for
A. finlandicus), assuming sex allocation by phytoseiid mites does not
depend on the prey species.

   r_m was estimated over a 10 day oviposition period, assuming that
oviposition rate and mortality of oviposition females were constant over
this period (cf. Sabelis 1981, 1985c). Oviposition periods of phytoseiid
mites usually last longer than 15 days at 25°C (Sabelis, 1985c) and
therefore this 10-day period is certainly not too long. A sensitivity
analysis for the effect of longer oviposition periods was carried out.
Because the estimate of r_m is calculated on the basis of an incomplete life
table, it will be referred to as r_m'.

Determination of life-history components.

   Predator eggs, collected from a culture fed on broad bean pollen were
transferred to leaf discs (2 cm diameter) that were placed with the adaxial
side on water-soaked cotton wool. Apple leaf discs were used for
A. potentillae and T. pyri and Prunus sp. leaf discs for A. finlandicus. On
each disc a rooflike structure (sides 0.5x0.5 cm) was placed to provide the
predators with a shelter (Overmeer, 1985c). Provision of food started just
before egg hatching was expected to occur. In this way emerging larvae
already encountered abundant food. New prey items were supplied at 8 h
intervals to guarantee a continuous ample prey supply. The number of prey
mites that was supplied each 8 h was tuned to the prey requirements of each
predator stage: 5, 10, 15 or 25 P. ulmi larvae, or 25, 25, 40 or 60
A. schlechtendali for larvae, protonymphs, deutonymphs and adults of the
predators respectively.

   To obtain prey of the right stage, leaves with P. ulmi eggs were taken
from cold storage and kept at 26°C; newly emerged larvae were collected and
transferred to the leaf discs. Active stages of A. schlechtendali were
collected from the cold-stored leaves and placed on the leaf discs
immediately. The leaf discs were observed at 8 h intervals to see whether
the predators had developed into the next stage. A moult was recorded only
if the exuvium was found, which was then discarded. When a female
deutonymph was 24 h old one or two adult males were placed on the disc to
allow for mating as soon as possible after the final moult. Oviposition was
recorded at 8 h intervals during 72 h after the first egg had been laid.

All observed eggs were removed from the disc.

Drowned predators were not included in the calculation of mortality.

The experiments were carried out in climatic rooms at 26±1°C and 50-70% r.h. For each predator species the experiments were carried out within a period of 3 months.

Effect of prey species on diapause induction.

Diapause induction in A. potentillae depends on food quality. Carotenoids were found to be indispensable (Van Zon et al., 1981; Veerman et al., 1983). When mothers feed on a carotenoid source, their offspring earn a carotenoid legacy and do not require dietary carotenoids to enter diapause (Van Zon et al., 1981). When feeding on spider mites or rust mites A. potentillae females enter reproductive diapause under short day conditions (Overmeer and Van Zon, 1983a; Dicke, unpublished data). To compare the effect of either P. ulmi or A. schlechtendali on diapause induction, A. potentillae was reared on either prey species for at least 3 weeks. Subsequently, adult females were transferred (2-3 h since the last prey supply) to a rearing unit on which V. faba pollen was the only food source (26±1°C, LD 24:0). Broad bean pollen is a carotenoid-poor food source for A. potentillae (Overmeer and Van Zon, 1983a). Eggs were collected at 24 h intervals and reared to adulthood on V. faba pollen at low temperature and short day conditions (18±1°C, LD 8:16). When matings were observed, the rearing plates were inspected for presence of eggs at two-day intervals. When eggs were found, females were individually isolated on plastic discs (diameter 2 cm) four days later in presence of V. faba pollen, to determine the percentage of predators that oviposited (non-diapause females). This isolation phase lasted for 7 days. When eggs had not been observed on a plate during 18 days since the first mating, the experiment was terminated and all females were classified as having entered diapause.

To investigate whether differential effects on diapause induction are a result of feeding from different plant tissues by rust mites and spider mites (Jeppson et al., 1975), another spider mite, Tetranychus urticae Koch was also used as prey during the initial phase.

A dietary effect on diapause induction has not been recorded for T. pyri (Dicke 1988a) nor has it been studied for A. finlandicus (Overmeer, 1985a). The current analysis was restricted to A. potentillae.

Table 12.2: Influence of diet on mortality of A. potentillae, A. finlandicus and T. pyri.

Predator species	Food species	% larvae becoming protonymph	% protonymphs becoming adult	% adult females surviving during first 72 h of oviposition period
A. potentillae	A. schlechtendali	100 (37)*	100 (35)	100 (20)
	P. ulmi (larvae)	100 (37)	100 (37)	100 (17)
T. pyri	A. schlechtendali	100 (39)	100 (36)	100 (22)
	P. ulmi (larvae)	100 (41)	100 (39)	100 (22)
A. finlandicus	A. schlechtendali	86 (42)	100 (28)	92 (13)
	P. ulmi (larvae)	46 (131)	86 (47)	96 (24)
	no food	6 (36)	0 (2)	−

* Numbers in parentheses indicate number of predators.

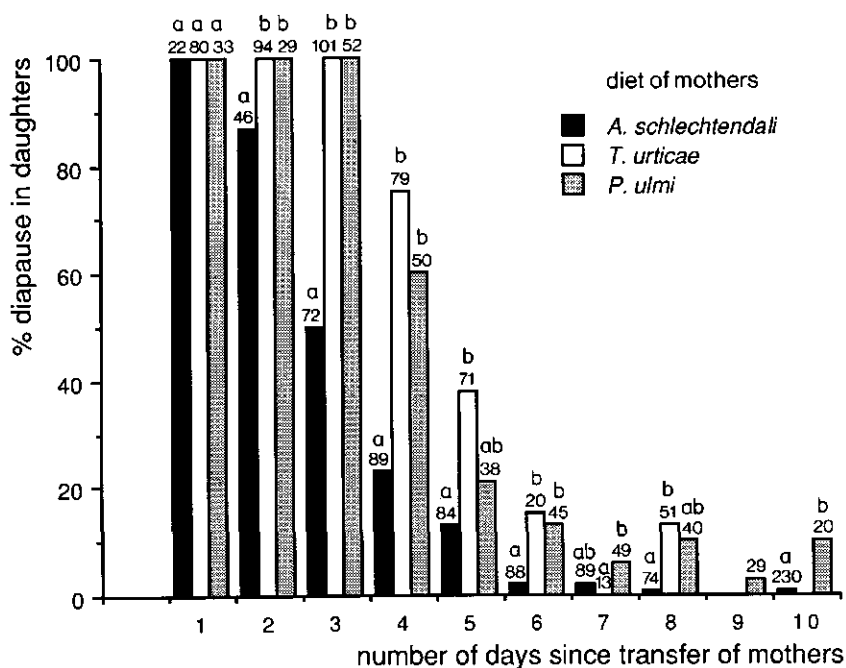Figure 12.1: Effect of diet of A. potentillae mothers on diapause induction in offspring, produced after transfer of mothers from a carotenoid-containing diet of phytophagous mites to the carotenoid-deficient diet of V. faba pollen. Numbers above bars indicate number of predators observed. Different letters above bars for the same day indicate significant differences (α =0.05, G-test, Sokal and Rohlf, 1981).

Table 12.3: Estimated rates of population increase (r_m') for A. potentillae, A. finlandicus and T. pyri when feeding on P. ulmi larvae or on A. schlechtendali adults.

Predator	Prey	l_x survival	n_x eggs/day	S sex ratio	development egg-egg (days)	r_m' day^{-1} 10	r_m' day^{-1} 20 day-period
A.potentillae	P.ulmi	1.0	2.7	0.69	8.63	0.221	0.228
		0.9*	2.7	0.69	8.63	0.212	0.220
	A.schlechtendali	1.0	2.9	0.69	7.92	0.240	0.247
		0.9*	2.9	0.69	7.92	0.231	0.239
T.pyri	P.ulmi	1.0	1.9	0.39	9.92	0.135	0.149
		0.9*	1.9	0.39	9.92	0.127	0.142
	A.schlechtendali	1.0	2.0	0.39	9.54	0.142	0.156
		0.9*	2.0	0.39	9.54	0.134	0.149
A.finlandicus	P.ulmi	0.39	2.3	0.62	9.33	0.125	0.140
	A.schlechtendali	0.84	2.4	0.62	9.13	0.175	0.186

* Observed survival was 1.0; calculations with l_x = 0.9 were made to investigate the effect of this more realistic value on r_m'.

rust mites than when they had fed on spider mites (Figure 12.1). When females fed on spider mites before transfer to broad bean pollen, a reduction in percentage diapause in their offspring was manifest after the third day since transfer to the carotenoid-deficient diet. When fed on rust mites before transfer, this effect occurred after the first day since transfer. No difference in effect was observed between experiments in which the mothers had fed on either of the two spider-mite species, T. urticae or P. ulmi, before transfer.

DISCUSSION

Life-history components.

Despite many literature data on development time and/or oviposition rate of the three phytoseiid species studied (Herbert, 1961; Kropczynska, 1970; Kropczynska-Linkiewicz, 1971; Zaher and Shehata, 1971; Van de Vrie, 1973; Rabbinge, 1976: Amano and Chant, 1977; McMurtry, 1977; Overmeer, 1981; Sabelis, 1981; Amano and Chant, 1986; Hayes and Ardle, 1987), we made a new investigation for the following reasons: (1) Changes in life-history parameters may occur with increasing time periods of laboratory rearing of a phytoseiid strain: e.g. during one year the oviposition rate of T. pyri, when feeding on V. faba pollen more than doubled for predators that had been reared on this food source (Overmeer, 1981; Overmeer and Van Zon, 1983b). Thus, for a comparative analysis of life-history components, experiments using one predator strain should be carried out within a short period of time. (2) Only Kropczynska (1970) and Kropczynska-Linkiewicz (1971) made a comparative analysis of development time and reproductive capacity for these predator species, when feeding on either P. ulmi or A. schlechtendali. However, no record was made of e.g. prey stages or time interval of observations made to determine development time. Drawing conclusions on the significance of the reported differences is therefore difficult (Sabelis, 1985a).

In spite of these reservations, the data of Kropczynska (1970) and Kropczynska-Linkiewicz (1971) on the development rates of A. potentillae,

A. finlandicus and T. pyri have been compared with our data (Table 12.4).
Although quantitative differences exist, qualitative conclusions on
relative development times of A. potentillae and T. pyri are similar in
both studies. However, a large discrepancy exists with respect to
A. finlandicus.

Larvae of many phytoseiid species do not need food to develop into the
protonymph stage (e.g., Chant, 1959; Amano and Chant, 1986), although
feeding may occur, as has been noticed for T. pyri and A. potentillae in
the present study. Recordings of phytoseiid larvae that need food to
complete the larval stages are only known to us for A. finlandicus (Chant,
1959; Amano and Chant, 1986). This raises the question of why
A. finlandicus larvae are obligate feeders. This question is also relevant
in the light of observations of large numbers of all stages of A.
finlandicus on trees on the leaves of which hardly any prey could be found
(Sabelis and Van de Baan, 1983; present observations on collection site of
A. finlandicus). Do A. finlandicus larvae rely on other food sources, that
are generally available? Present knowledge indicates that if leaf sap or
phyllosphere microorganisms are such a food source, they were
insufficiently available in the experimental setups (Chant, 1959; Amano and
Chant, 1986; present paper).

Effect of prey species on diapause induction in A. potentillae.

Amblyseius potentillae females that feed on T. urticae supply their
offspring with sufficient carotenoids to enter reproductive diapause (Van
Zon et al., 1981). In addition, daughters produced during the first 3 days
since transfer from a T. urticae diet to V. faba pollen all enter diapause
under short day conditions. In the offspring produced thereafter, the
percentage that enters diapause declines with time. When the mothers had
been feeding on the spider mite P. ulmi the effects are similar. However,
when the mothers had been feeding on the rust mite A. schlechtendali, the
decline occurs already after the first day. When predators are transferred
from one food source to another, the percentage of the former food in the
gut declines exponentially after transfer to a new diet (Sabelis, 1981).
This percentage amounts 8.2 ($e^{-2.50*1}$) after 1 day and 0.06 ($e^{-2.50*3}$)
after 3 days (relative rate of gut emptying obtained from Dicke et al.,

Table 12.4: Development times (h) of three phytoseiid species, when feeding on A. schlechtendali or P. ulmi at 25-26°C.

Predator species	Prey species	Development time (egg-adult) (h)	Dev. time on A.schlechtendali / Dev. time on P.ulmi	Reference
A. finlandicus	A. schlechtendali / P. ulmi	259 / 182	1.42	Kropczynska (1970)
A. finlandicus	A. schlechtendali (larvae) / P. ulmi (larvae)	164 / 175	0.94	present paper
A. potentillae	A. schlechtendali / P. ulmi	211 / 228	0.93	Kropczynska-Linkiewicz (1971)
A. potentillae	A. schlechtendali (larvae) / P. ulmi (larvae)	132 / 140	0.94	present paper
T. pyri	A. schlechtendali / P. ulmi	250 / 262	0.95	Kropczynska-Linkiewicz (1971)
T. pyri	A. schlechtendali (larvae) / P. ulmi (larvae)	163 / 170	0.96	present paper

1988b). These data show that spider mites and rust mites affect diapause induction in A. potentillae differently. It cannot be concluded from this experiment which prey component is involved. Because carotenoids are known to affect diapause induction, chemical analysis of quantitative and qualitative carotenoid content of spider mites and rust mites will be worthwile. Current knowledge indicates that only carotenoids with provitamin A function relieve the deficiency of the diet with respect to photoperiodic diapause induction (Veerman et al., 1983). However, no investigation has been made of the qualitative differences between such carotenoids regarding the effect on diapause induction. To date, other nutrients that affect diapause induction in mites are not known.

It will be interesting to investigate whether the differences in nutritious value of P. ulmi and T. urticae on the one hand and A. schlechtendali on the other are caused by differences in their feeding habits. Spider mites have much longer stylets and can penetrate the parenchyma, whereas rust mite stylets can only penetrate the epidermal cells (Jeppson et al., 1975). Plants infested by rust mites not only show damaged cells in the epidermis but also in deeper cell layers (Schliesske, 1977). However, it is not known whether this damage results in uptake of nutrients from the parenchyma by rust mites.

In conclusion, spider mites constitute a superior prey for A. potentillae in terms of diapause induction.

Prey preference and reproductive success.

Table 12.5 summarizes the data on prey preference (Dicke et al., 1988b) and reproductive success. For A. finlandicus, r_m' is much higher on A. schlechtendali than on P. ulmi larvae, because of high larval mortality of predators feeding on this latter prey species. This difference in reproductive success matches prey preference.

As a result of low development rates when feeding on P. ulmi larvae A. potentillae and T. pyri have lower r_m' values when feeding on this prey species. This is most obvious for A. potentillae. However, because the least vigorous P. ulmi stage was used, the difference in r_m' values is expected to be larger when other P. ulmi stages are used (Sabelis, 1985a). In terms of diapause induction, P. ulmi is a better prey species for

A. potentillae. This is of importance at the end of the season. Current data therefore indicate that A. schlechtendali is a better prey species for T. pyri and, at least during the beginning of the season, also for A. potentillae.

This conclusion implies that prey preference of these predator species does not match reproductive success. As argued in the 'Introduction' this may either be caused by prey availability as a prey species characteristic or by prey availability as a result of competition between prey species or competition between predator species. Prey characteristic availability is dependent on the intrinsic rate of population increase (r_m). Estimated values of r_m early in the season are higher for A. schlechtendali (based on data of Easterbrook, 1979) than for P. ulmi (based on data of Rabbinge, 1976) (P. van Rijn and M.W. Sabelis, unpublished data). From July onwards this difference is reversed because of production of overwintering females of the rust mites (deutogynes). This makes A. schlechtendali a better prey species during the first half of the season and P. ulmi during the last half. In addition, at the end of the season also prey effects on diapause induction render P. ulmi a better prey species for A. potentillae. Does this suggest that predatory mites change prey preference during the season? Investigation of field-collected predators are needed. Electrophoretic diet analysis of T. pyri, collected in August and September showed preference of P. ulmi (Dicke and De Jong, 1988), but no field data for the first half of the season are available.

Prey characteristic availability of P. ulmi and A. schlechtendali are

Table 12.5: Prey preference and best prey species in terms of reproductive success parameters of three species of phytoseiid mites.

Predator species	Preferred prey species	Best prey species in terms of	
		r_m'	Effect on diapause induction
A. finlandicus	A. schlechtendali	A. schlechtendali	
A. potentillae	P. ulmi	A. schlechtendali	P. ulmi
T. pyri	P. ulmi	A. schlechtendali	

also important to A. finlandicus. However, because of the large difference in r_m' values of A. finlandicus when feeding on P. ulmi or A. schlechtendali, the effect of a change in characteristic prey availability is less pronounced for A. finlandicus than for T. pyri or A. potentillae.

In conclusion, prey preference of A. finlandicus shows reasonable corrrespondence with reproductive success. For A. potentillae and T. pyri the situation is more complex. Reproductive success in terms of r_m' does not match prey preference of these predator species. Differences in characteristic prey availability can partly account for this discrepancy. To increase understanding of the conditions that have moulded prey preference of phytoseiid mites future investigations should concentrate on e.g. (1) possible role of competition between predator species or between prey species, (2) prey preference early in the season and (3) the effect of interspecific carotenoid content of prey species on overwintering ability of predatory mites.

ACKNOWLEDGEMENTS
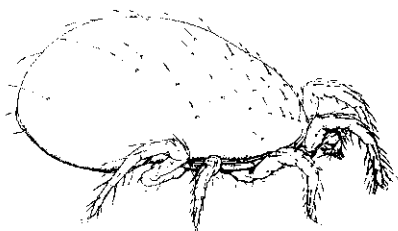
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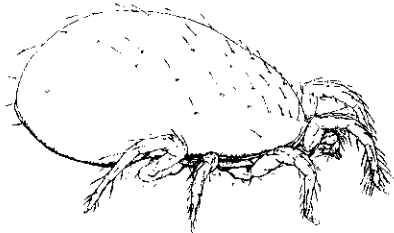
CURRICULUM VITAE

Marcel Dicke werd op 28 november 1957 geboren te Dordrecht. Hij behaalde in
1975 het eindexamen gymnasium-β (cum laude) aan het Marnix Gymnasium te
Rotterdam. In datzelfde jaar begon hij zijn studie Biologie aan de
Rijksuniversiteit te Leiden. In 1978 werd het kandidaatsexamen B4 (Biologie
met Scheikunde) afgelegd, waarna hij de volgende onderwerpen voor het
doktoraalexamen bewerkte:
- Chemische stimuli die een rol spelen bij het zoeken van het gastheermilieu
en het zoeken van de gastheer door Leptopilina heterotoma, een larvale
endoparasiet van Drosophila, onder begeleiding van Prof. Dr. J.C. van
Lenteren.
- Fungi in three main Negev desert plant communities (Artemisia herba-alba,
Hammada scoparia and Zygophyllum dumosum) and cellulose decomposition,
onder begeleiding van Prof. Dr. G. Buyanovsky en Prof. Dr. A. Quispel.
- De kwantitatieve bepaling van elongatiefaktor Tu in mutanten van
Escherichia coli veranderd in de genen tuf A en tuf B, onder begeleiding
van Drs. P.H. van der Meide en Prof. Dr. L. Bosch.

In april 1982 werd het doktoraal examen cum laude afgelegd. Tevens werd de
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