STELLINGEN

1. Bij een planteneter met een hoge populatiegroeisnelheid, zoals spint, kan het bepalen van alleen de sterfte of alleen de reproductie van de planteneter leiden tot verkeerde conclusies over de mate van resistentie van de waardplant.
   Lewontin, R. C. 1965. *In* "The genetics of colonizing species." (H. G. Baker and G. L. Stebbins Eds.), pp. 79-94.
   Caswell, H. & A. Hastings, 1980. Theoretical Population Biology 17: 71-79.
   Hstk. 1, dit proefschrift.

2. De eerder gepubliceerde r_m waarden van *Phytoseiulus persimilis* bij ± 25 °C zijn nooit boven de 0.4/dag uitgekomen, doordat ze niet zijn bepaald onder omstandigheden die voor de roofmijten optimaal zijn.
   References in appendix of Sabelis, M. W. and Janssen, A. 1994. *In* "Mites, ecological and evolutionary analyses of life-history patterns," (M.A. Houck, Ed.), pp. 70-98.
   Hance, T. 1988. Annls. Soc. r. zool. Belg. 118: 161-170.
   Hstk. 2, dit proefschrift.

3. Door de manier waarop een populatie spintmijten is verdeeld over gerberabladeren, is het bepalen van de dichtheid van spint binnen kolonies op de bladeren niet zinvol om inzicht te verkrijgen in de interactie tussen *T. urticae* en *P. persimilis* op gerbera.
   Hstk 3 en 6, dit proefschrift.

4. De grootste hiaat in onze kennis over geuren die planten met vraatschade produceren en die aantrekkelijk zijn voor natuurlijke vijanden van de planteneters, is dat we niet weten of de geurproductie uiteindelijk leidt tot minder vraatschade en daardoor een hogere fitness van de planten.

5. De teelt van een kasgewas wordt door het toepassen van biologische bestrijding niet ineens milieuvriendelijk.

6. Wie op de middelbare school biologie het leukste vak vindt, moet het bij haar of zijn carrière niet te ver van huis zoeken, maar gewoon zelf later die lessen gaan geven.

7. Statistische significantie wordt vaak verward met relevantie.

8. Het belang van tijdelijke personeelsleden voor een bedrijf of instelling zou overeen moeten komen met hun stem in de bedrijfsvoering.

9. Wanneer slechte schoolprestaties in het studiehuis leiden tot hervatting van controle op het huiswerk, wordt middelbare scholieren de kans ontnomen om zelf te leren omgaan met hun nieuwe verantwoordelijkheden.

10. In een echte democratie zou na het schrikbarende verlies van D66 bij de laatste verkiezingen de conclusie getrokken zijn dat de bevolking niet achter de standpunten van die partij staat en zou zijn afgezien van de invoering van het referendum of een gekozen burgemeester.

11. Goed statistiekonderwijs aan journalisten zou veel onzin uit de dagbladen houden.

12. Mensen die de kreet "Maar ik bedoelde er niets mee." bezigen, kunnen datgene waar ze toch niets mee bedoelen beter achterwege laten.

13. Het respect voor economische vluchtelingen is evenredig met de rijkdom van het land waar ze vandaan komen.

14. Gezien de grote maatschappelijke weerstand tegen het gebruik van hormoonpreparaten bij vee is het wijdverbreide gebruik van de anticonceptiepil door vrouwen onbegrijpelijk.

15. Zonder struikelblokken zou het afronden van promotieonderzoek lang niet zoveel voldoening opleveren.

STELLINGEN BEHOREND BIJ HET PROEFSCHRIFT:
Plant effects on biological control of spider mites in the ornamental crop gerbera

Olga Krips Wageningen, 10 april 2000

Plant effects on biological control of spider mites in the ornamental crop gerbera

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Plant effects on biological control of spider mites in the ornamental crop gerbera

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dr. C.M. Karssen
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CONTENTS

HOOFDSTUK 1

ALGEMENE INLEIDING EN SAMENVATTING

INLEIDING

Gerbera's

Tijdens mijn promotieproject heb ik gemerkt dat gerbera's tot de wat minder bekende siergewassen behoren. Wanneer ik vervolgens uitlegde dat het om een chrysantachtige bloem ging aan een steel zonder bladeren werd meer dan eens geroepen:" Oh, die afschuwelijke bloemen met die ijzerdraadjes!". Dat ze zonder die ijzerdraadjes niet overeind blijven staan is een misverstand en over het feit of ze al dan niet mooi zijn kun je lang twisten. Vast staat in ieder geval dat er veel mensen zijn die gerbera's wél mooi vinden, want dit siergewas staat in de top tien van de Nederlandse exportbloemen. Voor de economie van Nederland is de teelt van gerbera's dan ook erg belangrijk.

Bestrijding van plagen op siergewassen

Tijdens de teelt van siergewassen wordt doorgaans een grote hoeveelheid bestrijdingsmiddelen gebruikt. Zo protesteerde de milieubeweging enkele jaren geleden tegen het excessieve gebruik van pesticiden op chrysanten. Het hoge pesticidengebruik op siergewassen heeft voornamelijk te maken met strenge eisen op de bloemenveiling aan kosmetische kwaliteit. Daarnaast moeten bloemen die voor de export worden aangeboden geheel vrij zijn van plaagorganismen en vraatschade en mogen er ook geen resten van plaagorganismen zoals vervellingshuidjes op aangetroffen worden. Bij zulke hoge eisen aan de kwaliteit van siergewassen wordt het moeilijk om plaagorganismen op een andere manier dan met pesticiden te bestrijden.

Alternatieven voor plaagbestrijding met pesticiden zijn bijvoorbeeld biologische bestrijding, waarbij natuurlijke vijanden van de planteneters in het gewas worden uitgezet, of resistentieveredeling, waarbij geselecteerd wordt op plantenrassen waarop de planteneters zich slecht kunnen handhaven. Bij deze vormen van plaagbestrijding wordt een populatie van planteneters echter zelden geheel uitgeroeid. Zoals boven is uitgelegd, is zelfs de aanwezigheid van maar enkele plaagorganismen in de meeste gevallen bij het kweken van een siergewas niet acceptabel. Bij consumptiegewassen zoals tomaten en komkommers, waarbij men niet de hele plant verhandelt, ligt dit geheel anders. In deze gewassen is een bepaalde hoeveelheid schade aan de bladeren acceptabel en worden alternatieve methoden voor plaagbestrijding op grote schaal en met succes toegepast. Momenteel wordt in Nederland bij het kweken van de meeste vruchtgewassen zoals komkommers, paprika's, aubergines en tomaten biologische bestrijding toegepast. Maar bij het kweken van siergewassen worden dergelijke bestrijdingsmethoden slechts mondjesmaat gebruikt.

Bestrijding van plagen op gerbera's

In het gewas gerbera is het gebruik van alternatieve bestrijdingsmethoden beter mogelijk dan bij andere siergewassen. Gerberabloemen worden verhandeld zonder bladeren aan de steel. Een flink aantal plaagorganismen tast de bladeren van planten aan, maar laat de bloemen met rust. Aangezien de bladeren van gerbera's in de kas aan de planten achterblijven wanneer de bloemen naar de veiling gaan, is een zekere hoeveelheid vraatschade aan de bladeren te tolereren. Momenteel past al 75% van de gerberatelers biologische bestrijding toe tegen één of meer soorten planteneters.

De plaag: spint

Een belangrijk plaagorganisme in kasgewassen is de spintmijt *Tetranychus urticae,* in de volksmond 'spint' genoemd. Gerbera's worden in Nederland in kassen gekweekt en spint is ook in dit gewas een plaag. Spint tast voornamelijk de bladeren van planten aan. Bloemen worden pas in een zeer laat stadium aangetast, wanneer de rest van de plant al zwaar beschadigd is. In de praktijk zal een kweker nooit zo laat ingrijpen.

In veel groentegewassen onder glas wordt spint biologisch bestreden. Hierbij wordt de roofmijtensoort *Phytoseiulus persimilis,* die uitsluitend spintmijten eet, in de kassen uitgezet. Ook wordt in een aantal van deze groentegewassen gebruikgemaakt van plantenrassen waarop een spintpopulatie minder snel kan groeien. Ook in gerberakassen kunnen deze methoden toegepast worden, doordat spintschade aan de bladeren van gerberaplanten te tolereren is, zolang de productie van bloemen niet te sterk achteruit gaat.

Bestrijding van spint op verschillende cultivars van gerbera

Er zijn veel verschillende kleuren en vormen gerberabloemen. Ieder type gerberabloem komt van een afzonderlijke gerberacultivar. Een gerberaveredelingsbedrijf heeft al gauw meer dan honderd cultivars in de handel en per jaar komt er een flink aantal nieuwe op de markt. Bij de selectie van deze cultivars wordt voornamelijk gekeken naar de vorm, kleur en houdbaarheid van de bloemen. Maar de cultivars verschillen ook in diverse andere kenmerken, waar niet op geselecteerd wordt. Zo verschilt per cultivar de grootte en de hardheid van de bladeren en vertoont ook de dichtheid van beharing aan de onderzijde van de bladeren grote verschillen. Zoals ik hieronder zal uitleggen kunnen dit soort verschillen grote gevolgen hebben voor het succes van biologische bestrijding.

Zowel planteneters als hun natuurlijke vijanden kunnen sterk beïnvloed worden door kenmerken van planten. Dit kan zowel in het voordeel als in het nadeel werken van bestrijding van de planteneters door hun natuurlijke vijanden. Planten kunnen stoffen produceren die giftig of afstotend zijn voor bepaalde planteneters. Sommige plantensoorten maken nectar of stuifmeel wat als alternatief voedsel gebruikt kan worden door natuurlijke vijanden van planteneters, waardoor zij ook op de planten kunnen overleven wanneer er géén planteneters aanwezig zijn. Bepaalde plantensoorten zijn behaard, wat zowel de planteneters als hun natuurlijke vijanden kan beïnvloeden. En verder kunnen ook plantengeuren belangrijk zijn. Van een aantal soorten planten is bekend dat deze geuren gaan produceren, wanneer er door planteneters van hun bladeren gegeten wordt. Natuurlijke vijanden van de planteneters kunnen deze geuren gebruiken om planten met hun prooi te lokaliseren. Wanneer gerberacultivars verschillen in de bovengenoemde kenmerken, kan het succes van biologische bestrijding van bepaalde planteneters verschillen per cultivar.

Onderzoeksvragen

Het doel van dit promotieonderzoek was te bepalen welke verschillen tussen gerberacultivars van belang zijn voor bestrijding van de spintmijt *Tetranychus urticae* met de roofmijt *Phytoseiulus persimilis.* We hebben ons gericht op de volgende drie plantkenmerken:

1. Resistentie tegen spint.
2. Beharingsdichtheid aan de onderzijde van de bladeren.

3. Productie van geuren door de planten in reactie op vraatschade van spint.

We hebben van deze kenmerken bepaald of deze überhaupt verschillen per gerberacultivar. Vervolgens hebben we het effect van deze kenmerken bepaald op bepaalde aspecten van de interactie tussen de spintmijt *T. urticae* en de roofmijt *P. persimilis*.

De opbouw van het proefschrift

In hoofdstuk 3 wordt beschreven of gerberacultivars verschillen in resistentie tegen spint. Het effect van resistentie tegen spint op roofmijten wordt beschreven in hoofdstuk 4. Hoofdstuk 5 gaat over het effect van bladbeharing op het vermogen van roofmijten om hun prooi te vinden. In hoofdstuk 6 wordt beschreven of gerberaplanten geuren produceren in reactie op vraatschade door spint en of roofmijten door deze geuren worden aangetrokken. Of deze geurproductie en aantrekking van roofmijten verschilt per gerberacultivar is het onderwerp van hoofdstuk 7. Tenslotte laat hoofdstuk 8 zien hoe belangrijk de gevonden effecten zijn voor biologische bestrijding van spint op gerberaplanten.

HOOFDSTUK 3: CULTIVARVERSCHILLEN IN RESISTENTIE TEGEN SPINT

Cultivars van een plantensoort kunnen verschillen in resistentie tegen bepaalde planteneters. Hiervan kan gebruik worden gemaakt bij het veredelen op resistentie. Op deze manier is bij een aantal gewassen veredeld op resistentie tegen spint. Dit heeft zich echter voornamelijk beperkt tot een aantal groentegewassen en katoen.

Bij het bepalen van de mate van resistentie van cultivars kan je naar veel factoren kijken. Bijvoorbeeld naar beschadiging van de bladeren, naar ontwikkelingsduur van de planteneter, naar eilegsnelheid, naar sterfte, naar het percentage planteneters dat van plant gaat eten of er eieren op gaat leggen enzovoorts. Het is niet eenvoudig om uit dit veelvoud aan factoren juist díe factor te kiezen die representatief zal zijn voor de schade aan planten in de kas.

In het meeste werk aan resistentieveredeling wordt gekeken naar eiproductie of sterfte van de planteneters. Maar het is zeer de vraag of deze factoren bij spint een goede indicatie zijn voor wat er later in de kas zal gaan plaatsvinden. Spint is een planteneter waarvan de populatie zeer snel kan groeien. Een populatiegroeisnelheid van 25% per dag is geen uitzondering. Bij een populatie met zo'n hoge groeisnelheid wordt de populatiegroeisnelheid veel minder beïnvloed door een effect op de eilegsnelheid of sterfte dan door een effect op de snelheid waarmee eieren zich ontwikkelen tot volwassene die zelf eieren produceren.

Het effect van deze ontwikkelingssnelheid op de populatiegroeisnelheid is het makkelijkst te begrijpen met een getallenvoorbeeld. Spintmijten die 14 dagen doen over de ontwikkeling van ei tot eiprocucerende volwassenen, hebben na 84 dagen de zesde generatie bereikt. Wanneer deze ontwikkelingstijd met 2 dagen verkort wordt tot 12 dagen, bereiken de spintmijten in die 84 dagen al de zevende generatie. Wanneer de eilegsnelheid bij beide populaties spintmijten gelijk is zal de populatie spintmijten met een ontwikkelingstijd van 12 dagen dus veel sneller groeien dan de populatie waarvan de spintmijten zich in 14 dagen ontwikkelen van ei tot eiproducerende volwassenen. Bij spint kan een verlaging van 10% in ontwikkelingssnelheid onder bepaalde omstandigheden hetzelfde effect hebben op de populatiegroeisnelheid als een verdubbeling van de eilegsnelheid.

Wij hebben ervoor gekozen om zowel de ontwikkelingsduur als eiproductie en sterfte van spint te bepalen op negen cultivars bij een overmaat aan voedsel. Aan de hand van deze gegevens is de maximale populatiegroeisnelheid van spint op de cultivars berekend. De op deze wijze bepaalde populatiegroeisnelheid wordt de r_m genoemd. We hebben deze r_m bepaald op negen cultivars.

De r_m van spint bleek enorm te verschillen per cultivar. Zo nam de populatiegrootte van spint op de minst resistente cultivar Sirtaki met 27% per dag toe, terwijl dit op de meest resistente cultivar Bianca slechts 9% per dag was. Hoe groot dit verschil is wordt zichtbaar met een simpel rekenvoorbeeld. Wanneer je zou starten met 200 spintmijten op elk van beide cultivars dan zou dit na drie weken op Bianca gegroeid zijn tot iets meer dan 1100 individuen, terwijl de populatie op Sirtaki in die tijd al bijna 24000 spintmijten zou tellen.

De verschillen in r_m van spint op de negen cultivars werden veroorzaakt door verschillen in ontwikkelingstijd en eiproductie. In een aantal gevallen vonden we bij gelijke eiproductie toch verschillen in ontwikkelingssnelheid en daardoor een groot verschil in r_m. Dit geeft aan dat we verkeerde conclusies zouden hebben getrokken wanneer we alleen naar eiproductie hadden gekeken. Het is dus belangrijk om bij het bepalen van de mate van resistentie van cultivars de eventuele effecten op de ontwikkelingssnelheid van de planteneter niet over het hoofd te zien.

Naast de cultivarverschillen in resistentie hebben we bepaald of de leeftijd van de bladeren van invloed is op de r_m van spint. We hebben hiervoor de r_m bepaald op jonge en oude bladeren van de twee cultivars Sirtaki en Bianca. Hierbij bleek de populatiegroeisnelheid van spint op Sirtaki sterk afhankelijk te zijn van de leeftijd van de bladeren. Op jonge bladeren van Sirtaki had spint een veel hogere populatiegroeisnelheid dan op oude bladeren. Dit effect werd niet gevonden bij cultivar Bianca.

Planteneters kunnen zich aanpassen aan pesticiden en ook aan resistente planten. Ook spint blijkt zich aan te kunnen passen aan een resistente plantensoort en dit blijkt erg snel op te kunnen treden. Spint kan zich binnen tien generaties, wat ongeveer overeen komt met een half jaar, aanpassen aan tomatenplanten waar ze zich in eerste instantie slecht op kunnen handhaven.

Wij hebben gekeken of spint zich kan aanpassen aan de cultivar Bianca waarop een lage populatiegroeisnelheid gevonden was. We hebben spint ongeveer een half jaar op deze cultivar gekweekt. Hierna bleek te populatiegroeisnelheid op Bianca te zijn gestegen tot 23% per dag. Dit is nog steeds aanmerkelijk lager dan wat we in dat experiment vonden op de niet-resistente cultivar Sirtaki, namelijk 30% per dag. Spint kan zich dus binnen een half jaar gedeeltelijk aanpassen aan de resistentie van cultivar Bianca.

De gevonden cultivarverschillen in resistentie tegen spint zijn groot. Hierdoor zou het mogelijk moeten zijn om bij het veredelen ook te selecteren op resistentie tegen spint. Op de meest resistente cultivar is de populatiegroeisnelheid van spint echter niet zo laag dat er door de kweker nooit ingegrepen zal hoeven worden. Bovendien kan spint zich tenminste gedeeltelijk aanpassen aan de resistentie. Andere methoden van spintbestrijding zullen dus nodig blijven. Een combinatie van het gebruik van resistente cultivars met biologische bestrijding is een oplossing die voor de hand ligt. Maar dan moeten deze twee methoden wel compatibel zijn. Of dit in het gewas gerbera het geval is is het onderwerp van hoofdstuk 4.

HOOFDSTUK 4: EFFECT VAN RESISTENTIE OP BIOLOGISCHE BESTRIJDING

Intuïtief wordt vaak aangenomen dat een natuurlijke vijand een populatie van planteneters sneller onder controle zal krijgen op een resisténte plant dan op een niet-resistente plant. De snelheid waarmee planteneters in aantal toenemen is namelijk lager op een resistente plant dan op een niet-resistente plant. Wanneer natuurlijke vijanden even snel in aantal zouden toenemen op resistente en niet-resistente planten zou het uitmoorden van de planteneters dus het snelst gaan op resistente planten. Er wordt daarom vaak beweerd dat resistentie goed te combineren is met de inzet van biologische bestrijders.

In veel gevallen blijken resistentie en biologische bestrijding echter niet zo goed samen te gaan. Zo kunnen resistente planten stoffen produceren die niet alleen giftig zijn voor een planteneter, maar ook voor de biologische bestrijder. Verder kan een planteneter door te eten van een resistente plant giftige stoffen opslaan en daardoor ongeschikt worden als voedsel voor natuurlijke vijanden. Een bekend voorbeeld hiervan is de Monarchvlinder die oneetbaar wordt voor vogels door van zijdeplanten te eten. In de literatuur blijken veel voorbeelden te zijn dat resistentie compatibel is met biologische bestrijding. Maar er zijn evenveel voorbeelden waarbij de twee methoden juist níet compatibel zijn.

In hoofdstuk 4 hebben we onderzocht of resistentie tegen spint in gerbera effect heeft op de populatiegroeisnelheid van roofmijten. We hebben, net als bij spint, cultivareffecten bepaald op eiproductie, sterfte en ontwikkelingsduur van de roofmijten. Uit deze gegevens hebben we de maximale populatiegroeisnelheid, r_m, van de roofmijten berekend. We hebben dit bepaald op vier cultivars waarop de r_m van spint sterk verschilde. Als voer voor de roofmijten gebruikten we een overmaat aan eieren van spintmijten die op dezelfde cultivar waren gekweekt als waarop de r_m van de roofmijten werd bepaald.

De verschillen in r_m van de roofmijten waren minimaal. Deze populatiegroeisnelheid varieerde op de vier cultivars van 53% per dag tot 57% per dag, terwijl de populatiegroeisnelheid van spint op deze cultivars varieerde van 9% tot 27% per dag. Op allevier de cultivars was de populatiegroeisnelheid van de roofmijten dus veel hoger dan die van spint. Doordat roofmijten veel sneller in aantal toenemen dan spintmijten, zijn ze zulke succesvolle bestrijders van spint. Dat resistentie tegen spint geen effect heeft op de r_m van roofmijten geeft aan dat op bladeren van resistente gerberacultivars kennelijk geen stoffen voorkomen die giftig zijn voor roofmijten. Ook komen dergelijke stoffen niet voor in de eieren van spint op een resistente cultivar. Hierdoor is het gebruik van resistente gerberacultivars waarschijnlijk te combineren met biologische bestrijding van spint.

Omdat de r_m van roofmijten de maximale populatiegroeisnelheid vertegenwoordigt, wordt deze per definitie bepaald onder omstandigheden waarin voedsel in overmaat aanwezig is. Je kunt je afvragen of zo'n overmaat aan spint wel representatief is voor wat de roofmijten op planten aantreffen en of de r_m van roofmijten wel een goede maat is voor de groeisnelheid van een populatie roofmijten op gerberaplanten in een kas. Op deze vragen wordt in hoofdstuk 8 uitvoerig ingegaan.

HOOFDSTUK 5: EFFECT VAN BLADBEHARING OP ROOFMIJTEN

Bladbeharing kan een grote invloed hebben op natuurlijke vijanden van planteneters. Deze invloed kan zowel positief als negatief zijn. Zo heeft bladbeharing op *Poinsettia*, de kerstster, een negatief effect op het succes van een aantal natuurlijke vijanden van de kaswittevlieg. Aan de andere kant heeft bijvoorbeeld koolrabi juist zulke gladde bladeren dat bepaalde lieveheersbeestjes van de bladeren afvallen en daardoor bladluizen aan de onderkant van bladeren, in het midden, niet kunnen bereiken.

Ook voor roofmijten blijkt bladbeharing belangrijk te zijn. Bepaalde kleine soorten roofmijten hebben een sterke voorkeur voor plantensoorten die dichtbehaard zijn. Verder komen er op dichtbehaarde plantensoorten kleinere soorten roofmijten voor dan op die met een minder dichte beharing, wat samen hangt met de ruimte tussen de haren. Er blijkt een direkt verband te zijn tussen de ruimte tussen bladharen bij plantensoorten en de grootte van de roofmijtsoorten die van nature op deze planten voorkomen.

Gerberacultivars verschillen sterk in bladbeharing aan de onderkant van de bladeren. Zo heeft cultivar Bianca ongeveer 100 haren/ cm^2, vergeleken met 730 haren/ cm^2 bij cultivar Bourgogne. De haren op dichtbehaarde cultivars staan zo dicht bij elkaar dat volwassen vrouwtjes van de roofmijtensoort *Phytoseiulus persimilis* niet tussen de haren door kunnen lopen. Deze beestjes hebben een lichaamsbreedte van ongeveer 0.5 mm. Bij een beharingsdichtheid van 400 haren/ cm^2 is de gemiddelde afstand tussen de haren ongeveer 0.5 mm. Bij een hogere beharingsdichtheid en dus een kleinere afstand tussen de haren passen de roofmijtvrouwtjes er niet meer tussen en moeten ze over de haren heenlopen op zoek naar voedsel. Maar jonge stadia van spintmijten en met name de eieren, die ongeveer 0.1 mm klein zijn, kunnen wél tussen of onder de haren zitten. Het ligt dan ook voor de hand dat roofmijten bij het zoeken naar voedsel op gerbera gehinderd worden door bladbeharing.

Volwassen roofmijtvrouwtjes eten ongeveer vier keer zoveel spint als andere roofmijtstadia en zijn dus het belangrijkst voor de bestrijding van spint. Daarom hebben wij ons in veel hoofdstukken beperkt tot het onderzoeken van roofmijtvrouwtjes. Wij hebben het loopgedrag van volwassen roofmijtvrouwtjes bepaald op drie cultivars met sterke verschillen in beharingsdichtheid, deze dichtheid was 105, 400 en 730 haren/cm^2. De roofmijten bleken veel langzamer te lopen op de sterkst behaarde cultivar dan op de andere twee cultivars. Op de sterkst behaarde cultivar duurde het ook het langst voordat ze hun prooi gevonden hadden. Verder vonden we ook een effect van bladbeharing op de hoeveelheid prooi die in een bepaalde tijd gegeten werd. Maar dit effect was afhankelijk van de dichtheid waarin de prooi werd aangeboden. Bij lage prooidichtheid aten de roofmijtvrouwtjes het minst op de sterkst behaarde cultivar en het meest op de minst behaarde cultivar. Maar bij hoge prooidichtheid aten ze op alledrie de cultivars evenveel.

Om dit te begrijpen moet je je een roofmijt voorstellen die op een blad naar spintmijten op zoek is. Roofmijten zijn blind en kunnen op een blad alleen maar spintmijten vinden wanneer ze er tegenaan lopen. Wanneer er maar weinig spintmijten op het blad aanwezig zijn en de dichtheid dus laag is, wordt de hoeveelheid spint die een roofmijt in een bepaalde tijd eet bepaald door de snelheid waarmee het bladoppervlak wordt afgezocht en dus door de loopsnelheid van de roofmijten. Dichte bladbeharing verlaagt de loopsnelheid, waardoor in een bepaalde tijd een minder groot deel van het blad afgezocht kan worden. Een roofmijt zal dus binnen een bepaalde tijd dus minder spintmijten vinden op een dichtbehaard blad dan op een lichter behaard blad waarop de roofmijt sneller kan lopen. Maar wanneer er veel spintmijten op het blad aanwezig zijn en de dichtheid dus hoog is, zal de snelheid waarmee de roofmijt een spintmijt kan opeten beperkend worden. En bij nog hogere spintdichtheid wordt de hoeveelheid gegeten spint bepaald door de snelheid waarmee de darm van de roofmijten zich leegt. Dichte bladbeharing zal dan geen effect meer hebben op hoeveelheid spintmijten die in een bepaalde tijd gegeten worden. Dus: het effect van bladbeharing op de consumptiesnelheid van roofmijten is afhankelijk van de dichtheid van spint op gerberabladeren.

Hieruit blijkt dat het effect van bladbeharing op biologische bestrijding van spint op gerberaplanten afhangt van de dichtheid waarin spint op de planten voorkomt. In hoofdstuk 8 wordt hierop dieper ingegaan.

HOOFDSTUK 6: GEUREN VAN PLANTEN MET SPINTSCHADE

Plantengeuren kunnen een belangrijke rol spelen bij de interactie tussen planteneters en hun natuurlijke vijanden. Bij een flink aantal plantensoorten worden geuren geproduceerd nadat planteneters de bladeren beschadigd hebben. Natuurlijke vijanden kunnen gebruik maken van deze geuren om bladeren met hun prooi te lokaliseren.

Ook bij de interactie tussen spint en roofmijten blijken plantengeuren belangrijk te zijn. Bij veel plantensoorten worden roofmijten aangetrokken door geuren van bladeren met spint, terwijl ze niet worden aangetrokken door onbeschadigde bladeren. Roofmijten worden niet aangetrokken door geuren van de spintmijten zelf. En wanneer spintmijten verwijderd worden van de beschadigde bladeren, blijken roofmijten nog geruime tijd tot de geuren van deze bladeren aangetrokken te worden. Chemische analyse heeft aangetoond dat het om typische plantengeuren gaat. De planten produceren de geuren in reactie op vraatschade van spint en "roepen" met deze geuren als het ware de "hulp" van roofmijten in.

Wanneer roofmijten een plant met spint gevonden hebben, blijven ze in de spinthaard totdat deze volledig is uitgemoord. Het percentage roofmijten dat voor die tijd de plant verlaat is verwaarloosbaar klein. Dit komt waarschijnlijk doordat roofmijten door hun reactie op de geuren van de beschadigde bladeren in de spinthaard vastgehouden worden. Wanneer maar een fractie van de roofmijten de spinthaard vroegtijdig zou verlaten zou de populatie spint tot veel grotere aantallen toenemen en zou het langer duren voordat alle spint is uitgemoord. Hierdoor zouden de spintmijten veel meer schade aan de bladeren aanrichten voordat alle spint is gedood door de roofmijten. Dit geeft aan dat de reactie van roofmijten op geuren van bladeren met spintschade belangrijk is voor bestrijding van spint met roofmijten.

Wij hebben onderzocht of gerbera ook geuren produceert wanneer de bladeren beschadigd zijn door spint. Dit bleek inderdaad het geval te zijn, de geurproductie was minstens even groot als eerder gevonden bij andere plantensoorten. Verder was een aantal specifieke geurstoffen in het geurmengsel aanwezig waarvan bekend is dat deze roofmijten aantrekken. Maar roofmijten werden niet door de geuren van gerberabladeren met spint aangetrokken.

Het geurmengsel van gerberabladeren met spintschade had een aantal overeenkomsten met geurmengsels van andere plantensoorten, maar minstens evenzoveel verschillen. Iedere plantensoort blijkt in reactie op spintschade een specifiek mengsel van geuren te produceren. Het geurmengsel van verschillende plantensoorten zal voor de roofmijten verschillend "ruiken". Het is dan ook niet zo verwonderlijk dat roofmijten niet zonder meer tot ieder van deze geurmengsels worden aangetrokken.

Uit voorgaand onderzoek blijkt dat roofmijten inderdaad niet door geuren van bladeren met spint van alle plantensoorten worden aangetrokken. Maar honger, of ervaring met spint op de planten en dus met de plantengeuren, kan ervoor zorgen dat een zwakke reactie van de roofmijten versterkt wordt. Wij hebben daarom gekeken of de reactie van roofmijten op de gerberageuren beïnvloed wordt door een van deze twee factoren. Roofmijten die 24 uur niets te eten hadden gekregen bleken nog steeds niet te reageren op gerberageuren. Roofmijten die zes dagen ervaring hadden gekregen met spint op gerbera, en dus zes dagen aan gerberageuren waren blootgesteld, bleken daarentegen wél door deze geuren aangetrokken te worden.

Het voorafgaande roept de vraag op hoe roofmijten bij verschillende plantensoorten de bladeren met spint lokaliseren. Roofmijten die een spinthaard hebben uitgemoord op een bepaalde plantensoort reageren op geuren van bladeren met spint van dezelfde plantensoort. Daardoor zouden ze een nieuwe spinthaard kunnen vinden op dezelfde plantensoort. Wanneer het lang duurt voordat ze zo'n nieuwe spinthaard hebben gevonden zullen ze door honger gaan reageren op geuren van meer plantensoorten en neemt de kans op het lokaliseren van spint op een nieuwe plantensoort toe. Maar bij bepaalde plantensoorten, zoals bij gerbera, leidt 24 uur honger niet tot aantrekking tot geuren van de bladeren met spint. Mogelijk worden de roofmijten wél aangetrokken tot de geuren van gerberabladeren met spint, wanneer ze langer dan 24 uur niets gegeten hebben. Mogelijk ook is het arriveren van de roofmijten op sommige plantensoorten een kwestie van toeval. Wanneer roofmijten toevallig op bladeren met spint van zo'n plantensoort zijn beland zal de reactie van de roofmijten op de geuren van de bladeren met spint door ervaring toenemen. Dit kan ertoe leiden dat de roofmijten weer nieuwe spinthaarden op deze nieuwe plantensoort zullen vinden nadat oude zijn uitgemoord.

HOOFDSTUK 7: CULTIVARVERSCHILLEN IN GEURPRODUCTIE EN AANTREKKING VAN ROOFMIJTEN

Cultivars van bepaalde plantensoorten kunnen verschillen in de productie van geurstoffen nadat planteneters de bladeren hebben beschadigd. De aantrekkelijkheid van deze geurstoffen voor natuurlijke vijanden van de planteneters kan daardoor verschillen per cultivar. Hierdoor kan het succes van een natuurlijke vijand als biologische bestrijder van een planteneter verschillen per cultivar.

Wij hebben in hoofdstuk 7 gekeken of gerberacultivars verschillen in de productie van geuren in reactie op spintschade. We hebben het geurmengsel van een aantal cultivars chemisch geanalyseerd. Daarnaast is de aantrekkelijkheid van de geurmengsels voor roofmijten bepaald. Op deze manier is geprobeerd om eventuele verschillen in aantrekkelijkheid van de geurmengsels te relateren aan verschillen in chemische samenstelling.

Het vergelijken van de aantrekking van roofmijten tot geuren van verschillende gerberacultivars is minder simpel dan het op het eerste gezicht lijkt. In hoofdstuk 3 bleek dat gerberacultivars sterk verschillen in resistentie tegen spint. Wanneer een bepaalde hoeveelheid spintmijten wordt verdeeld over een aantal bladeren, zal de schade die hierbij aan de bladeren ontstaat sterk verschillen per cultivar. Spintmijten eten minder van bladeren van resistente cultivars en een groter percentage van de spintmijten zal van de resistente planten weglopen. Wanneer er nu cultivarverschillen blijken te zijn in aantrekking van roofmijten, kan dit komen doordat de bladeren van de ene cultivar meer beschadigd zijn door spint dan de bladeren van de andere cultivar, terwijl dit niet is wat je wilde onderzoeken. Het is dus belangrijk om bij het vergelijken van de geuren van verschillende gerberacultivars rekening te houden met verschillen in spintschade door verschillen in resistentie.

In eerste instantie hebben we geprobeerd om de spintschade op de bladeren van alle onderzochte cultivars gelijk te krijgen. Maar geen van de manieren die we bedachten leidde tot standaardisatie van spintschade. Uiteindelijk, na vele mislukte proeven en minstens even zo vele frustraties, werden de experimenten over een andere boeg gegooid. Bij ieder experiment werd de spintschade op de bladeren geschat. Op deze manier zouden we weten of eventuele verschillen in geurproductie en aantrekking van roofmijten verklaard konden worden door verschillen in spintschade of niet.

We hebben de chemische samenstelling van de geuren van bladeren met spint van de cultivars Sirtaki, Rondena, Bianca en Fame bepaald. Daarnaast hebben we roofmijten in tweekeuze-situaties laten kiezen tussen enerzijds de geuren van Sirtaki en anderzijds de geuren van Rondena, Bianca of Fame. Om een indruk te krijgen van de spintschade, werden de eieren geteld die de spintmijten op de bladeren hadden gelegd. De eiproductie van spintmijten is evenredig met de hoeveelheid voedsel die ze binnen hebben gekregen. Het tellen van de eieren was zeer tijdrovend, omdat dit aantal al snel in de duizenden per set bladeren liep en de eieren ongeveer 0.1 mm groot zijn. Maar het bleek alle moeite waard te zijn.

Wanneer roofmijten geen voorkeur zouden hebben voor de geuren van een bepaalde cultivar zou in een tweekeuze-situatie het aantal roofmijten dat voor de geuren van een van beide cultivars kiest gelijk moeten zijn bij gelijke hoeveelheid spinteieren. Met andere woorden, je verwacht dat bij gelijke spintschade de roofmijten even sterk worden aangetrokken door de geuren van elk van beide cultivars. En aan de andere kant zouden de roofmijten bij ongelijke hoeveelheid spintschade moeten kiezen voor de geuren van de meest beschadigde cultivar. We hebben met een statistische toets bepaald of de roofmijten volgens deze verwachting kozen.

Wanneer de keuze gegeven werd tussen de geuren van bladeren met spint van Sirtaki en Rondena bleken de roofmijten een voorkeur te hebben voor de geuren van Rondena, ondanks het feit dat de bladeren van Sirtaki meer spinteieren bevatten. Op dezelfde manier waren ook de geuren van Bianca aantrekkelijker dan die van Sirtaki, maar in dit geval was dat net niet statistisch significant. De geuren van Sirtaki en Fame verschilden niet in aantrekkelijkheid.

De chemische analyse van de geurmengsels liet grote cultivarverschillen zien. Zo was de productie binnen een bepaalde groep van organische verbindingen, de terpenen, erg laag bij cultivar Sirtaki. Dit verklaart mogelijk de lage aantrekkelijkheid van de Sirtakigeuren. Verder bleek de productie van de terpenen *(E)*-β-ocimeen en linalool laag te zijn bij zowel Sirtaki als Fame. Deze stoffen bleken in eerder onderzoek roofmijten aan te trekken. Het is interessant om in vervolgonderzoek te bepalen of de lage productie van deze twee stoffen samenhangt met de lage aantrekkelijkheid van de geuren van deze twee cultivars voor roofmijten.

HOOFDSTUK 8: HOE BELANGRIJK ZIJN DE CULTIVARVERSCHILLEN VOOR BIOLOGISCHE BESTRIJDING VAN SPINT OP GERBERAPLANTEN?

In de voorgaande hoofdstukken hebben we cultivarverschillen bepaald in resistentie tegen spint, bladbeharing en de productie van geuren in reactie op spintschade. Bovendien is van elk van deze plantkenmerken het effect op roofmijten bepaald. Het is echter nog nauwelijks aan bod gekomen hoe belangrijk de gevonden effecten zijn voor voor biologische bestrijding van spint op gerberaplanten. Dit hebben we in hoofdstuk 8 onderzocht door middel van een kleinschalige kasproef met planten van twee cultivars. We hebben spintmijten op de planten gezet en na verloop van tijd roofmijten uitgezet in een roofmijt:spint-verhouding van 1:40. Vervolgens is het verloop van de populaties spintmijten en roofmijten gevolgd op beide cultivars.

Om het effect van bladbeharing te bepalen hebben we de twee cultivars Bianca en Bourgogne gebruikt met een bladbeharing van respectievelijk 218 en 630 haren/cm^2. Om het effect van resistentie tegen spint te bepalen hebben we gebruik gemaakt van een bestaand, simpel, wiskundig model dat de dynamiek van spint- en roofmijtpopulaties op planten beschrijft. De populatiegroeisnelheid van spint moet in het model ingevoerd worden. Door de waarde van dit getal te varieren is het effect van resistentie op het verloop van de spint- en roofmijtpopulatie te simuleren. Verder hebben we de voorspellingen van het model vergeleken met het verloop van de populaties spint- en roofmijten op beide cultivars. Zoals verderop zal worden uitgelegd gaf dat onder andere informatie over het effect van de geuren die na spintschade geproduceerd worden.

Door de waarde voor de populatiegroeisnelheid van spint te variëren als invoer in het model, is bepaald of resistentie tegen spint effect heeft op de snelheid waarmee spint wordt uitgemoord door roofmijten. Deze factor bleek nauwelijks effect te hebben. Het is dus niet zo dat biologische bestrijding van spint sneller verloopt op een resistente cultivar. Dit lijkt op het eerste gezicht een argument te zijn tegen het gebruik van resistente cultivars. Maar het voordeel van resistentie moet meer gezocht worden in het effect op spint *voor*dat roofmijten worden uitgezet dan in het effect *daarna*. Zo deed de spintpopulatie er op de ene, niet-resistente cultivar negen dagen over om een omvang van 1500 individuen te bereiken terwijl dat op de andere, wél resistente cultivar 30 dagen duurde. Dus bij het gezamenlijk gebruik van resistentie en biologische bestrijding is het voordeel van resistentie dat het langer duurt voordat het nodig is om roofmijten uit te zetten.

In hoofdstuk 5 is al even aan de orde gekomen dat het belangrijk is om te weten in wat voor dichtheden spint voor kan komen op gerberabladeren, omdat het effect van bladbeharing op de consumptienelheid van de roofmijten hiervan afhangt. Op de plantensoorten boon en roos, die veel gebruikt zijn in het onderzoek aan spint en roofmijten, heeft spint de neiging om op bladeren kolonies te vormen met een hoge dichtheid, terwijl op andere delen van de bladeren soms helemaal geen spint zit. In zo'n situatie besteden de roofmijten veel meer tijd aan zoeken van spint binnen zulke kolonies dan aan het zoeken op de rest van het blad naar dergelijke kolonies. In dit geval is de dichtheid binnen de kolonies het belangrijkst voor de interactie tussen spint- en roofmijten. Deze dichtheid is op veel soorten planten erg hoog waardoor de roofmijten niet door voedsel gelimiteerd worden, waardoor de groeisnelheid van een roofmijtpopulatie maximaal is.

Maar op gerbera zijn de spintkolonies klein en sterk verspreid over de bladeren. Dit zou effect kunnen hebben op de consumptiesnelheid van roofmijten op gerbera. In hoofdstuk 5 is uitgelegd dat bladbeharing alleen effect heeft op de consumptiesnelheid van roofmijten wanneer de dichtheid van spint laag is. Wanneer deze consumptiesnelheid beperkt wordt door de dichtheid van spint, zal de populatiegroeisnelheid van de roofmijten niet maximaal zijn.

Wij hebben in onze modelberekeningen aangenomen dat de groeisnelheid van de roofmijtpopulatie maximaal was op beide cultivars. De maximale populatiegroeisnelheid van roofmijten op gerbera is de r_m die in hoofdstuk 4 is bepaald. In de modelberekeningen is een populatiegroeisnelheid van 53% per dag gebruikt. Deze waarde was bepaald bij een overmaat aan voedsel en dus bij een hoge spintdichtheid. We bekeken of het model goede voorspellingen gaf met deze maximale populatiegroeisnelheid van 53% per dag als invoer. Verder is op beide cultivars op gezette tijden het aantal roofmijten geteld en daaruit is de groei van deze populaties geschat.

Hiernaast hebben we bij onze modelberekeningen aangenomen dat roofmijten de planten met spint pas verlaten als alle spint is uitgemoord. In hoofdstuk 6 is al beschreven dat een populatie spint veel grotere aantallen bereikt en dat roofmijten er langer over doen om alle spint te doden, wanneer niet alle roofmijten tot het eind in de spinthaard blijven.

Volgens de modelvoorspellingen zouden de spintpopulaties op beide cultivars binnen 4 dagen na introductie van de roofmijten uitgemoord moeten zijn. In werkelijkheid duurde het wel twee weken. Een of meer van onze aannames in de modelberekeningen zijn dus niet geldig voor biologische bestrijding van spint op gerbera.

De groeisnelheid van de populatie roofmijten op beide cultivars bleek veel lager te zijn dan de 53% die we in hoofdstuk 4 hadden gevonden. Dit kan komen doordat spint in een lage dichtheid voorkomt op gerbera en de roofmijten daardoor niet met maximale snelheid kunnen eten. Maar het is ook mogelijk dat een deel van de roofmijten van de planten is weggelopen. Op dit moment is het niet mogelijk om uitsluitsel te geven of de lage populatiegroeisnelheid veroorzaakt wordt door een lage snelheid waarmee de roofmijten eten of door het verdwijnen van een deel van de roofmijten. Vervolgonderzoek zal dat uit moeten wijzen.

De populatiegroeisnelheid van de roofmijten was het laagst op de cultivar met de hoogste beharingsdichtheid. De roofmijtpopulatie groeide met 39% per dag op cultivar Bianca met 100 haren/cm^2 in vergelijking met 33% op cultivar Bourgogne met 730 haren/cm^2. Dit kan duiden op een negatief effect van bladbeharing op de consumptiesnelheid van de roofmijten. Maar het is ook mogelijk dat het verschil in populatiegroeisnelheid veroorzaakt is door een verschil in het percentage roofmijten dat van de planten is weggelopen. De aantrekking van de roofmijten tot de geuren van de twee cultivars kan verschillend zijn geweest. Ook hierover zal vervolgonderzoek uitsluitsel moeten geven.

Samengevat: Resistentie tegen spint heeft nauwelijks effect op de snelheid waarmee een populatie spint door roofmijten wordt uitgemoord, maar een groot effect op de tijd die het duurt voordat introductie van roofmijten nodig is. De populatiegroeisnelheid van roofmijten is niet maximaal op gerbera, wat veroorzaakt kan zijn door lage dichtheden van spint, maar ook door het verdwijnen van een deel van de roofmijten. De populatiegroeisnelheid van roofmijten is het hoogst op de cultivar met de laagste dichtheid van bladbeharing. Dit kan veroorzaakt zijn door een negatief effect van bladbeharing op de consumptiesnelheid van de roofmijten. Maar het is ook mogelijk dat het percentage roofmijten dat van de planten is verdwenen verschilde, doordat de geuren van de beschadigde bladeren niet bij beide cultivars even aantrekkelijk waren.

ANTWOORDEN OP VRAGEN ROEPEN NIEUWE VRAGEN OP

Door dit promotieonderzoek is een aantal belangrijke vragen over het effect van bepaalde plantkenmerken op biologische bestrijding van spint in gerbera beantwoord. Bepaalde antwoorden kunnen veredelaars en/of telers van gerbera's direct van nut zijn. Andere antwoorden zijn meer wetenschappelijk van aard en verhogen ons inzicht in de interactie tussen spint en roofmijten op planten. Maar wetenschap zou wetenschap niet zijn als bij het beantwoorden van de vragen geen nieuwe vragen zouden ontstaan. Daarom zal ik nu een overzicht geven van de waarde van de verschillende antwoorden en daarbij aangeven wat volgens mij logische en interessante vragen zijn voor vervolgonderzoek. Hierbij zal ik één voor één de verschillende plantkenmerken die onderzocht zijn behandelen.

Resistentie tegen spint

De gevonden verschillen tussen gerberacultivars in resistentie tegen spint zijn groot, veel groter dan bijvoorbeeld in vroeger werk aan cultivars van komkommer is gevonden. Het zou dan ook mogelijk moeten zijn om in de praktijk gebruik te maken van resistentie tegen spint in gerbera. Veredelaars zouden bijvoorbeeld bij selectie van cultivars rekening kunnen houden met resistentie tegen spint. Voor de gerberatelers heeft het gebruik van resistente cultivars als voordeel dat zij hun gewas minder vaak op de aanwezigheid van spint hoeven te controleren, doordat spint minder snel in aantal toeneemt. Verder zou er minder snel naar andere vormen van spintbestrijding uitgeweken hoeven te worden.

Momenteel weten we nog niet wat de cultivarverschillen in resistentie tegen spint veroorzaakt. Het kan zijn dat planten van resistente cultivars stoffen produceren die giftig zijn voor spintmijten. Maar het is ook mogelijk dat er cultivarverschillen zijn in hardheid van de bladeren, waardoor spint niet even goed van alle bladeren kan eten. Er zijn geen aanwijzingen dat bladbeharing een rol speelt bij resistentie tegen spint, omdat zowel de meest als de minst behaarde cultivar een hoge mate van resistentie heeft.

Het zou interessant zijn om te achterhalen welk mechanisme verantwoordelijk is voor resistentie tegen spint in gerbera. Daardoor zou het misschien makkelijker kunnen worden om de mate van resistentie van nieuwe cultivars te bepalen. Voor veredelaars is het veel te tijdrovend om de groei van een populatie spintmijten op iedere nieuwe cultivar te bepalen. Maar wanneer de resistentie op een bepaalde gifstof zou berusten, zou het zo kunnen zijn dat de aanwezigheid van zo'n stof makkelijker te bepalen is dan de groei van een populatie spintmijten.

Compatibiliteit van resistentie en biologische bestrijding

Resistentie tegen spint in gerbera heeft geen negatief effect op de maximale populatiegroeisnelheid, de r_m, van de roofmijt *Phytoseiulus persimilis*. Dit houdt in dat er op resistente cultivars geen stoffen op het bladoppervlak of in de spintmijten aanwezig zijn die schadelijk zijn voor de roofmijten. Het gebruik van resistente cultivars zou dan ook gecombineerd moeten kunnen worden met biologische bestrijding van spint. Resistentie lijkt daarbij geen invloed te hebben op de tijd die roofmijten nodig hebben om alle spintmijten te elimineren. Het zal op een resistente cultivar wel langer duren voordat het uitzetten van roofmijten nodig is.

Een aantal mogelijke indirecte negatieve effecten van resistentie op biologische bestrijding van spint is niet aan bod gekomen in dit proefschrift. Op resistente cultivars groeit het aantal spintmijten erg langzaam, waardoor er weinig schade aan de bladeren wordt aangericht. Mogelijk worden hierdoor bij resistente cultivars weinig geurstoffen geproduceerd die roofmijten aantrekken. Dit zou kunnen leiden tot het verdwijnen van een gedeelte van de roofmijten van de planten met spint, waardoor het langer duurt voordat alle spint is uitgemoord.

Verder is het mogelijk dat de dichtheid van spint op resistente cultivars lager is dan op niet-resistente cultivars. Hierdoor zou de snelheid waarmee spint gegeten wordt door de roofmijten lager kunnen zijn op resistente cultivars, wat een lagere groeisnelheid van de roofmijtpopulatie tot gevolg kan hebben. Ook in dit geval zou het langer duren voordat alle spint is geëlimineerd.

Uit het kasproefexperiment van hoofdstuk 8 bleek de populatiegroeisnelheid van de roofmijten op gerberaplanten lager te zijn dan de r_m die in hoofdstuk 4 was gevonden. Dit kon verklaard worden door het verdwijnen van een deel van de roofmijten van de planten of een lage dichtheid van spint. Beide cultivars uit het kasproefexperiment waren sterk resistent tegen spint. Het is mogelijk dat een van beide hierboven genoemde indirecte effecten van resistentie de oorzaak is van de lage groei van de roofmijtpopulatie.

Naar mijn mening zou onderzocht moeten worden wat precies de oorzaak is van de lage groei van de roofmijtaantallen op gerbera. Verder zouden de kasproeven uit hoofdstuk 8 herhaald kunnen worden, maar dan met niet-resistente cultivars, om te bepalen of de populatiegroeisnelheid van de roofmijten ook laag is op deze cultivars. Op deze manier kan bepaald worden of de lage groeisnelheden veroorzaakt worden door indirecte effecten van resistentie of niet.

Bladbeharing

We hebben in hoofdstuk 5 negatieve effecten gevonden van bladbeharing op de loopsnelheid van roofmijten en de hoeveelheid prooi die in een bepaalde tijd gevonden en gegeten wordt. In hoofdstuk 8 vonden we aanwijzingen dat de effecten van bladbeharing ook werkelijk van belang kunnen zijn voor biologische bestrijding van spint in een gerberakas. Maar het aantal onderzochte cultivars was te laag om hierover harde conclusies te kunnen trekken en het zou interessant zijn als er hiernaar vervolgonderzoek gedaan zou worden. De kasexperimenten uit hoofdstuk 8 zouden uitgebreid kunnen worden met meer cultivars die verschillen in bladbeharing.

Wanneer bladbeharing inderdaad effect blijkt te hebben op de populatiegroeisnelheid van roofmijten, zou hiermee rekening gehouden kunnen worden bij het veredelen en telen van gerberacultivars. Bladbeharing is makkelijk vast te stellen en het zal daarom niet moeilijk zijn om met deze factor rekening te houden bij de selectie van cultivars. Verder zouden gerberatelers zich moeten realiseren dat biologische bestrijding van spint langzamer gaat op een behaarde cultivar dan op een niet-behaarde. Ze zouden dit kunnen corrigeren door op behaarde cultivars meer roofmijten uit te zetten.

Geurproductie

Gerberabladeren produceren geuren wanneer ze door spint worden aangetast, maar roofmijten worden hierdoor in eerste instantie niet aangetrokken. De reactie van de roofmijten wordt niet beïnvloed door 24 uur honger. Na een aantal dagen ervaring met spint op gerbera gaan roofmijten wél op de geuren reageren.

Het feit dat roofmijten in eerste instantie niet reageren op geuren van gerberaplanten met spint kan van grote invloed zijn op biologische bestrijding van spint in gerbera. Mogelijk kan een groot deel van de roofmijten die op gerberaplanten worden uitgezet in eerste instantie de bladeren met spint niet vinden, doordat er niet op de geuren uit de spinthaard gereageerd wordt. Hierdoor zou dit deel van de roofmijten kunnen verdwijnen uit het gewas. Hierdoor zou de populatie spint tot veel grotere aantallen kunnen toenemen waardoor de bladeren veel meer beschadigd zouden worden voordat de roofmijten uiteindelijk de spintpopulatie elimineren.

Wanneer de roofmijten eerst ervaring zouden krijgen met de geuren van gerbera met spint voordat ze op gerberaplanten worden uitgezet, zou dat het succes van bestrijding van spint op gerbera weleens kunnen verhogen. Ook kunnen de roofmijten direct op de bladeren met spint worden aangebracht, waardoor ze niet naar de spinthaard hoeven te zoeken en zo vanzelf ervaring met spint op gerbera opdoen. Het zou erg interessant zijn om te onderzoeken of ervaring met geuren van gerbera met spint inderdaad leidt tot een snellere bestrijding.

Het feit dat roofmijten in eerste instantie niet reageren op geuren van gerberabladeren met spint biedt goede perspectieven om het effect van deze geuren op biologische bestrijding van spint te onderzoeken. Het feit dat bijna geen roofmijten uit een spinthaard vertrekken voordat deze is geëlimineerd wordt algemeen toegeschreven aan de reactie van de roofmijten op de geuren uit de spinthaard. Wanneer dit werkelijk het geval is, zouden meer roofmijten zónder ervaring met gerberageuren dan roofmijten mét ervaring van gerberaplanten met spint moeten verdwijnen. Hierdoor zou het langer duren voordat een spinthaard is uitgemoord wanneer roofmijten zónder ervaring worden uitgezet dan wanneer roofmijten mét ervaring worden uitgezet. Of dit klopt zou uitgezocht kunnen worden door een kasproef zoals beschreven in hoofdstuk 8 uit te voeren met roofmijten mét en zónder ervaring met geuren van gerbera met spint.

Cultivarverschillen in geurproductie

We hebben cultivarverschillen gevonden in de samenstelling van geuren die in reactie op spintschade geproduceerd worden. Ook verschillen cultivars in aantrekkelijkheid voor roofmijten. De minst aantrekkelijke cultivars hadden de laagste productie van de stoffen *(E)*-β-ocimeen en linalool. Of deze stoffen een rol spelen in de cultivarverschillen in aantrekkelijkheid zou in vervolgonderzoek verder onderzocht kunnen worden. Deze informatie kan vervolgens gebruikt worden bij de selectie van gerberacultivars door veredelaars. Het zal voor veredelaars niet eenvoudig zijn om arbeidsintensieve experimenten uit te voeren zoals die van hoofdstuk 7. Maar het zou wel eens veel eenvoudiger kunnen zijn om vast te stellen of bepaalde aantrekkelijke verbindingen door gerberacultivars geproduceerd worden.

Het feit dat roofmijten de geuren van de ene cultivar prefereren boven die van een andere cultivar zou kunnen betekenen dat er cultivarverschillen zijn in aantrekking van roofmijten in een spinthaard. Mogelijk blijven roofmijten op de aantrekkelijkste cultivar wél allemaal in de spinthaard totdat alle spint is uitgemoord, terwijl op een minder aantrekkelijke cultivar een deel van de roofmijten eerder van de planten verdwijnt. Hierdoor zou het op de minder aantrekkelijke cultivar veel langer duren voordat alle spint is opgegeten door de roofmijten dan op een aantrekkelijke cultivar.

Het is echter ook mogelijk dat de aantrekkelijkheid van de geuren bij alle cultivars boven een bepaalde drempel ligt, waardoor de roofmijten op alle cultivars in de spinthaard blijven totdat deze is uitgemoord. In dit geval leiden verschillen in aantrekkelijkheid van de geuren niet tot verschillen in het percentage roofmijten dat een spinthaard verlaat. Hierdoor zou de snelheid waarmee de spinthaard wordt uitgemoord niet verschillen per cultivar.

Oorspronkelijk was het de bedoeling een kasexperiment, zoals in hoofdstuk 8 beschreven is, uit te voeren met bijvoorbeeld de cultivars Sirtaki en Rondena, omdat de geuren van deze cultivars duidelijk verschillen in aantrekkelijkheid voor roofmijten. Op deze manier zou onderzocht kunnen worden of de gevonden verschillen in aantrekkelijkheid gevolgen hebben voor de bestrijding van spint. Praktische problemen en een beperkte hoeveelheid beschikbare tijd maakten dat echter helaas onmogelijk. Het ligt voor de hand om dit soort experimenten in vervolgonderzoek alsnog uit te voeren.

DE BELANGRIJKSTE CONCLUSIES

- Gerberacultivars verschillen sterk in resistentie tegen spint en er zijn geen aanwijzingen dat biologische bestrijding van spint negatief beïnvloed wordt door resistentie.
- Bladbeharing bij gerbera heeft een negatief effect op de snelheid waarmee roofmijten hun prooi vinden en opeten en mogelijk ook op de snelheid waarmee de roofmijten op planten in aantal toenemen.
- Gerberaplanten met spintschade produceren geuren, maar roofmijten moeten eerst een positieve ervaring met deze geuren opdoen voordat ze erdoor aangetrokken worden.
- Er zijn cultivarverschillen in aantrekking van roofmijten tot de geuren die geproduceerd worden door planten met spintschade. De minst aantrekkelijke cultivars hadden de laagste productie aan *(E)*-β-ocimeen en linalool. Het staat nog niet vast of de cultivarverschillen in aantrekkelijkheid van de geuren ook leiden tot verschillen in de snelheid waarmee een populatie spint wordt geëlimineerd door roofmijten.
- De populatiegroeisnelheid van roofmijten is niet maximaal op gerbera. Dit kan het gevolg zijn van een lage dichtheid van spint op gerbera of door een zwakke aantrekking van roofmijten tot de geuren van planten met spintschade.

CHAPTER 2

GENERAL INTRODUCTION AND SUMMARY

INTRODUCTION

The spider mite *Tetranychus urticae* Koch is an important pest in many greenhouse crops. In vegetables it can be successfully controlled with the predatory mite *Phytoseiulus persimilis* Athias-Henriot, a specialist predator of spider mites (Helle and Sabelis, 1985). However, on ornamental crops where aesthetic damage is not tolerated, biological control is more difficult. Pest control on ornamentals mainly relies on the use of pesticides (van de Vrie, 1985).

In the ornamental crop gerbera, *Gerbera jamesonii* Bolus, biological control stands a better chance, since gerbera cutflowers are sold without leaves. The leaves stay behind on the plants in the greenhouse, when flowers are harvested. Therefore, zero tolerance for aesthetic damage does apply for gerbera cutflowers but not for the leaves (van de Vrie, 1985).

In gerbera, spider mites usually only damage leaves and do not feed on flowers. The spider mites only migrate to the flowers when the leaves become overexploitated. Gerbera growers will interfere with control methods long before overexploitation takes place. If they apply biological control, there will be some damage by spider mites on the plant leaves. But spider mite damage on the leaves can be accepted, as long as it does not lead to an intolerable lower flower production by the plants. Therefore, biological control may be a suitable method to control spider mites in gerbera.

Many cultivars of gerbera are commercially available. At present these cultivars are mainly selected for the colour and shape of the flowers. However, the cultivars also differ in several plant characteristics that have not been selected for, such as leaf shape and density of trichomes on the undersurface of the leaves (Sütterlin and van Lenteren, 1997; Krips *et al.*, 1999). Earlier studies showed that the success of natural enemies in biological control can be influenced strongly by characteristics of the host plant (Bergman and Tingey, 1979, Boethel and Eikenbary, 1986; van Lenteren and de Ponti, 1990; Hare, 1992; Walter, 1996). If gerbera cultivars differ in such characteristics, biological control may not be equally successful on all cultivars.

The objective of this PhD study was to investigate which differences between gerbera cultivars result in differences in the success of biological control of *T. urticae* with the predatory mite *P. persimilis.* We have focussed on the following three host plant characteristics:

1. Host plant resistance to *T. urticae*
2. Density of leaf hairs on the under surface of leaves
3. Production of volatiles by leaves with spider mite damage

We determined to which extent gerbera cultivars differ in each of these characteristics. In addition, we assessed the effect of these characteristics on certain aspects of the interaction between spider mites and their predators on gerbera.

In chapter 3 we have investigated whether gerbera cultivars differ in host plant resistance to the spider mite *T. urticae.* The effect of host plant resistance to spider mites on predatory mites has been studied in chapter 4. In chapter 5 the effect of leaf hairs on the searching behaviour of the predators is studied. In chapter 6 we have investigated whether gerbera plants produce volatiles when damaged by spider mites and whether predatory mites are attracted to these volatiles. Cultivar differences in production of spider mite-induced volatiles and attractiveness of the volatiles to the predators have been described in chapter 7. Finally, in chapter 8 we have described the importance of the effects found in chapters 3 to 7 for predator-prey dynamics on gerbera plants. Below, a summary of each chapter is given.

CHAPTER 3: CULTIVAR DIFFERENCES IN HOST PLANT RESISTANCE TO SPIDER MITES

Cultivars of a certain host plant may differ in resistance to herbivores. Breeders may use such differences to select for new cultivars that are resistant to certain herbivores. In this way, resistance against spider mites has been developed in several vegetables and cotton (de Ponti, 1985).

When breeders screen new cultivars for resistance to herbivores, they have to choose which factor will be taken as a measure for resistance. They can simply determine the amount of damage on leaves or can, for example, assess cultivar effects on oviposition, mortality, or developmental time of the herbivore. They can also assess cultivar effects on the percentage of herbivores that accept the leaves for feeding or oviposition. It is important to focus on factors that will be representative for loss of plant quality and crop yield in a greenhouse.

Breeders commonly focus on oviposition or mortality of the herbivore in standard screening tests (e.g. de Ponti, 1977; Johnson *et al.*, 1980; Gimenez-Ferrer *et al.*, 1993; Aguilar *et al.*, 1996). For spider mites, it is questionable whether these factors are representative for future damage on plants. On suitable host plants, a spider mite population can double in two days at 25°C (Sabelis, 1985; 1991). For such fast growing populations, changes in oviposition rate or mortality are less important for the population growth rate than changes in developmental time (Lewontin, 1965; Caswell and Hastings, 1980).

We have determined the developmental time, oviposition rate and mortality of *T. urticae* on nine gerbera cultivars and used these data to calculate the intrinsic rate of population increase, r_m. The r_m differed largely per cultivar and varied from 0.09-0.23/day. In several cases we found cultivar effects on the r_m while no difference in oviposition rate was found. These cultivar effects were due to differences in developmental time and illustrate the importance of including this parameter in standard screening tests for host plant resistance.

In addition to determining the r_m of *T. urticae* on nine cultivars, we have investigated whether young and old leaves of gerbera differ in suitability for *T. urticae* and whether the spider mites are able to adapt to a resistant cultivar. On one cultivar, we found a large effect of leaf age on the r_m of *T. urticae*, the r_m differed from 0.239/day on young leaves to 0.154/day on old leaves. However, this effect was not found on another cultivar. The spider mites were able to adapt to some extent to a resistant cultivar. After they had been reared for half a year on this resistant cultivar, the r_m increased from 0.168/day to 0.208/day. The r_m on the most suitable cultivar was 0.252/day in these experiments. Hence, after the spider mites had been reared for half a year on a resistant cultivar, their r_m had increased but was still lower than the r_m on the most suitable cultivar before adaptation.

The large cultivar differences in suitability for *T. urticae* offer perspectives for breeding for host plant resistance to spider mites. However, even on the least suitable cultivar additional methods of spider mite control will still be necessary. An obvious additional method is biological control with the predatory mite *P. persimilis*. But this is only possible if plant resistance to spider mites and biological control are compatible in gerbera. This has been the topic of research in chapter 4.

CHAPTER 4: COMPATIBILITY OF HOST PLANT RESISTANCE TO SPIDER MITES AND BIOLOGICAL CONTROL

Host plant resistance and biological control were often considered compatible in the past (Adkinson and Dyck 1980; Kogan, 1982). One assumed faster extermination of herbivores by biological control agents on resistant plants, because the population growth rate of herbivores is low on resistant plants. However, this assumption is not always valid (Bergman and Tingey, 1979; Boethel and Eikenbary, 1986; Hare, 1992).

A biological control agent may be influenced by characteristics of resistant plants, which may result in lower performance of the natural enemies on these plants (Bergman and Tingey, 1979; van Lenteren and de Ponti, 1990; Dicke 1996, 1999). Secondary plant compounds that are responsible for host plant resistance may be harmful for natural enemies as well (Flanders, 1942; Boethel and Eikenbary, 1986; Hare, 1992). Furthermore, herbivores may accumulate toxic compounds by feeding on resistant plants which may make them unsuitable as prey or host for their natural enemies (De Moraes and McMurtry, 1987; Rogers and Sullivan, 1986). Literature reviews on the combined use of host plant resistance with biological control show as many examples of compatibility of the two methods, as examples of incompatibility (Boethel and Eikenbary, 1986; van Lenteren and de Ponti, 1990; Gould *et al.*, 1991; Hare, 1992).

We have investigated compatibility of host plant resistance to spider mites in gerbera with biological control. We determined the intrinsic rate of population increase, r_m, of *P. persimilis* on four gerbera cultivars on which the r_m of *T. urticae* varied from 0.09/day to 0.23/day. Cultivar differences in r_m of the predators were negligible, the r_m varied from 0.43/day to 0.45/day. This indicates that a combined use of resistance and biological control of spider mites might be possible in gerbera.

Since the r_m of predators represents the maximal population growth rate, it is assessed under optimal conditions where food is not limiting. Therefore, we used a surplus of food, i.e. a high prey density, in our experiments. One may wonder whether such a high prey density actually occurs on gerbera plants and whether the r_m is a good measure for the predator population growth rate on gerbera. This will be discussed in more detail in chapter 8.

CHAPTER 5: EFFECT OF LEAF HAIR DENSITY ON THE SEARCHING EFFICIENCY OF *P. PERSIMILIS*

The success of a biological control agent to exterminate a herbivore population can be influenced considerably by the presence of trichomes on the host plant. Several parasitoid species are affected negatively at high trichome densities. On Poinsettia, for example, four parasitoid species and one coccinellid predator of the silverleaf whitefly have the highest rate of parasitism or predation on the cultivar with the lowest trichome density (Heinz and Parella, 1994). On the other hand, positive effects of trichomes were, for example, reported by Grevstad and Klepetka (1992). On kohlrabi, a cruciferous plant with a very smooth leaf surface, coccinellid predators of aphids could not reach their prey that was located in the middle of the undersurface of the leaves. The predators could not grip the surface of the smooth waxy plants and fell off the leaves. On three other plant species, which do not have a smooth leaf surface, the predators were successful in locating their prey.

Predatory mites may also be influenced by trichomes. Several species of small predatory mites prefer leaf surfaces with a dense layer of trichomes (Downing and Moillet, 1967; Overmeer and van Zon, 1984; Duso, 1992; Karban *et al.* 1995; Walter, 1996). Furthermore,. Barrett (1994) suggests that the distribution of predatory mite species over different host plant species is dependent on the size of their body in relation to leaf hair density. In other words, small species of predatory mites occur on host plant species with a dense layer of trichomes whereas larger species of predatory mites occur on host plants with a less dense trichome layer.

Phytoseiulus persimilis is a large predatory mite species and may therefore be hampered by trichomes. Adult female *P. persimilis* have a body size of about 0.5 mm (Sabelis, 1981). At a trichome density of approximately 400 trichomes/cm^2, the predators just fit in between the trichomes. At higher trichome densities, the predators have to walk over the trichomes searching for prey, whereas especially eggs of spider mites with a size of about 0.1 mm may be situated between or under the trichomes. The ability of *P. persimilis* to find prey may, therefore, be lower on host plants with a dense trichome layer.

Gerbera cultivars differ largely in density of trichomes on the undersurface of leaves (Sütterlin and van Lenteren, 1997). We investigated whether prey searching behaviour of *P. persimilis* is affected on cultivars with a high density of trichomes. We determined the walking speed, walking activity and rate of encounter with prey on three cultivars with trichome densities of 105, 400 and 730 trichomes/cm^2. Furthermore, we assessed the predation rate on these three cultivars at three prey densities.

Walking speed of the predators was highest on the cultivar with the lowest leaf hair density. Walking activity, defined as the percentage of time the predators spent walking, was not dependent on leaf hair density of the cultivars. The rate of encounter with prey was inversely related to trichome density. Predation rate of adult female *P. persimilis* was affected negatively by trichome density when prey density was low. However, at high prey density such an effect was absent, because the predation rate is not limited by the rate of encounter with prey at high prey density.

The results of these experiments showed that the effect of trichome density on the predator-prey dynamics between *T. urticae* and *P. persimilis* on gerbera plants will be dependent on the density in which *T. urticae* occurs on these plants. This will be one of the subjects of study in chapter 8.

CHAPTER 6: SPIDER MITE-INDUCED VOLATILES

Many host plant species produce volatiles when herbivores damage the leaves. Natural enemies of the herbivores can use these volatiles to locate plants that are infested with their prey or hosts (see Vet and Dicke, 1992 and Dicke, 1994 for reviews). Such volatiles also are important in the interaction between *T. urticae* and *P. persimilis* (Sabelis and van de Baan, 1983; Sabelis *et al.*, 1984; Dicke and Sabelis, 1988; Dicke *et al.*, 1990a,b 1998; Sabelis and van der Weel, 1994; Sabelis and Afman, 1994). Plants of several species produce volatiles when spider mites damage their leaves. *Phytoseiulus persimilis* is attracted to these volatiles (Dicke and Sabelis, 1988). Once the predators have found spider mite-infested leaves, they stay there until all prey is exterminated, which is most likely the result of attraction of the predators to volatiles from the spider mite patch and arrestment by these volatiles (Sabelis and van de Meer, 1986; Sabelis and Afman, 1994).

Arrestment of predatory mites in a spider mite patch has a major influence on the population dynamics of both the prey and their predators, as demonstrated by van Baalen and Sabelis (1995) in simulation studies. If all the predators are arrested in the prey patch until all prey is exterminated, the prey population reaches much lower numbers than if part of the predators disperses from the patch before prey extermination. In addition, the prey population is exterminated faster when predator dispersal is absent. This illustrates the importance of the response of *P. persimilis* to volatiles from spider mite-damaged leaves.

We have investigated whether gerbera leaves with spider mite damage also produce volatiles that attract *P. persimilis*. In contrast to undamaged gerbera leaves that had a low production of volatiles, spider mite-damaged gerbera leaves produced many volatiles in large quantities. However, *P. persimilis* from our standard culture on Lima bean leaves with *T. urticae* was not attracted to these volatiles.

The response of *P. persimilis* to spider mite-induced volatiles from certain plant species can be enhanced by starvation (Sabelis and Dicke, 1985; Dicke and Sabelis, 1988). In addition, Takabayashi et al. (1994a) found indications that the response could also be enhanced by experience with the volatiles. We investigated whether starvation or experience also influenced the response of *P. persimilis* to volatiles from spider mite-damaged gerbera leaves. Predators that were starved for 24 hours still did not respond to the volatiles from gerbera leaves with spider mite damage. In contrast, predators that had been exposed to the volatiles for 6 days, responded strongly.

CHAPTER 7: CULTIVAR DIFFERENCES IN THE PRODUCTION OF SPIDER MITE-INDUCED VOLATILES AND ATTRACTIVENESS OF THE VOLATILES TO *P. PERSIMILIS*

Plant cultivars can differ in the production of herbivore-induced volatiles and the attractiveness of the blends to natural enemies of the herbivores can differ as well (Dicke *et al.*, 1990a; Takabayashi *et al.*, 1991; Loughrin *et al.*, 1995; Loughrin *et al.*, 1996; Takabayashi and Dicke, 1996; Souissi, 1999). Therefore, we have investigated whether the chemical composition of the spider mite-induced volatiles differs between gerbera cultivars. Furthermore, we determined whether cultivar differences exist in attractiveness of the spider mite-induced volatiles to *P. persimilis.*

Comparing cultivars in production and attractiveness of spider-mite induced volatiles is not as easy as it as it may seem. Results presented in chapter 3 showed that gerbera cultivars differ largely in resistance to *T. urticae.* As a consequence, placing a standardised number of spider mites on gerbera leaves for a standardised amount of time will not result in equal damage on all cultivars, because the consumption by spider mites differs per cultivar.

We present a method to compare cultivar differences in the production and attractiveness of volatiles, based on the assumption that a higher amount of damage leads to higher production of volatiles and and higher attractiveness. With this method, we assessed cultivar differences in attractiveness in two-choice olfactometer tests, in which the predators were given the choice between the spider mite-induced volatiles of cultivar Sirtaki and the spider mite-induced volatiles of the cultivars Rondena, Fame or Bianca. In addition, the chemical composition of the blends of these four cultivars was determined.

The predators preferred the spider mite-induced volatiles of cultivar Rondena over those of cultivar Sirtaki. Similarly, the volatiles of cultivar Bianca were preferred over those of Sirtaki, but this was not significant. The volatiles of Sirtaki and Fame leaves with spider mite damage did not differ in attractiveness to *P. persimilis.*

The blend emitted by spider mite-damaged Sirtaki leaves consisted of a much lower relative amount of terpenes than the blends of the other three cultivars, which was mostly due to a low production of *cis*-α-bergamotene, *trans*-α-bergamotene, *trans*-β-bergamotene and *(E)*-β-farnesene. Furthermore, the production of *(E)*-β-ocimene and linalool was lower by Sirtaki and Fame leaves than by leaves of Bianca and Rondena. These two compounds attract *P. persimilis* when offered in a Y-tube olfactometer (Dicke *et al.*, 1990b). Whether the poor attractiveness of the spider mite-induced volatiles of Sirtaki and Fame is related to the limited production of these compounds should be investigated in future studies.

CHAPTER 8: CONSEQUENCES OF CULTIVAR EFFECTS ON *P. PERSIMILIS* FOR PREDATOR-PREY DYNAMICS ON GERBERA

In chapter 3 to 7 we have described several host plant characteristics of gerbera that differ between cultivars. Furthermore we have determined the effect of these characteristics on *P. persimilis*. But we have not yet determined the importance of each of these effects for the predator-prey dynamics on gerbera plants. This is the topic of chapter 8.

We have performed a population experiment with *T. urticae* and *P. persimilis* on gerbera plants of two cultivars, i.e. Bianca (218 hairs/cm^2) and Bourgogne (630 hairs/cm^2). We introduced spider mites and predatory mites on the plants in a predator: prey ratio of 1: 40. Subsequently, we have monitored dynamics of the spider mite and predator populations on both cultivars. We compared the predator-prey dynamics on both cultivars with predictions of a model that was previously developed to describe the predator-prey dynamics of spider mites and predatory mites on plants (Diekmann *et al.*, 1988; Janssen and Sabelis, 1992; Sabelis, 1992; van Baalen and Sabelis, 1995).

The population growth rates of the spider mites and their predators are important input parameters in this model. The values of these parameters were derived from chapters 3 & 4. We have simulated the effect of host plant resistance to spider mites by varying the value for the spider mite population growth rate. The effect of this parameter on the predator-prey dynamics was negligible. This indicates that extermination of spider mites by their predators is not faster on resistant cultivars than on susceptible cultivars. Therefore, the advantage of resistant cultivars for biological control does not lie in the effect *after* introduction of predators, but in the time *before* introduction of predators becomes necessary. Hence, the moment of predator introduction will be postponed on resistant cultivars.

The model is based on the assumption that the predator population growth rate is maximal, which indicates that prey density is not a limiting factor. Another assumption is that predators do not disperse until after prey extermination. The maximal population growth rate of *P. persimilis* is the r_m that was assessed in chapter 4 at high prey density. We determined whether the assumption of maximal predator population growth rate was valid, by estimating the predator population growth rates from the monitored numbers of predators on both cultivars. In addition, the predator-prey dynamics were compared with model predictions when the predator r_m of 0.43/day was used as input value.

The model predicted extermination of spider mites on both cultivars in four days after predator introduction while this took two weeks in reality. This indicates that at least one of the assumptions in the model simulations is not valid for the predator-prey dynamics on gerbera. The population growth rates of the predators appeared to be much lower than the r_m of 0.43/day, they were 0.285/day on Bourgogne (730 hairs/cm^2) and 0.326/day on Bianca (100 hairs/cm^2). Possibly the spider mite densities on gerbera plants were lower than used in the experiments of chapter 4 in which the r_m of the predators was assessed. These spider mite densities may have been limiting for the population growth rates of the predators. However, it is also possible that the low population growth rates are caused by dispersal of predators before all prey is exterminated.

The difference in predator population growth rates on the two cultivars indicates an effect of leaf hair density because this growth rate is the highest on the cultivar with the lowest leaf hair density. It is, however, also possible that the difference in population growth rates is the result of a difference in predator dispersal. The response of the predators to the spider mite-induced volatiles from the two cultivars may not be equal, which may have led to a difference in predator dispersal. The exact cause of the low predator population growth rates and the difference in these growth rates on the two cultivars will have to be investigated in future studies.

In conclusion, host plant resistance to spider mites in gerbera has little effect on the time in which *T. urticae* is exterminated by *P. persimilis,* but does prolong the time before predator introduction becomes necessary. The population growth rate of *P. persimilis* is not maximal on gerbera, which may be the result of low prey densities, or dispersal of predators before prey extermination. The population growth rate of the predators is highest on the cultivar with the lowest leaf hair density. This may be caused by a negative effect of leaf hair density on the predation rate of the predators. However, it is also possible that predator dispersal differs on the two cultivars as a result of unequal attractiveness of the spider mite-induced volatiles.

DISCUSSION

The results of this PhD study show that the dynamics between *P. persimilis* and *T. urticae* on gerbera plants may be affected by host plant characteristics that differ between cultivars. The effects of host plant resistance to *T. urticae,* leaf hair density and the production of spider mite-induced volatiles on these dynamics were investigated. We demonstrated that host plant resistance does not affect the dynamics between *T. urticae* and *P. persimilis,* but does prolong the time before predator introduction becomes necessary. In addition, we found indications that cultivar differences in leaf hair density and spider mite-induced volatiles are important for the predator-prey dynamics on gerbera. Below, we will discuss the results presented in this thesis which will be done for each host plant characteristic separately.

Host plant resistance to *<u>T. urticae</u>*

The differences between gerbera cultivars in host plant resistance to *T. urticae* are large; the r_m ranges from 0.09/day on the most resistant cultivar to 0.23/day on the most susceptible one. In studies on susceptible and resistant cucumber varieties, the r_m of *T. urticae* ranged from 0.218/day to 0.272/day (Sabelis, 1985). Hence, the r_m of *T. urticae* is much lower on the most resistant gerbera cultivar than on a cucumber variety that was classified as resistant. This offers good perspectives to breed for host plant resistance in gerbera.

The spider mites were able to adapt partly to the host plant resistance in gerbera. Fast adaptation of *T. urticae* to unsuitable host plants was also found in other studies (Gould, 1979, Fry, 1989; Bruin *et al.,* 1992). However, after the spider mites had been reared for half a year on a resistant cultivar the difference in r_m on the most susceptible and most resistant gerbera cultivar was still larger than the above mentioned difference in r_m on susceptible and resistant cucumber varieties without adaptation of *T. urticae.* In addition, the r_m of *T. urticae* that had been reared for half a year on the resistant gerbera cultivar was 0.208/day, which is still lower than the r_m on the resistant cucumber variety. Hence, even after adaptation of *T. urticae,* the cultivar that was least suitable for *T. urticae* can still be classified as resistant.

The advantage of the use of resistant gerbera cultivars lies in postponing the moment that additional methods of spider mite control are necessary. As an example, on the most suitable gerbera cultivar a population of 100 spider mites will increase to 1000 individuals in 10 days, whereas this will take more than 25 days on the most resistant cultivar. However, even on the most resistant cultivar it will eventually be necessary to apply other methods of spider mite control, especially since the r_m of *T. urticae* on resistant cultivars may increase due to natural selection.

Compatibility of host plant resistance and biological control

We found no effect of host plant resistance to spider mites in gerbera on the r_m of *P. persimilis*. Therefore, biological control of spider mites should be possible as additional method of spider mite control on resistant gerbera cultivars. In contrast to what is commonly assumed, the speed at which spider mites are exterminated by *P. persimilis* is not higher on resistant cultivars than on susceptible cultivars. At first this seems to be an argument against breeding for resistance to spider mites in any crop. However, the advantage of resistant cultivars lies more in the time *before* predator introduction is necessary, so, to delay the moment that additional control methods are necessary, than in the success of biological control *after* predators are introduced.

Two possible negative indirect effects of host plant resistance on biological control have not been investigated in this PhD study because of time constraints. First, host plant resistance may lead to a low level of damage on leaves of resistant plants, which may result in a low production of spider mite-induced volatiles. Attraction of predators to volatiles from spider mite-damaged leaves may, therefore, be lower on resistant cultivars than on susceptible cultivars. If this leads to a higher predator dispersal rate on resistant cultivars, the spider mite population will reach higher numbers and time until spider mite extermination will be longer on resistant cultivars than on susceptible ones. This will result in a higher amount of spider mite damage on the plants. An example that such a phenomenon may indeed exist is given by Souissi (1999), who found cultivar differences in attractiveness of volatiles from cassava plants to a parasitoid of the cassava mealybug. However, the most attractive cultivars were also the most susceptible ones. Therefore, the differences in attractiveness may have been the result of differences in the amount of damage on the leaves.

The second possible indirect effect of host plant resistance is related to the density of *T. urticae* on gerbera. This density may be lower on resistant cultivars than on susceptible ones, which may result in lower population growth rates of *P. persimilis*. This will also lead to higher spider mite numbers, later extermination of spider mites and more damage on resistant cultivars. The possibility of such an effect was pointed out by Yanes and Boethel (1983) for the effectivity of a parasitoid of the soybean looper on a resistant soybean variety. Besides a negative effect of resistance on parasitoid survival, the availability of hosts was much lower on the resistant variety. This may result in a negative effect of host plant resistance in soybean on biological control of the soybeen looper.

The results of the population growth experiment of chapter 8 showed that the measured population growth rates of *P. persimilis* on the two gerbera cultivars were lower than the r_m of the predators estimated from life-history studies on gerbera. This could be explained by dispersal of predators from the spider mite-damaged leaves, or prey densities on the gerbera plants that were limiting the predator population growth rates. Both cultivars from the population growth experiment had a high level of resistance to *T. urticae*. Possibly, the low predator population growth rates were caused by one of the above mentioned indirect effects of resistance. The exact cause of the low predator population growth rates requires further study.

Leaf hair density

The walking speed of *P. persimilis* and the rate of encounter with prey are negatively related to the density of leaf hairs on the undersurface of gerbera leaves. This results in a negative effect of leaf hair density on the predation rate of the predators, when the rate of encounter with prey is limiting the predation rate. The fact that the predator population growth rates in the population experiment of chapter 8 are lower than the r_m found in chapter 4 indicates that it is indeed possible that the rate of prey encounter limits the predation rate of *P. persimilis.* However, these low population growth rates could also be explained by dispersal of part of the predators from the plants with spider mites. Future studies are necessary to determine whether the low predator population growth rates are caused by low encounter rates with prey or by dispersal of predators.

The difference in predator population growth rates in the population experiment of chapter 8 supports our hypothesis that leaf hair density affects the dynamics between *P. persimilis* and *T. urticae* on gerbera plants. However, since we used only two gerbera cultivars, it is uncertain whether the difference in predator population growth rates is really caused by the cultivar difference in trichome density. In future studies the population experiments of chapter 8 may be repeated with more gerbera cultivars that differ in leaf hair density, to determine whether the effects of leaf hairs on the predator-prey dynamics are consistent.

To our knowledge no studies with other host plants than gerbera have been done in which the effects of leaf hair density on the foraging behaviour of *P. persimilis* were investigated. There is a study with tomato plants in which the dispersal of *P. persimilis* from leaf to leaf was found to be hampered by trichomes on the stems of the plants (van Haren *et al.*, 1987). However, whereas the trichomes of gerbera plants are non glandular, tomato plants have glandular trichomes that produce a sticky exudate upon contact with, for example, a predatory mite. Predators run a high risk to become trapped in the exudate, which explains the negative effect of tomato trichomes.

Spider mite-induced volatiles

Gerbera leaves with spider mite damage produce a large amount of volatile compounds in high quantities. Like in other plant species the major part of the volatiles consists of terpenes (Dicke and Sabelis, 1988; Dicke *et al.*, 1990b; Turlings *et al.*, 1990, 1993, Dicke, 1994; Takabayashi *et al.*, 1991, 1994b). Furthermore, of the four compounds that are known to attract *P. persimilis* when offered in a Y-tube olfactometer (Dicke *et al.*, 1990b), three are present in the blend of spider mite-damaged gerbera leaves, the terpenoids linalool, *(E)*-β-ocimene and 4,8-dimethyl-1,3 *(E)*, 7-nonatriene.

Predatory mites from our standard culture on Lima bean leaves with *T. urticae* are initially not attracted to the blend of gerbera leaves with spider mite damage, which cannot be explained by a low production of volatiles by gerbera or absence of active compounds in the blend. Similarly, *P. persimilis* from the same culture initially did not respond to spider mite-induced volatiles from leaves of cucumber, apple and pear. However, the predators could be induced to respond to the apple and pear volatiles by 24 hr of starvation (Sabelis and Dicke, 1985; Dicke and Sabelis, 1988). And the response to the cucumber volatiles could be induced by 7 days of experience with these volatiles (Takabayashi *et al.*, 1994a). The response of *P. persimilis* to volatiles of spider mite-damaged gerbera leaves was not influenced by 24 hr starvation. In contrast, 6 days of experience with the spider mite-induced gerbera volatiles resulted in a strong response of the predators to these volatiles. It seems that the response of the predators to spider mite-induced volatiles from some plant species can be induced by 24 hours of starvation, whereas this does not induce a response to volatiles from other species of plants. Possibly a longer starvation time, or experience is necessary to induce a response to the volatiles from these other host plant species.

The fact that *P. persimilis* does not have a fixed response to the spider mite-induced volatiles of all plant species raises the question how the predators find their prey on different plant species. Most likely, predators that have exterminated a spider mite patch on a certain host plant will try to find a new prey patch by responding to the spider mite-induced volatiles of the same plant species. If it takes long before they find such a prey patch, their starvation level will increase, which will induce them to respond to spider mite-induced volatiles of more plant species. If they coincidentally arrive in a prey patch on another host plant species, the response to the volatiles of this plant species will increase due to experience. If they now exterminate the prey patch, they may find ones on the same new plant species because they now respond to the volatiles of this species.

Cultivar differences in production and attractiveness of spider mite-induced volatiles

The spider mite-induced volatiles of gerbera cultivars differ in attractiveness to *P. persimilis.* The predators preferred the volatiles of cultivars Rondena and Bianca over those of cultivars Sirtaki and Fame. Comparing the chemical composition of the blends of these four cultivars gave indications that the attractiveness of the blends may partly be related to the production of linalool and *(E)*-β-ocimene. These two compounds attract *P. persimilis* when offered in a Y-tube olfactometer (Dicke *et al.*, 1990b). Furthermore, Loughrin (1996) found a relation between the production of linalool by several apple cultivars and their attractiveness to the Japanese beetle *Popillia japonica.* However, he did not find indications that *(E)*-β-ocimene was also involved in attractiveness. It would be interesting to further investigate whether cultivar differences in attractiveness are indeed related to differences in the production of these two compounds and which other compounds are important.

The cultivar differences in attractiveness of the spider mite-induced gerbera volatiles may have large consequences for the amount of damage caused by spider mites before a spider mite patch is exterminated by *P. persimilis* on the different cultivars. It may be that the less attractive volatile blends of Sirtaki and Fame lead to a lower arrestment response of the predators on these cultivars. The percentage of predators that leaves a spider mite patch before all prey is exterminated may be larger on these two cultivars than on cultivars with a more attractive blend. Dispersal of predators from the prey patch results in much higher *T. urticae* numbers on the plants and postpones prey extermination (van Baalen and Sabelis, 1995). Therefore, *T. urticae* may cause much more damage on cultivars that produce a blend with low attractiveness to *P. persimilis.*

On the other hand, it is possible that the production of volatiles by all four cultivars is above a threshold above which no dispersal of *P. persimilis* takes place until prey extermination. If this is the case, cultivar differences in attractiveness of the volatiles will not lead to cultivar differences in the speed at which a spider mite patch is exterminated. Therefore, it will be necessary to investigate in future studies whether the arrestment response of the predators is related to the attractiveness of the blends.

CONCLUSIONS

We have shown that plant cultivars can differ in host plant characteristics that affect the dynamics between *T. urticae* and *P. persimilis*. In addition, we have described how the dynamics between the spider mites and their predators are affected by different host plant characteristics. In summary, the main conclusions that can be drawn from the work described in this thesis are that:

- Large differences exist between gerbera cultivars in host plant resistance to *T. urticae.*
- There are no indications the success of *P. persimilis* as a biological control agent is affected on gerbera cultivars that are resistant to *T. urticae.*

- The walking speed of *P. persimilis*, the rate of encounter with *T. urticae* and possibly also the population growth rate of the predators are affected negatively on gerbera cultivars with a high density of leaf hairs on the undersurface of the leaves.
- Gerbera plants with spider mite damage produce a large amount of volatiles in high quantities, but *P. persimilis* only responds to these volatiles after 6 days of experience.
- Gerbera cultivars differ in attractiveness of the volatiles that are produced by leaves with spider mite damage. The cultivars with the lowest attractiveness had the lowest production of *(E)*-β-ocimene and linalool. Whether the cultivar differences in attractiveness will result in cultivar differences in the speed at which a spider mite patch will be exterminated by *P. persimilis* has to be investigated in future studies.
- The population growth rate of *P. persimilis* is not maximal on gerbera leaves. This may be the result of low densities of spider mites on gerbera leaves or of a low response of *P. persimilis* to the volatiles from spider mite-damaged leaves.

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CHAPTER 3

INTRINSIC RATE OF POPULATION INCREASE OF THE SPIDER MITE *TETRANYCHUS URTICAE* ON THE ORNAMENTAL CROP GERBERA: INTRASPECIFIC VARIATION IN HOST PLANT AND HERBIVORE

SUMMARY

Eight cultivars of the ornamental crop *Gerbera jamesonii* Bolus (Asteraceae) were compared in host plant suitability for the two spotted spider mite *Tetranychus urticae* Koch (Acarina: Tetranychidae). This was done by determining the intrinsic rate of population increase, r_m, of spider mites on leaf discs of plants from each of the cultivars. Large differences in r_m values were found, ranging from 0.088/day on cultivar Bianca to 0.242/day on cultivar Sirtaki. This variation in r_m was mainly caused by differences in developmental time of the spider mites.

We assessed the performance of spider mites on young and old leaves of the two gerbera cultivars Bianca and Sirtaki. On Sirtaki the spider mites had a shorter developmental time and higher peak oviposition rate on young leaves than on old leaves. However, on Bianca such an effect was not found.

We also determined the performance of two spider mite strains on the resistant gerbera cultivar Bianca. We compared the r_m of a strain that had bean reared on this cultivar for approximately half a year with the r_m of a strain that was reared on bean. The r_m of the strain that was reared on cultivar Bianca increased to 0.208/day, which is however still substantially lower than the r_m on the susceptible cultivar Sirtaki.

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INTRODUCTION

The spider mite *Tetranychus urticae* Koch is an important pest in greenhouses. A spider mite population can expand rapidly with up to 40% per day (Shih *et al.*, 1976). This explosive population growth usually ends abruptly due to overexploitation of the host plant or local extermination by natural enemies. Spider mites that colonise new host plants start a new fast growing population. Hence, *T. urticae* populations are generally longer in the exponential growth phase than in the phase in which the population approaches the carrying capacity. This makes *T. urticae* typically r_m-selected (Caswell and Hastings, 1980; Sabelis, 1985).

A rapid population increase can be achieved by a high reproduction rate or a short developmental time. However, the relative contribution of those two parameters is not equal. How much a change in either parameter affects the r_m depends on the actual rate of population increase. (Lewontin, 1965, Caswell and Hastings, 1980). With the models from Caswell and Hastings (1980), one can demonstrate that for r_m values higher than approximately 0.1/day a change in developmental time has a larger effect on the population growth rate than an equal proportional change in oviposition rate. For lower r_m values the opposite is true. On suitable host plants, spider mites have an r_m between 0.22 and 0.34/day (Sabelis, 1985; 1991). Hence, for spider mites, changes in developmental time are more important for the population growth rate than similar changes in oviposition rate (Sabelis, 1985).

One of the methods to control spider mites is breeding for host plant resistance. Standard laboratory tests to screen for host plant resistance usually consist of determining oviposition rate or amount of damage on breeding lines (e.g. de Ponti, 1977; Johnson *et al.*, 1980; Gimenez-Ferrer *et al.*, 1993; Aguilar et al. 1996). However, as explained above, the r_m of spider mites is more sensitive to effects on developmental time than to changes in oviposition rate. Therefore, one should not overlook effects on the developmental time.

Breeding for host plant resistance to spider mites is applied in a small number of vegetable crops and cotton (de Ponti, 1985). In ornamentals aesthetic damage is not tolerated, which make alternative methods of pest control difficult. In these crops the use of pesticides is usually the only option (van de Vrie, 1985). The ornamental crop gerbera (*Gerbera jamesonii* Bolus) is a positive exception, since gerbera cut flowers do not have leaves at their stem and leaves are not harvested. This allows some damage on the leaves, without exceeding the economic damage threshold (van de Vrie, 1985). Since spider mites only damage leaves while flowers remain undamaged, alternative methods of spider mite control are possible in gerbera.

Gerbera is an economically important crop of which a large number of cultivars is grown commercially. At this moment, selection for gerbera cultivars mainly focusses on colour and shape of flowers and not on host plant resistance. However, cultivars differ largely in plant characteristics that have not been selected for, such as leaf shape and leaf hair density (Klein *et al.*, 1996; Sütterlin *et al.*, 1997; Krips *et al.*, 1999). Gerbera cultivars may differ as well in suitability for spider mites, which would offer good perspectives for resistance breeding. However, since spider mites can rapidly adapt to unsuitable host plants (Gould, 1979; Fry, 1989, Bruin *et al.*, 1992), resistance to spider mites in gerbera may not be durable.

Within plants from one gerbera cultivar, differences in suitability for spider mites may exist as well. Leaf age may affect suitability for spider mites. Kielkiewicz and van de Vrie (1990) found lower spider mite densities on young leaves of chrysanthemum than on old leaves. The opposite was found by Rodriguez et al (1983) for spider mites on soybean.

The objective of this study was to determine whether gerbera cultivars differ in suitability for spider mites and we assessed the r_m of spider mites on eight cultivars. In addition, we determined whether leaf age affects the r_m of spider mites. Furthermore, we investigated whether spider mites are able to adapt to an unsuitable cultivar.

MATERIAL AND METHODS

Plant material

Gerbera plants were obtained from commercial breeding companies, Prego Rijsenhout bv (Rijsenhout, The Netherlands) and Terra Nigra bv (De Kwakel, The Netherlands). They were subsequently grown in a greenhouse at 20-30°C, 50-70 % RH and at least 16 h light per day. Plants used in experiments were between six and twelve months old and cultivars used were Bianca, Bourgogne, Donga, Estelle, Fame, Rondena, Sardana and Sirtaki. In experiment 1 we used the youngest fully unfolded leaves and classified these as leaves of "middle"-age. To assess effects of leaf age (experiment 2), "young" and "old" leaves were used. Young leaves were not yet fully unfolded. Old leaves had a horizontal position in the plant and were tougher and less glossy than young and middle leaves. In experiment 3, "middle" leaves were used.

Spider mites

Gerbera leaves (cv. Sundance) that were well-infested with spider mites (*Tetranychus urticae*) were collected in a commercial gerbera greenhouse at Mijdrecht, The Netherlands, in the spring of 1994. With several hundred spider mites from these leaves a stock colony was started on gerbera cultivar Sirtaki. By transferring Sirtaki leaves that together contained hundreds of spider mites of all stages, subcolonies were started on Lima bean and the gerbera cultivar Bianca in December 1995. All subcolonies were kept in a greenhouse at 20-30°C, 50-70 % RH and at least 16 h light per day . The spider mite populations in the subcolonies were never below several hundred individuals We used different subcolonies for different experiments and we will descibe below per experiment which ones were used. Three days prior to the start of all experiments, adult female spider mites were transferred to the leaf material on which the r_m value was going to be determined. This was done to ensure that eggs with which we started the experiments were produced from food that was ingested from the leaf type on which we determined the r_m.

Experiment 1: Suitability of gerbera cultivars for spider mites

Experimental procedure

Spider mites were used from the subcolony on Sirtaki. From leaves of each cultivar leaf discs of 2 cm in diameter were cut which were placed upside down on wet cotton wool. On each leaf disc one spider mite egg was placed. Eggs differed maximally 8 hours in age and were allowed to hatch after which the developmental time, age specific survival and age specific reproduction were recorded. This was done once a day during the start and the end of the experiment and two to three times a day at the onset of reproduction. Furthermore, we determined the secondary sex-ratio on six of the gerbera cultivars. The experiment took place in a climate cabinet at 25 ± 0.5 °C, ≥ 65% RH and a photoperiod of L16:D8.

In seven days most of the eggs had hatched after which the mites and remaining eggs were transferred to fresh leaf discs for the first time. From this moment onwards, leaf discs were replaced every Monday, Wednesday and Friday. After 25 days the experiment was terminated, but one replicate on the cultivars Sirtaki , Rondena and Sardana was terminated after 21 days. For this specific replicate we assumed the reproduction after 21 days to be zero. This results in an underestimation of the r_m which is however negligable since reproduction late in life has a much smaller effect on r_m than reproduction early in life (Lewontin, 1965).

Calculation of r_m values

We calculated r_m values using the equation given by Carey (1993): $\sum \exp(-r_m x) l_x n_x s_x = 1$, where x is the age, l_x is the age specific survival, n_x is the age specific reproduction and s_x is the proportion of females in the offspring of a female at age x. According to Carey (1993), pivotal age, which is the average of age x and x-1, was used in the calculation of r_m values. For each pivotal age class the average of l_x and l_{x-1} was taken as age specific survival. We assumed that the proportion of females was not affected by the age of the mother.

Mites that dispersed from the leaf discs to the surrounding wet cotton wool were replaced on the leaf discs only once. If they were found trapped in the wet cotton wool for a second time, they were discarded. These data are presented as the percentage of loss by dispersal in 21 days. Only data obtained from these mites before they walked into the wet cotton wool for the second time were used in the analysis. Since it is unknown whether these mites would have died if they would not have dispersed, they were not used for determining the age-specific mortality. An initial number of 30 to 45 eggs per cultivar was used and the experiments were repeated until data for a total number of at least 15 adult females were obtained.

Experimental groups

The experiment was divided into three major groups in which cultivar Sirtaki was used as a reference cultivar. The first group consisted of the cultivars Sirtaki, Donga, Estelle and Fame. Data gathered in this group on cultivar Sirtaki will be referred to as data for Sirtaki 1. The second group consisted of Sirtaki (=Sirtaki 2) , Rondena and Sardana. The third group consisted of Sirtaki (=Sirtaki 3), Bianca and Bourgogne.

Experiment 2: Suitability of young and old leaves

Following the same procedure as described for experiment 1, the r_m of spider mites on young and old leaves of the cultivars Sirtaki and Bianca was calculated (see paragraph *Plant material* for our definition of "young" and "old" leaves). For this experiment we used the spider mite subcolony on Lima bean.

Experiment 3: Ability of spider mites to adapt to an unsuitable cultivar

To determine whether spider mites adapt to an unsuitable cultivar, three colonies were used. The colony on Lima bean will be referred to as the NA-population (Not Adapted). The colonies on Sirtaki and Bianca will be called AD-populations (ADapted). The r_m of the NA-population was determined on Sirtaki and Bianca. The r_m of the AD-population on Bianca was determined on Bianca and the r_m of the AD-population on Sirtaki was determined on Sirtaki. Experiments started 5 months after the AD-populations were started, which corresponds with approximately 10 generations. The experimental procedure and calculation were identical to that described for experiment 1.

Egg mortality

Because eggs could easily be damaged during transfer to the leaf discs, egg mortality, which represents the age-specific mortality between day 0 and day 1, was measured in separate experiments. To determine egg mortality in experiment 1, approximately 60 adult females were placed on leaf discs from leaves of the respective cultivars that were placed upside down on wet cotton wool. After 8 h the adult females were removed and the eggs were counted and kept at 25 ± 0.5 °C, ≥ 65% RH and a photoperiod of L16:D8. The fraction of eggs that hatched was determined. Eggs that did not hatch within 10 days were assumed to be dead.

To determine egg mortality in experiment 3, approximately 25 adult females were placed on pieces of leaves that were placed upside down on wet cotton wool. After 24 h the adult females were removed and the eggs were counted and kept at 23 ± 1 °C, ≥ 65% RH and a photoperiod of L16:D8. The fraction of eggs that hatched within ten days was determined.

As we did not find any correlation between r_m and egg mortality in experiment 1 and 3, the egg mortality was not determined in a separate experiment for experiment 2. We used the values for Sirtaki and Bianca obtained in experiment 1 instead.

Statistical analysis

We subjected the data to statistical analysis on egg-to-egg developmental time and peak oviposition rate. For the developmental time in experiment 1 we used a Kruskal-Wallis test and a nonparametric multiple comparison test with Bonferroni correction (Siegel and Castellan, 1988). The two spider mite populations in experiment 3 were compared with Wilcoxon tests. Since the experimental approach of experiment 2 required a two-way analysis, it was not possible to use a non-parametric statistical method. Therefore, a two-way analysis of variance was used with subsequent contrasts. Since the data for developmental time were not normally distributed, while their reciprocals were, the latter were used in the ANOVA.

The peak oviposition rate was defined as the mean oviposition over 24 h from day three to day four after the average start of reproduction. For these data in experiment 1 we used a Kruskal-Wallis test and a nonparametric multiple comparison test with Bonferroni correction (Siegel and Castellan, 1988). For experiment 3 Wilcoxon-tests were used for both cultivars. The experimental approach of experiment 2 required a two-way analysis. Since the data were not normally distributed, we used a generalized linear model with contrasts, using a Poisson distribution, a log-link and a multiplicative scale parameter (Crawley, 1993).

The sex-ratio was analysed with a six by two contingency table on the proportion of females compared to the proportion of males on six cultivars. We assessed relations between r_m and either % egg hatch or % loss by dispersal in experiment 1 with analyses of covariance. On % egg hatch and % loss by dispersal angular transformations were performed.

RESULTS

Experiment 1: Suitability of gerbera cultivars for spider mites

The age specific fecundity (total eggs/ total females/ day) of *T. urticae* on gerbera is clearly affected by gerbera cultivar (Fig. 1a). The shortest developmental time was found in the first replicate on Sirtaki, with the absolute start of reproduction (the moment when the *first* female produces the *first* egg) between day 10 and 11. The reproduction reached a maximum at approximately 9 eggs/female/day. On Bianca the developmental time was longest and first reproduction took place between day 14 and 15. Maximal reproduction was 2.8 eggs/female/day only. The developmental time and maximal reproduction on the other cultivars were in between those two extremes, which is presented in detail in Fig. 1b and 1c.

The average egg-to-egg developmental time of *T. urticae* on the gerbera cultivars is presented in ascending order in Fig. 1b. This variable represents the *average* moment of first reproduction, which was calculated per cultivar over *all* females (in contrast to the *absolute* first reproduction that can be read from Fig. 1a). The developmental time varies considerably with cultivar, ranging from 11.4 days on Sirtaki 1 to 17.0 days on Bianca. No significant variation in developmental time was found in the three replicates on Sirtaki (Fig.1b).

We defined the mean peak oviposition rate, as the *average* reproduction over 24 h between 3 and 4 days after the *average* start of reproduction. As an example, the average start of reproduction on Bourgogne is 15.6 days (Fig. 1b). Hence, as the average peak oviposition rate, the average reproduction between the moments of observation closest to 18.6 days and 19.6 days after the start of the experiment was used. If the observation interval was not exactly 24 h, the number of eggs produced in 24 h was calculated from the reproduction over the time interval. In Fig. 1a, the approximate time-intervals over which we have calculated the oviposition rate are indicated with thick lines. For all cultivars the reproduction was at or was close to its maximum in that time interval. The mean peak oviposition rate varied from 9.1 eggs/female/day on Sirtaki 2 to 1.6 eggs/female/day on Bianca (Fig.1c). No significant differences were found in peak oviposition rate in the three replicates on Sirtaki.

When the data on developmental time on the different cultivars are compared with those on the peak oviposition rate, one finds differences in the consecutive order of the cultivars. However, these differences are all within groups that show no significant differences in peak oviposition rate. Hence, significant differences in developmental time are not contradicted by opposite effects on oviposition rate.

The proportion of females on the first six cultivars tested was not affected by cultivar (six by two contingency table, $P>0.05$) and was 0.77 when data from these cultivars were pooled ($N=391$). We used this value of 0.77 for s_x in all calculations of r_m values, in experiment 1 as well as in experiment 2 and 3. These r_m values vary largely with cultivar with a range from 0.088 day^{-1} on Bianca to 0.242 day^{-1} on Sirtaki 3 (Table 1). The values found in the three replicates on Sirtaki are all within the same order of magnitude. Egg mortality and loss of spider mites by dispersal from the leaf discs show considerable variation, but are both not correlated with r_m values (ANCOVA: $P=0.64$ for egg mortality and $P=0.73$ for loss by dispersal).

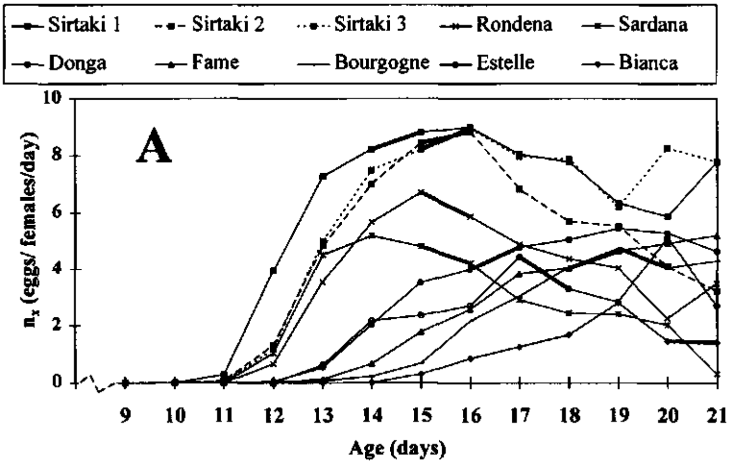

Fig. 1: Age specific reproduction (n_x: per age class total number of eggs divided by the number of living females) (A), egg-to-egg developmental time (B) and reproduction from day 3 to day 4 after the average start of reproduction (C) of *T. urticae* on leaf discs of eight gerbera cultivars. In Fig. 1A the approximate time intervals over which the numbers from Fig. 1C are calculated are indicated with thick lines. Sirtaki 1, 2 and 3 represent three separate determinations on cultivar Sirtaki. Vertical lines represent standard errors, numbers above the vertical lines show the number of replicates. Different letters indicate statistical differences; one or more letters in common means that differences are not significant (Kruskal-Wallis, followed by a non parametric multiple comparison test which includes Bonferroni correction; corrected $\alpha = (0.05/45) = 0.0011$).

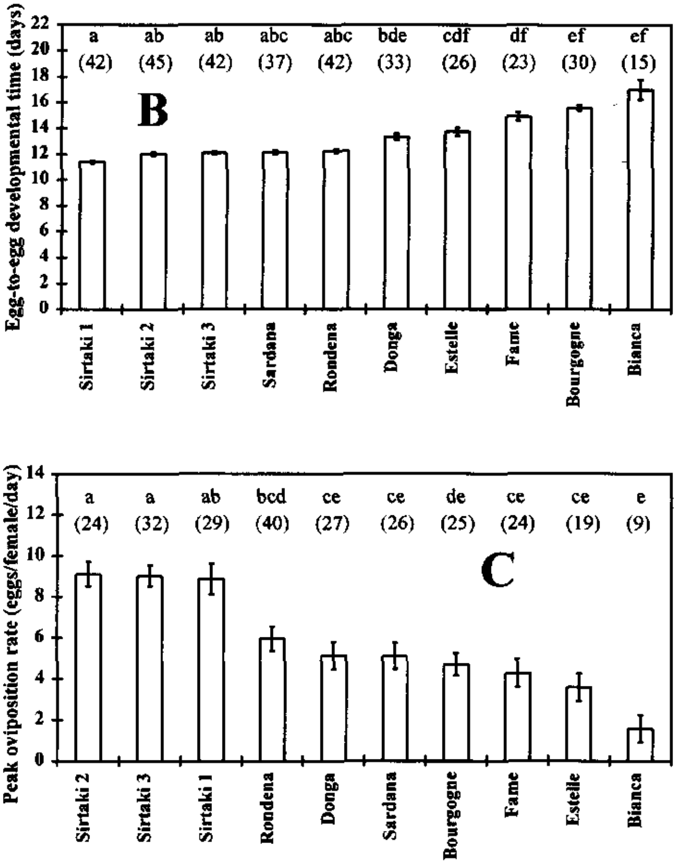

Fig. 1: Continued

Experiment 2: Suitability of young and old leaves

A two-way ANOVA on the reciprocals of the developmental time on young and old leaves of two cultivars reveals a significant effect of cultivar, leaf age and a significant interaction ($P<0.001$ for all three factors). Subsequent contrasts show that on Sirtaki the developmental time of *T. urticae* is significantly shorter on young leaves than on old leaves ($P<0.001$). On Bianca, the same pattern can be seen, which is, however, not significant ($P=0.061$) (Fig. 2).

For analysis of the peak oviposition rate we used a generalized linear model with a Poisson distribution and a log-link. Peak oviposition rate is significantly affected by leaf age ($P<0.001$), cultivar ($P<0.001$) and there is a significant interaction ($P=0.031$). Contrasts show that the peak oviposition rate is significantly higher on young leaves than on old leaves of Sirtaki ($P<0.001$). On Bianca, this parameter is not affected by leaf age ($P=0.31$) (Fig. 2).

Table 1: Intrinsic rate of population increase (r_m) of *T. urticae* on leaf discs of eight gerbera cultivars, the number of females on which r_m values are based, percentage egg mortality and the percentage loss by dispersal in 21 days. Numbers in brackets give the total numbers on which the percentages are based. Sirtaki 1, 2 and 3 represent replicates of the r_m value on the gerbera cultivar Sirtaki.

Cultivar	r_m (day^{-1})	Number of females	% Egg mortality (total eggs)	% Loss by dispersal in 21 days (total)
Sirtaki 3	0.242	43	19 (n=112)	61 (n=64)
Sirtaki 1	0.235	44	11 (n=87)	49 (n=68)
Sirtaki 2	0.225	49	9 (n=93)	42 (n=79)
Rondena	0.205	46	13 (n=165)	33 (n=76)
Sardana	0.191	40	21 (n=117)	32 (n=59)
Donga	0.183	35	6 (n=94)	31 (n=54)
Fame	0.155	27	9 (n=74)	33 (n=54)
Bourgogne	0.144	32	14 (n=80)	49 (n=67)
Estelle	0.132	26	11 (n=42)	41 (n=56)
Bianca	0.088	20	21 (n=76)	49 (n=63)

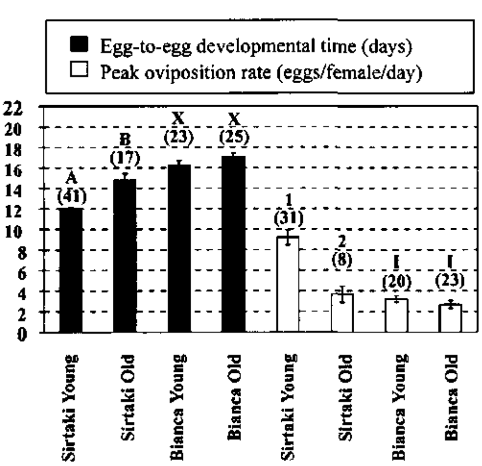

Fig. 2: Egg-to-egg developmental time and reproduction from day 3 to day 4 after the average start of reproduction (peak oviposition rate) of *T. urticae* on leaf discs of young and old leaves of two gerbera cultivars. Vertical lines represent standard errors, numbers above the vertical lines show the number of replicates. For each cultivar separately, different letters or numbers above the bars indicate that differences are significant, On data on developmental time a two-way ANOVA on the reciprocals of the developmental time was performed followed by contrasts (α= 0.05). For data on peak oviposition rate a generalised linear model was used using a Poisson distribution and log-link, followed by contrasts (α= 0.05).

Table 2: Intrinsic rate of population increase (r_m) of *T. urticae* on leaf discs from young and old leaves of the gerbera cultivars Sirtaki and Bianca, the number of females on which r_m values are based and the percentage loss by disperal in 21 days. Numbers in brackets represent the total numbers on which the percentages are based.

Cultivar	r_m (day^{-1})	number of females	% Loss by dispersal in 21 days (total)
Sirtaki Young	0.239	42	52 (n=56)
Sirtaki Old	0.154	18	21 (n=24)
Bianca Young	0.137	26	38 (n=47)
Bianca Old	0.118	25	9 (n=46)

Since the developmental time and the peak oviposition rate of *T. urticae* on Sirtaki are both significantly affected by leaf age, the intrinsic rate of population increase (r_m) is much lower on old leaves than on young leaves of this cultivar (Table 2). On Bianca the r_m on young and old leaves is in the same order of magnitude. However, the r_m of *T. urticae* is lower on both types of leaves of Bianca than on old leaves of Sirtaki.

Experiment 3: Ability of spider mites to adapt to an unsuitable cultivar

No difference in egg-to-egg developmental time was found between the NA and AD-population on Sirtaki (Fig. 3). In contrast, on Bianca the AD-population develops significantly faster than the NA-population (Wilcoxon; P=0.032). This AD-population on Bianca, however, still has a considerably longer developmental time than both populations on Sirtaki.

There are no differences between the NA-populations and the AD-populations in peak oviposition rate on both cultivars, (Wilcoxon; P=0.85 on Sirtaki and P=0.38 on Bianca) (Fig. 3). However, both populations have a lower peak oviposition rate on Bianca than on Sirtaki.

The intrinsic rate of population increase of *T. urticae* on Sirtaki is almost the same for the NA-population and the AD-population (Table 3). On Bianca the r_m of the AD-population is higher than the r_m of the NA-population. However, the AD-population still has a considerably lower intrinsic rate of population increase on Bianca than both populations on Sirtaki.

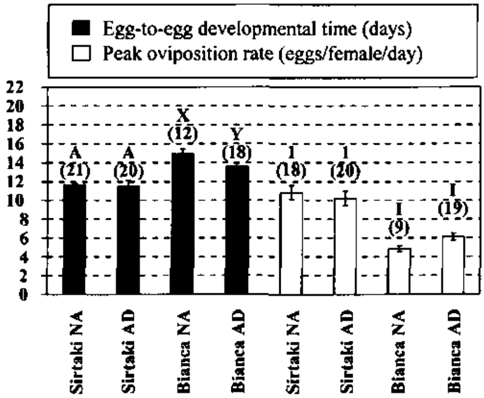

Fig. 3: Egg-to-egg developmental time and reproduction from day 3 to day 4 after the average start of reproduction (peak oviposition rate) of *T. urticae* on leaf discs of two gerbera cultivars. Spider mites originated either from a population on bean (NA)=not adapted) or from a population that was reared for at least five months on the respective gerbera cultivar (AD=adapted). Vertical lines represent standard errors, numbers above the vertical bars show the number of replicates. For each cultivar separately, different letters or numbers above the bars indicate that differences are significant, (Wilcoxon; $\alpha = 0.05$).

Table 3: Intrinsic rate of population increaese (r_m) of *T. urticae* on leaf discs of the gerbera cultivars Sirtaki and Bianca, the number of females on which r_m values are based, egg mortality and the percentage loss by dispersal in 21 days. Numbers in brackets give the total numbers on which the percentages are based. Spider mites came either from a population on bean (NA=not-adapted) or from a population that was kept on the respective gerbera cultivar for at least five months (AD=adapted).

Cultivar	r_m (day^{-1})	Number of females	% Egg mortality (total eggs)	% Loss by dispersal in 21 days (total)
Sirtaki NA	0.252	23	5 (n=134)	42 (n=31)
Sirtaki AD	0.260	21	5 (n=136)	46 (n=35)
Bianca NA	0.168	12	16 (n=67)	33 (n=21)
Bianca AD	0.208	19	5 (n=81)	24 (n=29)

DISCUSSION

Sabelis (1991) gives an extensive review for life history data of tetranychid mites. For *T. urticae* he found a range in r_m values at 25°C from 0.219 to 0.336 day^{-1}. The r_m of 0.242 day^{-1} on our most suitable cultivar falls well within this range and is comparable with the r_m value of *T. urticae* on cotton (Guttierez, 1976). The large differences in our r_m values show that there is substantial variation between gerbera cultivars in suitability for spider mites. Rauwerdink et al. (in Sabelis, 1985) determined the r_m of *T. urticae* on a susceptible and resistant line of cucumber. The r_m on the resistant line was 0.218 day^{-1}, which was 0.064 day^{-1} lower than on the susceptible line. In our studies the r_m of 0.088 day^{-1} on the least suitable cultivar was 0.15 day^{-1} lower than on the most suitable cultivar. Thus, next to the fact that cultivar differences in suitability are large, the unsuitability of some cultivars is pronounced. This may offer possibilities for breeding for resistance to spider mites in gerbera.

Throughout all three experiments we found low variation in r_m values on the cultivar Sirtaki. This indicates that the determination of r_m is repeatable and can thus be used to determine suitability for spider mites. The r_m values on Bianca were much more variable between experiments. Seasonal influences may have affected the outcome of experiments since they were not conducted simultaneously. De Kogel (1997) found a clear difference between two cucumber accessions in resistance to thrips during the winter season. During the summer season this effect was almost absent. The determination of the r_m on Bianca in experiment 1 was carried out during early spring of 1995, while experiments 2 and 3 were performed during the summer of 1996. Another factor that may have affected the outcome of experiments is that we used different batches of plants for different experiments. It is possible that the different batches of cultivar Bianca were not equally resistant to *T. urticae.*

The r_m of spider mites was higher on young leaves of Sirtaki than on old leaves. On Bianca no such an effect of leaf age was found. Similarly, de Kogel (1997) found a negative effect of leaf age on the reproduction of thrips on some cucumber accessions, while on other accessions this effect was absent. Effect of leaf age on suitability for herbivores has been reported for several plant species. Rodriguez et al. (1983) found higher densities of *T. urticae* on young leaves of soybean than on older leaves, possibly because these leaves have a higher nutrient concentration. In contrast, Kielkiewicz and van de Vrie (1990) reported lower densities of *T. urticae* on young leaves of chrysanthemum, which they explain by a higher phenolic content of these leaves.

We have shown that *T. urticae* can adapt to the unsuitable cultivar Bianca. The colony on Bianca had been kept on that cultivar for five months before experiments were started, which corresponds with approximately 10 generations. A significant decrease in developmental time led to an increase in r_m of 0.040 day^{-1}. Several authors have shown that *T. urticae* can adapt to an unsuitable host plant species within approximately 10 generations (Gould, 1979; Fry, 1989; Bruin et al., 1992). This adaptation is fast considering the fact that *T. urticae* is haplo-diploid which means that reproduction is necessary for the production of daughters. However, in our experiments the adapted population had an r_m of 0.208 day^{-1} on Bianca, which is still 0.052 day^{-1} lower than on Sirtaki. Hence, the absolute value of the r_m on Bianca is lower than what Rauwerdink et al. (in: Sabelis, 1985) found for *T. urticae* on resistant cucumber. Moreover, after adaptation the difference in r_m values on Bianca and Sirtaki is still in the same order of magnitude as the difference in r_m of *T. urticae* on susceptible and resistant cucumber (Rauwerdink *et al.,* in Sabelis, 1985). Therefore, Bianca can still be classified as resistant to *T. urticae* after the mites have been able to adapt for five months.

Plant breeders quite often focus on host plant effects on oviposition rate only while breeding for resistance. A close look at our data shows that this may result in overlooking important differences in host plant suitability. A clear example gives a comparison between the cultivars Rondena and Bourgogne in experiment 1. The difference in peak oviposition rate on these two cultivars is approximately 1 egg/female/day only and is not significant. On the other hand, there is a difference of more than three days in developmental time resulting in an 0.061 day^{-1} difference in r_m value, which is considerable. Since the intrinsic rate of population increase is very sensitive for changes in developmental time (Lewontin, 1965; Caswell and Hastings, 1980), we argue that when breeding for resistance to spider mites the effects on developmental time should be incorporated.

In conclusion, gerbera cultivars differ largely in suitability for spider mites and, on one of the two cultivars tested, spider mites perform better on young leaves than on old leaves. Spider mites are able to adapt to an unsuitable cultivar but throughout our experiments the r_m of *T. urticae* remained lower on the unsuitable cultivar Bianca than on the suitable cultivar Sirtaki. However, since the resistance present in some gerbera cultivars is only partial and some adaptation has been found, breeding for resistance alone may not be sufficient for control of spider mites. Possibly a combination with other methods for spider mite control, such as the introduction of natural enemies, will be necessary to keep the spider mite population under the economic damage threshold. However, biological control is not always compatible with host plant resistance (Boethel and Eikenbary, 1986; Van Lenteren and De Ponti, 1990; Gould et al. 1991; Hare, 1992). Therefore, the compatibility of biological control and resistance to spider mites in gerbera is presently investigated.

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CHAPTER 4

COMPATIBILITY OF HOST PLANT RESISTANCE AND BIOLOGICAL CONTROL OF THE TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* IN THE ORNAMENTAL CROP GERBERA

SUMMARY

We investigated the compatibility of host plant resistance to the spider mite *Tetranychus urticae* Koch in the ornamental crop gerbera with the use of the predatory mite *Phytoseiulus persimilis* Athias-Henriot for biological control. We used four gerbera cultivars on which the intrinsic rate of population increase (r_m) of *T. urticae* was previously found to differ largely. We performed life-history studies on those cultivars with *P. persimilis*, and determined whether the developmental time, peak oviposition rate and r_m of the predator was influenced by host plant resistance.

Because the first egg of *P. persimilis* is usually a male and therefore does not contribute to the population growth rate, we discarded the first egg of all predators from our analyses. The egg-to-second-egg developmental time of the predator was approximately 5.2 days. This developmental time was slightly but significantly shorter on gerbera cultivar Fame than on the other three cultivars. However, this shorter developmental time did not lead to a higher r_m of the predators on this cultivar. No cultivar effect was found on the peak oviposition rate, which varied from 5.1 to 5.4 eggs/female/day. On the cultivars used, the r_m of the spider mites was previously found to vary from 0.09 to 0.23 per day. We did not find any effect of spider mite resistance in gerbera on the r_m of the predators, which ranged from 0.43 to 0.45 per day. Hence, the capacity for population increase of *P. persimilis* is not influenced by host plant resistance to its prey.

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INTRODUCTION

Biological control and host plant resistance are often combined in integrated pest management programs and are clasically considered to be compatible. Because the population growth of a pest species is low on resistant plants, one commonly assumes that resistance will increase the capacity of a biological control agent to keep the pest population under the economic damage threshold. However, this assumption is not always valid (Bergman and Tingey, 1979; Boethel and Eikenbary, 1986; Hare, 1992).

A biological control agent is influenced by several host plant characteristics, which may result in a lower performance of natural enemies on resistant plants (Bergman and Tingey, 1979; van Lenteren and de Ponti, 1990; Dicke 1996, 1998). As early as in 1942, Flanders described negative effects of secondary plant chemicals on parasitic Hymenoptera, which showed that toxic compounds that are responsible for resistance may affect the natural enemies of herbivores as well. Furthermore, herbivores may accumulate toxic compounds while feeding on resistant plants and this may make them unsuitable as prey or hosts for natural enemies (De Moraes and McMurtry, 1987; Rogers and Sullivan, 1986). Literature reviews on the combined use of host plant resistance and biological control give several examples of compatibility. However, many examples of incompatibility are found as well (Boethel and Eikenbary, 1986; van Lenteren and de Ponti, 1990; Gould *et al.*, 1991; Hare, 1992).

Gerbera jamesonii Bolus is among the ten economically most important ornamentals grown in greenhouses in the Netherlands. One of the main pest species on gerbera is the spider mite *Tetranychus urticae* Koch (Nucifora and Calabretta, 1986). Gerbera cultivars differ largely in resistance to *T. urticae* (Krips *et al.*, 1998). The mechanism of this resistance is not known, but the large effects on the herbivores offer potential for resistance breeding. However, although the population growth rate of *T. urticae* is low on the most resistant cultivars, the spider mites will still exceed the economic damage threshold if left undisturbed. Therefore, gerbera growers will remain dependent on other methods of pest control, such as the introduction of the predatory mite *Phytoseiulus persimilis* Athias-Henriot, which is commonly used for biological control of spider mites in many greenhouse crops.

Biological control of *T. urticae* with *P. persimilis* may not be equally successful on different gerbera cultivars. The walking speed of *P. persimilis* is lower on cultivars with a high trichome density on the undersurface of the leaves, which leads to a lower predation rate when prey density is low (Kleijn *et al.*, 1997; Krips *et al.*, 1999). Most likely the trichomes have a mechanical effect on the walking behaviour of the predators (Krips *et al.*, 1999). The resistance of certain gerbera cultivars to *T. urticae* is, however, not correlated with trichome density and therefore not likely to be mechanical. Possibly these cultivars produce toxins that affect *T. urticae* and such toxins may be harmful for *P. persimilis* as well.

Some secondary plant chemicals are not only present in the plants but also on the plant surface (Hugentobler and Renwick, 1995). *Phytoseiulus persimilis* do not consume leaf material but may be exposed to secondary plant compounds while walking over the leaf surface or by feeding on prey. While searching for prey, the predators continuously tap the leaf surface with their palps (Sabelis and van de Baan, 1983; Jackson and Ford, 1973; Dicke *et al.*, 1991). Subsequent cleaning of these legs palps to ingestion of compounds that may be present on the leaf surface which may lead to negative effects on the predators.

This study focused on the effect of host plant resistance to *T. urticae* in gerbera on the performance of *P. persimilis*. The ability of a natural enemy to suppress its prey is affected by its intrinsic rate of population increase (r_m) relative to that of the herbivore (van Lenteren and Woets, 1988). Hence, we determined the r_m of *P. persimilis* on four gerbera cultivars on which the r_m of spider mites was previously shown to differ largely (Krips *et al.*, 1998).

Because the r_m represents the maximum rate of population increase, the r_m of the predators should be determined at high prey density. The results of this study therefore indicate whether cultivar effects exist on predators that search for prey within spider mite colonies with a high prey density. If a negative effect of resistance to spider mites on the r_m of the predators is found it can be explained by two factors. First, toxic compounds that may be present on the leaf surface have a negative effect on the predator. Second, the predator is affected because prey has accumulated toxic compounds from the leaves while eating, which makes them less suitable as a food source for *P. persimilis* on a resistant cultivar.

MATERIAL AND METHODS

Plant Material

Gerbera plants were obtained from commercial gerbera breeders, Prego Rijsenhout private company and Terra Nigra private company. They were subsequently grown in a greenhouse at Wageningen Agricultural University at 20-30°C, 50-70% RH and a photoperiod with at least 16 hours of light. Plants used for the experiments were between six and twelve months old. Only the youngest, fully unfolded leaves were used. Cultivars used were: Bianca, Fame, Rondena, and Sirtaki.

Spider Mites

Spider mites (*Tetranychus urticae*) were collected from a commercial gerbera greenhouse at Mijdrecht (The Netherlands) in the spring of 1994 and were subsequently reared in our laboratory on bean. They were kept under the same conditions as the gerbera plants. Two weeks before the experiments started, we transferred adult female spider mites to the respective cultivars on which we determined the intinsic population growth rate (r_m) of the predators. This was done to ensure that spider mite eggs that were used as food for the predators were produced from plant material that was ingested from the cultivar on which the r_m value was determined.

Predatory mites

Phytoseiulus persimilis individuals were originally obtained from Entocare limited partnership, a commercial mass rearing company for biological control. In our laboratory, they were reared on *Tetranychus urticae* that had been kept for many generations on lima bean leaves. For this purpose, pieces of bean leaves that contained all *T. urticae* stages were put in Petri dishes of 9 cm diameter. Five adult female *P. persimilis* were placed in each Petri dish after which the dishes were closed with parafilm. Two times a week the predatory mites were offered fresh pieces of bean leaves with spider mites. Once a week, five adult female predatory mites were transferred from each Petri dish to new ones and the rearing was continued with these new dishes. The Petri dishes were kept in a climate room at 23±1°C, 60±5% RH and a photoperiod of 16:8 L:D.

Assessment of r_m of Predatory Mites

Preparation of leaf discs with spider mite eggs

Leaf discs of 4 cm^2 were cut from leaves of the four gerbera cultivars and placed upside down on wet cotton wool in small vials that had the same diameter as the leaf discs. Vials were closed by lids with openings that were covered with fine gauze. In this way the predators could neither drown in the wet cotton wool, nor escape from the vials. The space between the leaf discs and the lids was approximately 2 cm.

Depending on the cultivar, between 10 and 30 adult female spider mites were allowed to oviposit on the discs for two or three days, after which the majority of the adults were removed. A maximum number of two adult females was left on the leaf discs to produce spider mite eggs during the experiment. The spider mite eggs were counted and more eggs were added if needed. These eggs came from spider mite colonies on the respective gerbera cultivars that were used in the experiments, as described above under *"Spider Mites."* A minimum number of 60 spider mite eggs per leaf disc was used, which resulted in an egg density of at least 15 eggs/cm^2. During the experiment, the eggs consumed by the predators were replaced twice a day. This resulted in a maximum variation in egg density of 20%, due to consumption. However, this does not imply that the predation rate varies with 20%. At the densities of spider mite eggs used in our experiments, predation rate is limited by the turnover rate of food in the gut, instead of by prey density. This means that the 20% variation in prey density that we had in our experiments results in only a slight variation in egg consumption by the predators (Sabelis, 1981). Leaf discs were replaced in the experiment every Monday, Wednesday and Friday.

Experimental setup

Predator eggs that were produced within a time interval of 8 hours were collected from predatory mites that had fed for at least three days on spider mites on the gerbera cultivar that was used for the experiment. In every vial one predator egg was placed on the leaf disc and two days later one male predatory mite was introduced in each vial, to ensure that mating could take place immediately after females matured. The development of the predators from egg to adult was followed. For each of the four cultivars the age-specific reproduction and the age-specific survival of the female predatory mites was determined by counting the number of surviving mites and the number of eggs per adult female. This was done twice a day during which spider mite eggs were added until a total number of at least 60 eggs per leaf disc was reached and hence the prey density was re-established at 15 eggs/cm^2. For this purpose we used eggs from spider mite colonies on the respective cultivars that were used in the experiment. The vials with predators were kept in a climate cabinet at 25 ± 0.5 C, 65% RH and a photoperiod of 16:8 L:D. After 12 days the experiment was terminated.

Experimental groups

The experiment was carried out in three blocks. In the first block 30 individual predators were observed on cultivar Sirtaki and 30 were observed on Bianca. In the second block 15 predators were observed on Sirtaki, 45 on Fame and 15 on Bianca. In the third block 15 predators were observed on Sirtaki along with 45 on Rondena. In this way, Sirtaki was used as a reference cultivar. The replicates on cultivars Sirtaki and Bianca will be named Sirtaki 1, 2 and 3 and Bianca 1 and 2, referring to the respective experimental blocks.

Secondary sex ratio

The proportion of daughters in the offspring was determined in a separate experiment on the cultivars Sirtaki and Rondena. For this purpose, predators were reared individually in vials from egg to adult using the same experimental setup as used for determining the r_m. From these adults, eggs were collected for three days and these were kept in Petri dishes on bean leaves with spider mites, after which the proportion of females was determined.

Calculating the r_m values

For each cultivar the intrinsic rate of population increase (r_m) of the predatory mites was calculated by using the equation given by Carey (1993): $\exp(-r_m x) l_x n_x s_x = 1$, where l_x is the age specific survival; n_x is the age specific reproduction; and s_x is proportion of daughters in the offspring of a female at age x. The moment in between two measurement points was used as the x value, which is called the pivotal-age. Following Carey

(1993) the pivotal age x' was used in the equations, which is the moment in between age class x and x-1. For each pivotal-age class, the average of l_x and l_{x-1} was taken as age specific survival wich is also entirely according to Carey (1993).

Data obtained in different blocks were pooled for cultivars Sirtaki and Bianca. According to Amano and Chant (1977) and Nagelkerke (1993) the first egg produced is usually a male. Therefore, these first eggs do not contribute to the intrinsic rate of population increase. For this reason, we excluded the first egg of each individual from the calculations, which leads to lower r_m values. As mentioned above, we determined the r_m values over a period of 12 days which means that we measured the oviposition over a period of approximately 7 days and that the oviposition period had not yet ended at the end of the experiment. This would have resulted in an underestimation of the r_m values. However, early produced eggs have a much larger contribution to the r_m than eggs that are produced later in life (Lewontin, 1965). Therefore, the underestimation of the r_m values is low and will be similar for all cultivars under study.

Statistical Analysis

We analysed the developmental time of the predators on the four gerbera cultivars and for this purpose we used a two-way ANOVA with cultivar and block as independent variables. Differences between cultivars were analysed with Tukey contrasts. We defined developmental time as the time between the start of the experiment and the moment the adult females produced their *second* egg.

The peak oviposition rate was analysed, and we defined this variable as the reproduction from day three to day four after the average start of reproduction. At this moment the reproduction of the predators was at or close to its maximum. For the analysis we used a generalized linear model with a Poisson distribution and a log-link (Crawley, 1993). Cultivar and block were the independent variables.

We analysed the sex ratio on Sirtaki and Rondena with a two-by-two contingency table in which we compared the number of females and males on both cultivars.

RESULTS

The egg-to-second-egg developmental time of *P. persimilis* varied from 5.1 days on Rondena to 5.3 days on Bianca (Fig. 1). A two-way ANOVA showed that there was no significant effect of cultivar (F=0.58, df=3, P=0.62), or block (F=0.55, df=2, P=0.58) and that there was no significant cultivar-block interaction (F=0.14, df=1, P=0.71). However, on both Fame and Sirtaki we found one predator with an exceptionally long egg-to-second-egg developmental time of 8.2 and 9.7 days, respectively. The egg-to-second-egg-developmental time of all the other predators together was 5.2 ± 0.28 days (mean ± sd). The two exceptions were possibly due to mistakes while we supplied males. Because males are very difficult to distinguish from juvenile predators, we may have added juveniles instead of males. Therefore, these two specific females may have remained unmated for a long time and therefore unable to produce eggs. The reproduction of these females was normal, once it started.

When we excluded the two outliers, the two-way ANOVA revealed a significant effect of cultivar (F=3.10, df=3, P=0.030). There was no effect of block (F=1.36, df=2, P=0.26) and no significant cultivar-block interaction (F=0.35, df=1, P=0.55). When we subsequently removed the cultivar-block interaction from our model we could perform Tukey contrasts, which showed that there was only a significant difference in egg-to-second-egg developmental time between Bianca and Fame (P=0.022) (Fig.1).

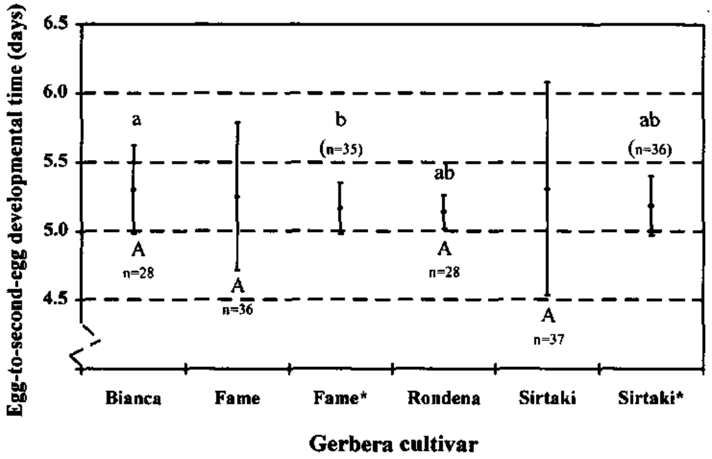

Fig. 1: Egg-to-second-egg developmental time of *P. persimilis* on leaf discs of four gerbera cultivars, containing approximately 15 *T. urticae* eggs/cm^2. Sirtaki* and Fame* = data from Sirtaki and Fame with one outlier with an exceptionally long developmental time excluded per cultivar. Numbers show the number of replicates, vertical bars represent standard deviations. Data were analysed with a two-way ANOVA and subsequent Tukey contrasts (α=0.05) with cultivar and block as independent variables. Letters below bars represent the result of the statistical analysis when the outliers were included and letters above the bars represent the same when the outliers were excluded. Different letters indicate significant differences.

The average peak oviposition rate of *P. persimilis* varied between 5.1 and 5.4 eggs/female/day (Fig. 2). A generalized linear model with a Poisson distribution and a log-link showed that there was no effect of cultivar (X^2=1.44, *df*=3, *P*=0.70), or block (X^2=0.52, *df*=2, *P*=0.77) and there was no significant cultivar-block interaction (X^2=0.01, df=1, *P*=0.91). Exluding the two outliers mentioned above, did influence the *P* values, but not the conclusions of the statistical analysis.

In Fig. 3 the age specific reproduction of *P. persimilis* on the four gerbera cultivars was compared with this parameter for its prey *T. urticae*. Data for *T. urticae* came from Krips et al. (1998). While the developmental time and fecundity of *T. urticae* were clearly affected by gerbera cultivar, these parameters remained unaffected for *P. persimilis*. The peak-oviposition rate *of P. persmilis* was lower than that of *T. urticae* on the most susceptible gerbera cultivar. However, *P. persimilis* developed between two and three times faster from egg to adult than its prey.

The sex ratio (proportion of females) of *P. persimilis* was 0.89 on Sirtaki and 0.87 on Rondena (Table 1). There was no significant difference in sex ratio on the two cultivars (two-by-two contingency table on numbers of males and females on both cultivars, *P*=0.54). For calculation of the intrinsic rate of population increase of *P. persimilis* on the four gerbera cultivars we used the pooled value of 0.88 (Table 1) for the proportion of daughters in the offspring.

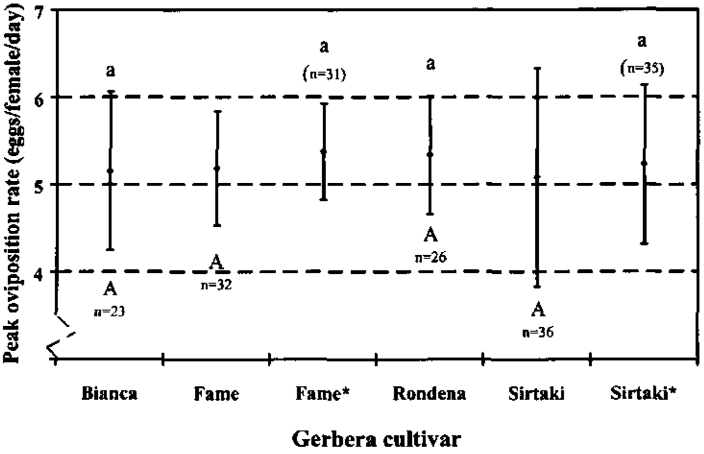

Fig. 2: Reproduction from day 3 to day 4 after the average start of reproduction of *P. persimilis* on leaf discs of four gerbera cultivars, containing approximately 15 *T. urticae* eggs/cm^2. Sirtaki* and Fame* = data from Sirtaki and Fame with one outlier with an exceptionally long developmental time excluded per cultivar. Numbers show the number of replicates, vertical bars represent standard deviations. Data were analysed with a generalized linear model with Poisson distribution and log-link and cultivar and block as independent variables (α=0.05). Letters below bars represent the result of the statistical analysis when the outliers were included and letters above the bars represent the same when the outliers were excluded. Different letters indicate significant differences.

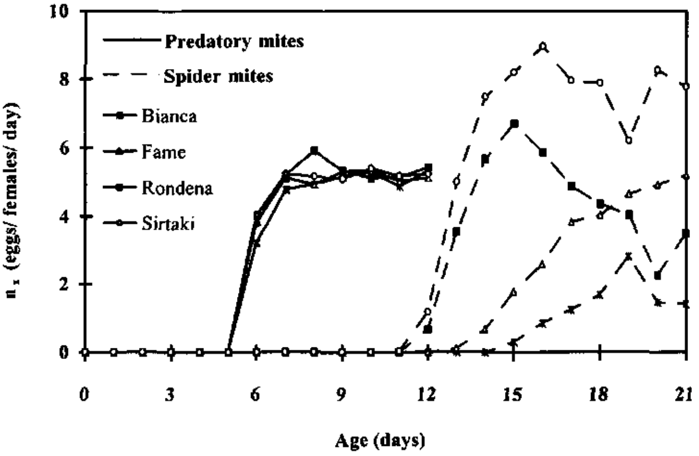

Fig. 3: Age specific reproduction (n_x: per age class total number of eggs divided by the number of living females) of *T. urticae* on leaf discs of four gerbera cultivars (data from Krips *et al.*, 1998) and *P. persimilis* on leaf discs of the same gerbera cultivars that contain at least 15 *T. urticae* eggs/cm^2. Age zero is moment of oviposition by mother.

Table 1: Sex-ratio of *P. persimilis* on leaf discs of two gerbera cultivars containing approximately 15 *T. urticae* eggs/ cm^2. Numbers followed by the same letter are not significantly different (two-by-two contingency table on the number of females compared to the number of males on both cultivars, α=0.05).

Cultivar	**Females**	**Males**	**Sex-ratio**
Sirtaki	190	23	0.89 a
Rondena	275	41	0.87 a
Total	465	64	0.88

Out of the 195 eggs used in the entire experiment, only 4 were found dead. In 4 cases we did not find an egg or larva at the first moment of observation. As the few eggs that did not hatch could have been killed by transfer to the leaf discs, we assumed egg mortality to be zero and excluded these eggs from the calculation of the r_m values. Including or excluding these eggs had a negligible effect on the r_m values.

The intrinsic rate of population increase (r_m) of *P. persimilis* varied little with gerbera cultivar, although a large cultivar effect on r_m of its prey *T. urticae* was found previously (Table 2) (data for *T. urticae* from Krips *et al.,* 1998). The r_m of *P. persimilis* varied from 0.424/day on Fame to 0.448/day on Sirtaki. Excluding the outliers with the exceptionally high developmental time on Fame and Sirtaki led to an increase in r_m of 0.004/day and 0.003/day, respectively. The r_m of *P. persimilis* was much higher than that of its prey, even on the most susceptible gerbera cultivar Sirtaki. This was due to the fact that the developmental time of *P. persimilis* was so much shorter than that of its prey (Fig. 3).

Table 2: Intrinsic rate of population increase (r_m) and number of females on which r_m values are based of *T. urticae* on leaf discs of four gerbera cultivars (data from Krips *et al.,* 1998) and of *P. persimilis* on leaf discs of four gerbera cultivars containing approximately 15 *T. urticae* eggs/cm^2. Fame* and Sirtaki*: same data as for fame and sirtaki, with the only difference that one outlier with an exceptionally late start of reproduction has been discarded for each cultivar.

	Spider mites		**Predatory mites**	
Gerbera cultivar	**r_m (day^{-1})**	**females**	**r_m (day^{-1})**	**females**
Bianca	0.088	32	0.431	28
Fame	0.155	27	0.424	37
Fame*			0.427	36
Rondena	0.205	46	0.432	28
Sirtaki	0.234	136	0.448	38
Sirtaki*			0.451	37

DISCUSSION

Literature on the r_m of *P. persimilis* is scarce and according to our knowledge only one publication exists in which the r_m of *P. persimilis* was determined while using *T. urticae* as prey. Hance (1988) found an r_m value at 24°C of 0.294, which is much lower than what we found in our study. However, while we replaced consumed prey eggs once every twelve hours, Hance replaced leaf discs with *T. urticae* eggs once every four days. This would have resulted in a large variation in prey density. Possibly prey densities were low during parts of that study, which may have resulted in a lower consumption rate and therefore a lower developmental rate, oviposition rate and r_m.

Comparing the r_m values obtained in this study with other studies on phytoseiids showed that we found higher r_m values than previously reported for phytoseiids in general and for *P. persimilis* in particular (Takafuji and Chant, 1976; Badii and McMurtry, 1984; Hance, 1988; see Sabelis and Janssen, 1994 for a review). Takafuji and Chant (1976) and Badii and McMurtry (1984) studied *P. persimilis* and used *Tetranychus pacificus* as prey. They found r_m values of 0.317 and 0.374 respectively. The former authors used mobile stages as prey while the latter used prey eggs. The lower r_m values as compared to our study may be explained by a lower suitability of *T. pacificus* as prey, or maybe a lower predation rate of *P. persimilis* when *T. pacificus* was offered as food source. Furthermore, differences in experimental conditions such as replacement of consumed prey may have played a role. The authors did not mention how often they replaced consumed prey.

There are two factors that make the difference between our high r_m values and previously published values even more remarkable. First, we *underestimated* the r_m values by terminating the experiments one week after the predators had reached the reproductive period. We assumed in our calculations that the reproduction was zero from this moment onwards, while in fact the predators could still have a high reproduction rate for more than a week. However, this reproduction late in life has a much smaller effect on r_m than reproduction early in life (Lewontin, 1965). For example, assuming for the predators on Rondena that the reproduction continues at its maximum until they are 50 days old while no further mortality takes place, results in the slight increase in r_m from 0.432/day to 0.438/day. It will be clear that the latter value is an *overestimation*, because a reproductive period of about 45 days is unrealistically long. Hence, our underestimation of the r_m values is negligible.

The second factor that increases the discrepancy between our data and the data in literature is the fact that we excluded the first egg from each adult female predator from our calculations, which decreases the r_m values. This was not done by the authors mentioned above, although first eggs are usually males (Amano and Chant, 1977, Nagelkerke, 1993) and therefore do not contribute to the population growth rate. Discarding these eggs from the analyses results in an increase in developmental time of approximately 7 h (=~6%) and an approximate decrease in r_m values of 0.013/day (=~3%).

Our high r_m values, as compared to previously published values for phytoseiids, can be explained by either a short developmental time, a high oviposition rate, a high survival rate, a high sex ratio, or a combination of these factors. A literature survey by Sabelis and Janssen (1994) showed that the sex ratio of *P. persimilis* varies from 0.800 (M.W. Sabelis, unpublished data) to 0.910 (C. Nagelkerke, unpublished data). The sex ratio we found falls well within this range and therefore does not explain our high r_m values. A high survival rate cannot explain our high r_m values either, as mortality of *P. persimilis* is described to be negligable until the end of the oviposition phase (Takafuji and Chant, 1976; Sabelis, 1985). As expected, mortality was low throughout our experiments.

In contrast to the lack of data available on the intrinsic rate of increase of *P. persimilis* when fed upon *T. urticae*, much has been published about the predator's oviposition rate and developmental time. But again, there is a large variation in experimental methods such as the stage of the prey offered as food and if and how often consumed prey is replaced. Sometimes this information is even lacking. Peak oviposition data range from 3.3 eggs/female/day (at 24°C) (Hance, 1988) to 5.2 eggs/female/day (at 26°C) (McClanahan, 1968), while we found an average peak ovipostition rate ranging from 5.1 to 5.4 eggs/female/day on the four gerbera cultivars. Data on egg-to-*first*-egg-developmental time range from 5.0 (at 26°C) (McClanahan, 1968) days to 5.9 days (at 24°C) (Hance, 1988). We found egg-to-*second*-egg developmental times between 5.1 and 5.3 days and the egg-to-*first*-egg developmental times ranged from 4.9 to 5.1 days. Thus, our data for peak oviposition rate and developmental rate are high, but are very well comparable with what has been found by McClanahan (1968). McClanahan did not calculate the r_m of *P. persimilis*, but it is likely that the r_m would have been similar to our r_m values.

Sabelis and Janssen (1994) provided a number of equations describing linear relationships between developmental time (first egg), peak oviposition rate and r_m of phytoseiid mites. The equations are based on a large number of data from about 50 species of phytoseiid mites. Comparing our data with the equations shows a good fit, with our data deviating from the predicted values with a maximum of 13%. Our data for developmental rate, peak oviposition rate and r_m are among the highest of the data used by Sabelis and Janssen (1994) to formulate the above mentioned equations. That our data do not deviate from the predicted values shows that there is no indication that the relations between developmental rate, oviposition rate and r_m level off above a certain point. Hence, there is no reason to believe that *P. persimilis* has reached a physiological limit for any of those parameters.

Comparing the demographic data for *P. persimilis* on the different gerbera cultivars shows that the developmental time is slightly but significantly shorter on cultivar Fame than on cultivar Bianca. However, this shorter developmental time does not lead to a higher r_m on Fame, which indicates that the effect of a shorter developmental time is compensated by a slightly higher mortality or lower oviposition rate. Oviposition rate was not affected by gerbera cultivar. On Fame and Rondena, 83% of the predatory mites were still alive at the end of the experiment, while 93% survived on Bianca and no mortality occurred on Sirtaki.

In our studies, we did not find any effect of resistance of the specific gerbera cultivar to *T. urticae* on the r_m of *P. persimilis*. A similar phenomenon was found by Wheatley and Boethel (1992), who determined the population growth of *T. urticae* on susceptible and resistant soybean, in presence or absence of *P. persimilis*. They found no indication that the effect of *P. persimilis* on the spider mite population was affected by host plant resistance. Because we followed the predators in our study for only one generation, we cannot rule out the possibility that negative effects of resistance occur in later generations. Greenhouse experiments that we are running at present will reveal whether such an effect actually occurs.

The results obtained in our study indicate that a predator in a patch with a high density of prey eggs is not affected negatively by plant characteristics that may be responsible for a decreased suitability for *T. urticae*. Furthermore, prey eggs on the different cultivars are of equal quality as food for *P. persimilis*. In our experiments, eggs of *T. urticae* were used as food source. If secondary plant compounds are responsible for the differences between gerbera cultivars in suitability for spider mites these compounds are either not toxic for *P. persimilis*, or these compounds are not present in the eggs of *T. urticae* in concentrations that affect *P. persimilis*.

It is possible that, in contrast to prey eggs, mobile stages of *T. urticae* do differ in suitability as food source for *P. persimilis* on the different gerbera cultivars, as they contain undigested leaf material in their gut. This was found for *T. urticae* from nightshade (De Moraes and McMurtry, 1987) as compared to *T. urticae* from bean. Weight gain of *P. persimilis* was less when fed adult female spider mites from nightshade. When the prey was starved, this effect disappeared, suggesting that toxic leaf material was present in the gut of the spider mites. Weight gain was not affected when the predators were fed upon prey eggs.

We used spider mite eggs as food source in our experiments because *P. persimilis* preferentially feeds upon the egg stage of its prey (Sabelis, 1981; 1990). A lower suitability of mobile stages would lead to only a slight decrease in the predator's population growth rate as under natural conditions the majority of their food consists of prey eggs. Furthermore, in pilot studies we found no indication that mobile stages of *T. urticae* on the resistant gerbera cultivar Bianca are less suitable as food source for *P. persimilis* than mobile stages on the susceptible cultivar Sirtaki. (O.E. Krips and P.E.L. Willems, unpublished data).

Hence, there is no reason to believe that the suitability of a gerbera cultivar for population increase of spider mites affects the rate of population increase of *P. persimilis,* once it is present in a patch with a high density of prey. Densities of spider mite eggs in prey patches on gerbera are generally lower than what has been described for other host plants (e.g. rose) (Sabelis, 1986; R. Gols and O.E. Krips, unpublished data). However, egg densities in prey patches on gerbera fall within the range where the functional response of the predator to the density of the prey has levelled off (Sabelis, 1986). Therefore the r_m values obtained in this study give a realistic estimate for the performance of *P. persimilis* on gerbera cultivars. Other aspects of this predator-prey interaction on gerbera, such as cultivar effects on location of prey-infested leaves are currently under investigation.

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CHAPTER 5

LEAF HAIRS INFLUENCE SEARCHING EFFICIENCY AND PREDATION RATE OF THE PREDATORY MITE *PHYTOSEIULUS PERSIMILIS* (ACARI: PHYTOSEIIDAE)

SUMMARY

The effect of leaf hairs on searching efficiency of adult female *Phytoseiulus persimilis* was investigated. For this purpose we used the ornamental crop *Gerbera jamesonii* and determined the predator's searching efficiency on three cultivars that differ largely in the density of leaf hairs on the undersurface of the leaves. Walking speed of the mites was highest on the cultivar with the lowest leaf hair density. Walking activity, defined as the percentage of time spent walking, was not dependent on leaf hair density of the cultivars. At both prey densities tested, time until first predation increased with leaf hair density.

Predation rate of adult female *P. persimilis* is affected by trichome density, especially when prey density is low. At prey densities of 1.3 and 2.5 *Tetranychus urticae* eggs/cm^2, predation rate was inversely related to leaf hair density. At a prey density of 8.0 eggs/cm^2 no significant effect of leaf hair density on predation rate was found.

These negative effects on searching efficiency and predation success at low prey density of *P. persimilis* suggest that biological control of *T. urticae* on gerbera may be hampered by leaf hairs.

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INTRODUCTION

Plant structures can largely influence the ability of natural enemies to suppress herbivore populations (Bergman and Tingey, 1979; van Lenteren and de Ponti, 1990; Dicke 1996, 1998). Plants may form special structures that are used by natural enemies for shelter (Walter, 1996) or supply natural enemies with alternative food (Bakker and Klein, 1992). In addition, plant trichomes may influence natural enemy species. Trichomes on leaves can have positive or negative influences on herbivores as well as their natural enemies. Many natural enemy species, however, are much more mobile than herbivores and cover larger distances over the leaf surface in search for their prey or host. This causes these natural enemies to be more affected by leaf hairs than herbivores.

Several parasitoid species are affected negatively at high trichome densities. Walking speed of *Trichogramma exiguum*, for example, is inversely related to trichome density of three plant species, which affects the rate of host encounter (Keller, 1987). On Poinsettia, four parasitoid species and one coccinellid predator of the silverleaf whitefly have the highest rate of parasitism or predation on the cultivar with the lowest trichome density (Heinz and Parrella, 1994). Less clear-cut is the effect of cucumber leaf hairs on *Encarsia formosa*, a parasitoid of the greenhouse whitefly. Walking speed of *E. formosa* is inversely related to the density of leaf hairs on the undersurface of cucumber leaves (Hulspas-Jordaan and van Lenteren, 1978; van Lenteren *et al.*, 1987, 1995; de Ponti *et al.*, 1988). On leaves of a hairless cucumber variety, however, walking speed of *E. formosa* is so high that the parasitoid walks over its hosts without detecting them. At equal whitefly densities the number killed through either parasitism or predation by *E. formosa* is highest on a cucumber cultivar with intermediate trichome density (van Lenteren *et al.*, 1995).

Positive effects of trichomes on the performance of natural enemies have been reported by Grevstad and Klepetka (1992). They determined the effect of leaf morphology on four coccinellid predators of cabbage aphids. On kohlrabi, a cruciferous plant with a very smooth leaf surface, none of the four predators could reach aphids that were located in the middle of the undersurface of the leaves. The predators could not grip the surface of the smooth waxy plants and fell off the leaves. On the other three plant species, which do not have a smooth leaf surface, this effect was absent. For these predators trichomes enhance foraging success.

Predatory mites may also be influenced positively by trichomes. Several species of small mites prefer leaf surfaces with a dense layer of trichomes (Downing and Moillet, 1967; Overmeer and van Zon, 1984; Duso, 1992; Karban *et al.*, 1995; Walter, 1996). This preference may be explained by higher humidity in between leaf hairs. Eggs of predacious mites suffer high mortality at low humidity (Sabelis, 1981). Furthermore, leaf surfaces that are densely covered with trichomes may protect predacious mites against their predators.

As predatory mite species differ in size it may well be that larger species are hampered at a certain trichome density, while smaller species are not. Smaller species may prefer leaf surfaces with trichomes, in contrast to larger species whose performance is decreased at higher trichome densities. Barrett (1994) suggested that the distribution of predatory mite species over different host plant species is dependent on the size of their body in relation to leaf hair density. Predacious mites known to have a preference for leaf surfaces with a dense layer of hairs are all small compared to the predatory mite *Phytoseiulus persimilis* (Walter, 1996). The width of the searching path of *P. persimilis* varies from 0.42 mm when the predator is resting to 0.84 mm when walking. The smaller species *Metaseiulus occidentalis*, for example, has a searching path varying from 0.27 to 0.52 mm wide (Sabelis, 1981). Therefore, adult female *P. persimilis* may have more difficulty penetrating a dense trichome layer than smaller predacious mites. Whether this predatory mite species prefers leaf surfaces with trichomes is unknown.

Van Haren *et al.* (1987) found that dispersal of *P. persimilis* from leaf to leaf is hampered by trichomes on stems of tomato plants. Tomato has glandular trichomes that produce a sticky exudate upon contact with, for example, a predatory mite. When *P. persimilis* disperse from one leaf to another, they run a high risk of getting trapped and killed in the exudate. Nothing is known about the effect of non-glandular trichomes on dispersal and walking behaviour of *P. persimilis.*

Phytoseiulus persimilis search randomly within a prey patch. The rate at which they encounter prey depends on the speed at which they search the leaf surface (Sabelis, 1981). Our paper addresses the question whether searching efficiency of *P. persimilis* is affected by the density of leaf hairs on the undersurface of leaves of *Gerbera jamesonii.* Gerbera is an ornamental plant of which many cultivars are grown commercially. The cultivars are mainly selected for colour and shape of the flowers. However, they differ largely in other characteristics such as leaf shape, leaf size, and hair density on leaf undersurface (Sütterlin and van Lenteren, 1997; O.E. Krips, unpublished data).

We used three cultivars that differ largely in hair density on the undersurface of the leaves and determined whether searching efficiency of *P. persimilis* differs on those cultivars. We determined walking speed of the predators, and walking activity, defined as percentage of time spent walking. Furthermore, we determined the time until first predation at a given prey density. To determine whether an effect of leaf hairs on searching behaviour would affect the effectiveness of the predator we also measured predation rate on the three gerbera cultivars.

MATERIAL AND METHODS

Plants

Gerbera plants were obtained from two commercial gerbera-growing companies, Prego Rijsenhout bv and Terra Nigra bv. The plants were subsequently grown in a greenhouse at the Laboratory of Entomology, Wageningen Agricultural University (20-30°C, 50-70% RH and a photoperiod of at least 16h). The youngest fully unfolded leaves of plants between six and twelve months old were used. Three cultivars were used in each experiment: Bianca, Sirtaki and Bourgogne. The hair densities on the undersurface of the leaves were 105±17 hairs cm^{-2} for Bianca, 400±75 hairs cm^{-2} for Sirtaki and 730±78 hairs cm^{-2} for Bourgogne (mean ± SD) – the cultivars will be referred to as LOW, MEDIUM and HIGH, respectively.

Spider mites

History of the spider mites (*Tetranychus urticae*) used differed between experiments. For determination of the time until first predation (experiment 2) spider mite eggs were used, taken from a colony that was kept on Lima bean plants for at least 5 years. For determination of the predation rate (experiment 3) a spider mite colony was used that originated from spider mites collected in 1994 from a commercial gerbera glasshouse. The spider mites were reared on the gerbera cultivar Sirtaki and were kept on the particular cultivars used in the experiment for at least one generation. All spider mite colonies were kept in a glasshouse in our department, at 20-30°C, 50-70% RH and a photoperiod of at least 16h.

Predatory mites

Predatory mites (*Phytoseiulus persimilis*) were obtained from Entocare CV, a commercial producer of natural enemies for biological control. They were subsequently reared on spider mites (*Tetranychus urticae*) on Lima bean leaves (*Phaseolus lunatus*). Predatory mites were kept in Petri dishes containing pieces of

Lima bean leaves with *T. urticae*. Once a week, predatory mites were transferred to new Petri dishes. Petri dishes were kept in a climate room at 23 ±1°C and a photoperiod of L:D 16:8. All experiments were carried out with mated adult female predators of 6 to 7 days old, which means that they were approximately 2 days in the adult stage.

Experiment 1: Walking behaviour

Leaf discs of 16 cm^2 were cut from plants of each of the three gerbera cultivars and placed upside down on agar (1% w/v). No prey were present on the leaf discs. A disc was placed above a ring of fluorescent light and under a video camera. The images from the camera were digitized by a computer which recorded the position of the predatory mite every 1.04 s. Walking speed and walking activity of the predatory mites were calculated using the image analysis programme Ethovision version 1.70 (Noldus Information Technology Inc.), which records *x,y* coordinates of the mite in the horizontal plane. Walking speed was determined over the periods that the predator was actually walking. Walking activity was defined as the percentage of time the predatory mite moved during the total time of the track.

Predators were starved for 24 h at 22±2°C before start of the experiment. A predatory mite was placed on a leaf disc after which its position was recorded for 10 min. For each predatory mite a different leaf disc was used and every predatory mite was used only once. Tracks of predatory mites that left the leaf disc within 3 min after start of the experiment and did not return within 30 s were omitted from analysis. The track of a predatory mite that left the leaf disc after 3 min was analysed until the moment the predatory mite had left.

The experiment was carried out at 23±1°C. Twenty adult female predatory mites were tested per cultivar. Differences among treatments were tested using the Kruskal-Wallis test. Differences between treatments were analysed using a non-parametric multiple comparison test, which includes Bonferroni correction (Siegel and Castellan, 1988).

Experiment 2: Time until first predation

Leaf discs of 4 cm^2 were cut from plants of each of the three gerbera cultivars and placed upside down on wet cotton wool. Spider mite eggs were transferred with a small paint brush and were distributed evenly over the leaf discs. Two densities of spider mite eggs were used: 2.5 and 5.0 eggs/cm^2. One female predatory mite was placed on a leaf disc after which the time until first predation was determined. Prior to the start of the experiment predators were starved for 24 h at 22±2°C.

The experiment was carried out at 22±2°C and 20 predatory mites were used per treatment. For each predatory mite a different leaf disc was used and each predatory mite was used only once. The data were analysed using a two-way ANOVA on log-transformed data. For both egg densities the overall effect of leaf hair density on the time until first predation was analysed with contrasts. If a significant effect was found further contrasts were used for multiple comparisons within each egg density. For these multiple comparisons the Bonferroni correction factor was applied.

Experiment 3: Predation rate

Leaf discs of 4 cm^2 were cut from plants of each of the three gerbera cultivars and placed upside down on wet cotton wool. Spider mite eggs were placed on the leaf discs in rows to facilitate determination of the numbers of eggs preyed upon. Rows were spread evenly over the surface of the leaf discs.

Three densities of spider mite eggs were used: 1.3, 2.5 and 8.0 eggs cm^{-2}. One predator was placed on each leaf disc.The experiment lasted 16 h during which prey eggs that had hatched or been preyed upon were replaced at a time interval that allowed egg densities to vary with a maximum of 5%. These time intervals were 0.5 h at egg density 1.3 egg cm^{-2}, 1 h at 2.5 eggs cm^{-2} and 2 h at 8.0 eggs cm^{-2}. The first 8 h of the experiment served as a conditioning period, which is required to allow the food content of the gut of the predators to reach a steady state (Rabbinge, 1976; Sabelis, 1981). During the last 8 h of the experiment the predation rate was determined.

The experiment was carried out in a climate room at 23±1°C and 65±5% RH, with 20 replicates per treatment. The data were analysed using a two-way ANOVA. For each egg density the overall effect of leaf hair density on predation rate was analysed with contrasts. If a significant effect was found further contrasts were used for multiple comparisons within each egg density. For these multiple comparisons the Bonferroni correction factor was applied.

RESULTS

Walking behaviour

Walking speed of the predators is 0.10 ± 0.030 cm s^{-1} (mean ± SD) on cultivar LOW, which is approximately twice as high as on the other two cultivars (Fig. 1). There is a significant overall effect of cultivar on walking speed (Kruskal-Wallis; KW=29.620; $P<0.001$). Following the method descibed by Siegel and Castellan (1988), differences between average ranks should exceed 13.22 at $\alpha=0.05$, 3 treatments and n=20 per treatment. This is the case for walking speed on cultivar LOW compared to the other two cultivars. There is no significant difference in walking speed on the cultivars MEDIUM and HIGH.

Walking activity of the predators on the three cultivars ranges from 70 to 90% (Fig. 2). There are no significant differences in walking activity of the predators on the three cultivars (Kruskal Wallis; KW=4.541; P=0.103).

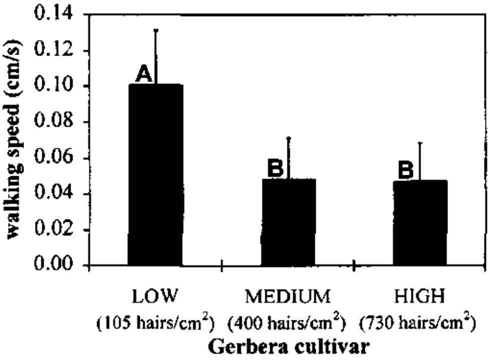

Fig. 1. Walking speed of adult female *P. persimilis* on leaf discs of three gerbera cultivars. Vertical lines represent standard deviations. Bars with different letters differ significantly (Kruskal-Wallis with subsequent multiple comparison test which includes Bonferroni correction ($P<(0.05/3=)0.017$), n=20 per cultivar.

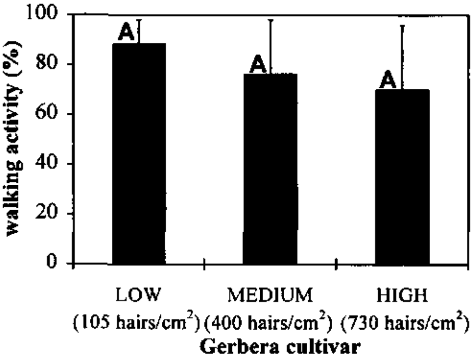

Fig. 2. Walking activity of adult female *P. persimilis* on leaf discs of three gerbera cultivars, defined as the percentage of time the predator walks on those cultivars. Vertical lines represent standard deviations. Bars with different letters differ significantly (Kruskal-Wallis, $P<0.05$), n=20 per cultivar.

Time until first predation

At both densities of spider mite eggs the time until the predator first preys upon a spider mite egg depends on gerbera cultivar (Fig. 3). The speed at which the predator preys upon its first prey is inversely related to leaf hair density of the cultivar. The difference in time until first encounter on the cultivars LOW and HIGH is 3-fold at egg density 2.5 eggs cm^{-2} and 4-fold at 5.0 eggs cm^{-2}.

The two-way ANOVA on the log-transformed data on time until first predation shows that there is a significant effect of egg density (df=1, F=11.17, P=0.001), of cultivar (df=2, F=36.66, P<0.001) and that there is no interaction between egg density and cultivar (df=2, F=0.60, P=0.55). For both egg densities, contrasts show that differences among all three cultivars in effect on time until predation are significant (df=2, P<0.001 for both egg densities, with F=23.16 for density 2.5 and F=14.10 for density 5.0). Within both egg densities all pairwise comparisons in time until first predation show significant differences (corrected α= 0.05/3=0.017).

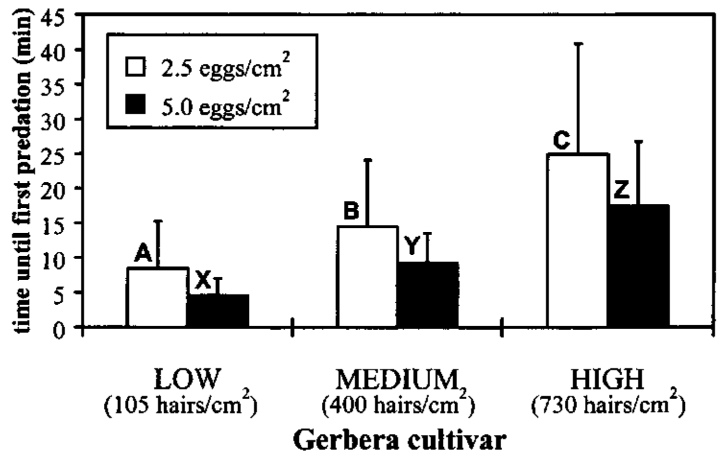

Fig. 3. Time until an adult female *P. persimilis* first preys upon a *T. urticae* egg on leaf discs of three gerbera cultivars at two densities of prey eggs. Predators have been starved for 24 h prior to the experiment. Vertical lines represent standard deviations. At each egg density bars with different letters differ significantly (Two-way ANOVA with subsequent contrasts on log-transformed data (P<(0.05/3=)0.017)), n= 20 per bar.

Predation rate

The predation rate of *P. persimilis* on the three gerbera cultivars at three densities of spider mite eggs is shown in Fig. 4. The two-way ANOVA for these data shows that there is a significant effect of egg density (df=2, F=37.23, P<0.001), of cultivar (df=2, F=3.73, P=0.026) and that the interaction between egg density and cultivar is significant as well (df=4, F=5.05, P=0.001). Contrasts show that differences in predation rate among cultivars are significant for egg densities 1.3 and 2.5 eggs cm^{-2} (df=2, F=4.18, P=0.017 for density 1.3 and df=2, F=7.74, P=0.001 for density 2.5).

At egg density 1.3 eggs cm^{-2}, predation rate on cultivar LOW differs significantly from predation rate on cultivar HIGH (df=1, F=8.36, P=0.004). Predation rate on cultivar MEDIUM is intermediate to predation rate on the other two cultivars.

At egg density 2.5 eggs cm^{-2}, no difference was found in predation rate on the cultivars LOW and MEDIUM (df=1, F=0.38, P=0.54). On cultivar HIGH the predation rate is significantly lower than on cultivars LOW (df=1, F=9.46, P<0.001) and MEDIUM (df=1, F=13.33, P=0.003).

There is no significant difference in predation rate of the predators on the three gerbera cultivars at egg density 8.0 egg cm^{-2} (df=2, F=1.90, P=0.15). At this egg density the rate of encounter between predator and spider mite eggs is high when compared to the actual predation rate. Therefore a decrease in encounter rate on a cultivar with high leaf hair density does not affect predation rate.

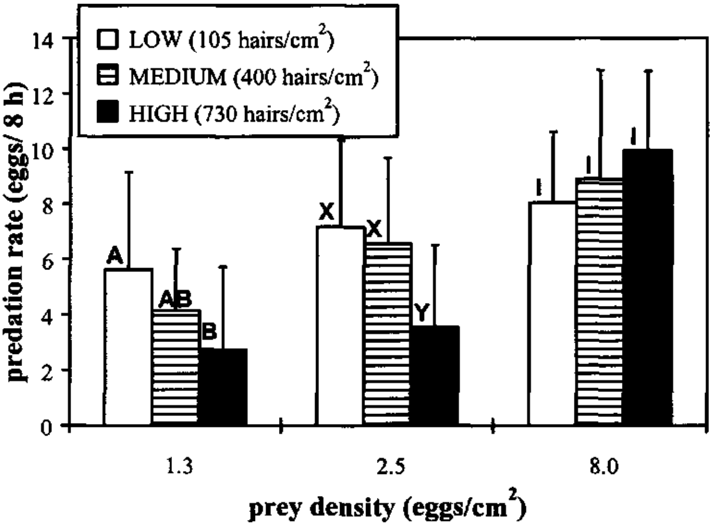

Fig. 4. Predation rate of adult female *P. persimilis* on leaf discs of three gerbera cultivars at three densities of *T. urticae* eggs. Vertical lines represent standard deviations. At each egg density bars with different letters differ significantly (Two-way ANOVA with subsequent contrasts (P<(0.05/3=)0.017), n=20 per bar.

DISCUSSION

The results of this study are well in accordance with our hypothesis that searching efficieny of *P. persimilis* on gerbera is influenced by the density of trichomes on the undersurface of leaves. Walking speed of the predators is highest on cultivar LOW and time until first predation increases with increasing leaf hair density. In addition, a lower predation rate is found at higher trichome densities.

Walking speed of the predatory mites is approximately 0.10 ± 0.030 cm s^{-1} on cultivar LOW. Sabelis (1981) found a walking speed of 0.12 ± 0.015 cm s^{-1} on rose, which is a plant without trichomes on the undersurface of the leaves. This indicates that up to a certain density of trichomes walking speed is not affected.

On the gerbera cultivar with 105 hairs cm^{-2} the predators walk approximately two times faster than on the cultivars with a leaf hair density of 400 hairs cm^{-2} or higher. This is in agreement with our hypothesis that walking behaviour of the predators is negatively affected by presence of trichomes. Comparing walking speeds at leaf hair densities of 400 and 730 hairs cm^{-2} shows no difference. Above a certain density of trichomes the effect on walking speed may have reached a maximum. The space between hairs on the cultivar with 400 hairs cm^{-2} is on average approximately 0.50 mm, which is less than the width of the searching path of an active adult female *P. persimilis* (Sabelis, 1981). Furthermore, because gerbera hairs are long and curled over the leaf surface (Sütterlin and van Lenteren, 1997) the actual space between hairs is even smaller, which forces the predators to walk more over the hairs instead of in between them. This is also the case on leaf surfaces with even higher hair densities, which may result in a constant effect on walking speed above a certain trichome density.

Walking speed of the parasitoid *Encarsia formosa* on cucumber is three times higher on a hairless variety than on a variety with 400 hairs cm^{-2}. On a cultivar with intermediate leaf hair density the walking speed is intermediate as well (van Lenteren *et al.*, 1995). In contrast, Sütterlin and van Lenteren (1997) found no effect of leaf hair density on walking speed of *E. formosa* on ten gerbera cultivars, which ranged in leaf hair density from 80 to over 1000 hairs cm^{-2}. The difference in structure of the trichomes on those two plant species probably accounts for this difference in effect on walking speed. Cucumber has straight trichomes, whereas gerbera has long trichomes that are curled over the leaves, which results in less space between trichomes.

Walking activity of *P. persimilis* is between 70 and 90% and does not depend on leaf hair density. Sabelis (1981) found a walking activity of 68% on rose leaves. When the data on walking speed and walking activity are combined it is clear that the surface that a predator explores in a certain amount of time is largest on the gerbera cultivar with the lowest trichome density.

Time until first predation increases with leaf hair density, indicating that searching efficiency of the predators is affected at higher trichome densities. Time until first predation was not only lowest on cultivar LOW, but it was also found to differ on cultivars MEDIUM and HIGH. Walking speed and walking activity of the predators on these two cultivars did not differ. The difference in time until first predation on cultivars MEDIUM and HIGH can be explained by the fact that the image analysis system can only measure the speed at which the predator moves horizontally. On leaf surfaces with dense trichome layers some eggs are hidden between hairs, where predators cannot reach them easily. This results in an increase in time until first predation.

Predation rate at the lowest density of prey eggs differs on the three gerbera cultivars. It decreases as hair density on the undersurface of the leaves increases. This effect disappears at higher densities of prey eggs. Predation rate depends on the rate of encounter between predator and prey and the motivation of predators to consume an encountered prey. This motivation depends on the food content of the predators' gut (Sabelis, 1981). Our data on the time until first predation indicate that the rate of encounter between predator and prey is inversely related to trichome density. However, at high prey densities the rate at which the predators encounter prey is high and predators will not consume all prey eggs encountered. Therefore, a decrease in encounter rate will not have a large effect on predation rate at those high egg densities. An effect of leaf hair density on searching efficiency of the predators will therefore only affect the predator's success in suppressing its prey at low prey densities.

Mean densities of *T. urticae* eggs on gerbera leaves are low, particularly on cultivars that have high spider mite resistance. Within spider mite colonies on leaves, however, egg densities generally exceed the densities used in our experiments. The densities of spider mite eggs in colonies are between 12 and 18 eggs cm^{-2} on the three cultivars used in our experiments. However, the size of these colonies is small. When ten adult female *T. urticae* are given the opportunity to deposit eggs for 4 days, the number of eggs per colony is 15 on average. In addition, up to 14% of the eggs of *T. urticae* are not laid in groups, but singly on the leaf (G.J.Z. Gols and O.E. Krips, unpublished data). Within spider mite colonies the predators are most likely not much affected by leaf hairs. Predators are more likely to be affected in locating eggs outside colonies and in moving between colonies. Since colony size is small, predators will have to move between colonies regularly. Predators search for prey colonies by walking over veins and leaf edges with excursions to the remainder of the leaf surface (Sabelis and Dicke, 1985). In gerbera, leaf veins are the leaf structures that are most densely covered by trichomes. As our data indicate that walking speed is affected by leaf hairs, predators may have more difficulties finding prey colonies on cultivars with high leaf hair density.

A number of phytoseiid mite species such as *Amblyseius aberrans*, *Typhlodromus pyri*, *Metaseiulus occidentalis*, *Neoseiulus caudiglans* and some *Phytoseius* species predominate on leaf surfaces that are densely covered with trichomes or have a preference for these surfaces (Downing and Moillet, 1967, Overmeer and van Zon 1984; Duso, 1992; Walter, 1992). However, these species are all small compared to *P. persimilis,* which enables them to penetrate higher densities of trichomes. Eggs of predatory mites are sensitive to drought. In a dense layer of trichomes humidity is likely to be relatively high, which might explain the preference of these predatory mites for leaf surfaces with a high density of leaf hairs.

Nothing is known about a preference of *P. persimilis* for any density of leaf hairs. It may well be that there is a relation between the performance of *P. persimilis* on leaf surfaces and its preference. Furthermore, this predator feeds predominantly on *Tetranychus* species, which form a dense web on the leaf surface, in contrast to the smaller phytoseiid mites mentioned above which feed on mites that do not produce a dense web. Humidity in the web of *Tetranychus* species is likely to be relatively high compared to the surrounding leaf surface. Therefore *P. persimilis* may be less dependent on leaf surfaces with a dense layer of trichomes for depositing its eggs. It may well be that this predator has a lower preference for surfaces with a high density of leaf hairs because of the negative effects on its feeding behaviour.

In conclusion, the data presented are well in accordance with our expectation that searching efficiency of the predatory mite *P. persimilis* is decreased by the presence of leaf hairs on the undersurface of gerbera leaves. In addition, at low densities of prey eggs, predation success decreases with increasing trichome density. Therefore the success of *P. persimilis* in biological control of *T. urticae* on gerbera may be influenced by leaf hairs.

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CHAPTER 6

THE RESPONSE OF *PHYTOSEIULUS PERSIMILIS* TO SPIDER MITE-INDUCED VOLATILES FROM GERBERA: INFLUENCE OF STARVATION AND EXPERIENCE

SUMMARY

When leaves of the ornamental crop *Gerbera jamesonii* are damaged by the spider mite *Tetranychus urticae*, they produce many volatile compounds in large quantities. Undamaged gerbera leaves produce only a few volatiles in very small quantities. In the headspace of spider mite-damaged gerbera leaves many terpenoids are present, comprising 65% of the volatile blend. In addition, a number of nitrogen containing compounds, such as oximes and nitriles, are produced.

We studied the attraction of *P. persimilis* to the volatiles from spider mite-damaged gerbera leaves and how attraction is affected by starvation and previous experience. *Phytoseiulus persimilis* that were reared on spider mites (*T. urticae*) on Lima bean were not attracted to spider mite-induced volatiles from gerbera. Starvation did not influence the predator's response to these volatiles. In contrast, predators that were reared on spider mites on gerbera leaves were strongly attracted to volatiles from spider mite-infested gerbera. This was also found for predators that originated from a culture on spider mite-infested bean and were offered six days of experience with spider mites on gerbera leaves.

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INTRODUCTION

Phytoseiulus persimilis Athias-Henriot is a specialist predator of the herbivorous spider mite *Tetranychus urticae* Koch. Once it is present in an environment with an abundant presence of prey it gives rise to an exponentially growing population that can increase up to 57% per day (Krips *et al.*, 1999). This capacity for population increase of the predator is much higher than that of its prey, which leads to rapid extermination of the herbivore population (Sabelis, 1985a).

Unlike many other species of predatory mites that can survive on alternative food sources, *P. persimilis* feeds almost exclusively on spider mites from the genus *Tetranychus* (Sabelis, 1985a). Hence, the ability to find patches where these spider mites are present is a matter of life or death for this predator. Any behavioral characteristic that would enable this predator to locate such patches from a distance would clearly be adaptive.

Several species of host plants respond to damage by spider mites with the production of volatiles that attract *P. persimilis* (Sabelis and van de Baan, 1983; Sabelis *et al.*, 1984; Dicke, 1988a; Dicke and Sabelis, 1988; Dicke *et al.*, 1990a, 1998; Sabelis and van der Weel, 1994; Sabelis and Afman, 1994). *Phytoseiulus persimilis* uses those odors to find leaves with colonies of its prey. Once *P. persimilis* is present on such leaves the speed at which they find prey colonies is enhanced by odors from the damaged spot (Garms *et al.*, 1998). Predators do not leave a prey patch until all prey is exterminated locally. This is most likely the result of arrestment in response to volatiles from damaged plants (Sabelis and van der Meer, 1986; Sabelis and Afman, 1994). The arrestment of the predators in a prey patch has a major influence on the population growth of the predators as well as their prey (van Baalen and Sabelis, 1995), which indicates the importance of the behavioral response to the volatiles.

Volatiles that are produced by plants in response to herbivore damage vary between plant species, between plant cultivars and also between leaves of different age (Dicke *et al.*, 1990b; Takabayashi *et al.*, 1990; 1991, 1994a, b; Turlings *et al.*, 1993; Loughrin *et al.*, 1995; see Takabayashi and Dicke, 1996 and Dicke *et al.*, 1998 for reviews on variability of spider mite-induced plant volatiles). Since *T. urticae* is a herbivore with a very wide range of host plant species (Sabelis, 1985b), a predator like *P. persimilis* is faced with a large variation in volatile information that indicates the presence of prey. It is very unlikely that *P. persimilis* has a fixed response to all these volatile blends.

The response of the predators varies largely with host plant species (Dicke and Sabelis, 1988; Takabayashi and Dicke, 1992; Takabayashi *et al.*, 1994b) and an initially low response can be enhanced by starvation, experience or selection. For example, the predators are only attracted towards spider mite-damaged pear leaves after starvation (Dicke and Sabelis, 1988). The response to spider mite-damaged cucumber leaves increased significantly after the predators were given several days of experience with spider mites on cucumber (Dicke *et al.*, 1990c; Takabayashi and Dicke, 1992; Takabayashi *et al.*, 1994b). Furthermore, with selection for several generations on high or low responding predators it has been possible to establish lines that have a significantly different response from the original population. This indicates that, besides phenotypic plasticity, a genetic component determines the predators' response towards spider mite-induced volatiles (Margolies *et al.*, 1997).

Based on our current knowledge, we formulate the following hypothesis regarding the foraging behavior of *P. persimilis*. Predators are arrested in a prey patch by spider mite-induced volatiles. Local extermination of the spider mites will decrease the production of volatiles by the plant and increase the starvation level of the predators. This will increase the response of predators to volatiles from a wider range of host plants. When a predator finds a prey patch on a plant of a new species, the initially low response to the spider mite-induced volatiles will increase due to experience. This will keep the predators in the new prey patch until all prey is exterminated.

Phytoseiulus persimilis is commonly and successfully used for biological control of *T. urticae* in vegetable crops in greenhouses. In ornamental crops, where aesthetic damage cannot be tolerated, biological control is usually more difficult. The ornamental gerbera (*Gerbera jamesonii*) is a positive exception to this rule since gerbera cutflowers are sold without leaves, which allows some damage on leaves. Spider mites usually only damage leaves, which makes biological control of spider mites possible on gerbera (van de Vrie, 1985).

The present paper reports on the production of spider mite-induced volatiles by gerbera and the attraction of *P. persimilis* towards these volatiles. Furthermore, we investigated whether the attraction is affected by starvation or experience in a spider mite patch on gerbera. With the results of this study the hypothesis on the foraging behavior of *P. persimilis* will be evaluated.

MATERIAL AND METHODS

Plant material

Gerbera (*Gerbera jamesonii*) plants (cv. Sirtaki) were obtained from a commercial gerbera grower, Terra Nigra bv, The Netherlands. They were subsequently grown in a greenhouse at the Laboratory of Entomology (Wageningen Agricultural University, The Netherlands) at 20-30°C, 50-70 % RH and a photoperiod of at least 16 hr of light. High pressure mercury lamps switched on when the light intensity outside dropped below 150 watt/m^2 and switched off when it increased above 250 watt/m^2. Plants used for the experiments were 6 - 12 months old. Only the youngest, fully unfolded leaves were used in experiments.

Spider mites

Spider mites (*Tetranychus urticae*) were collected from a commercial gerbera greenhouse at Mijdrecht, The Netherlands in the spring of 1994 and were subsequently reared in our laboratory on gerbera cultivar Sirtaki. The spider mite culture was kept under the same conditions as the gerbera plants.

Predatory mites

Predatory mites (*Phytoseiulus persimilis*) were originally obtained from Entocare CV, The Netherlands, a commercial mass rearing company for biological control. In our laboratory they were reared on spider mites (*T. urticae*) on either Lima bean leaves or leaves of the gerbera cultivar Sirtaki, depending on the experiment. Predators that were reared on spider mites on Lima bean had been kept on this plant species for many generations. Predators that were reared on spider mites on gerbera were kept on this plant species for at least two generations. The predators were kept in closed Petri-dishes of 9 cm diameter and were offered pieces of leaves with spider mites three times a week. Once a week, a new colony was started by transferring five gravid female *P. persimilis* from each Petri-dish to new dishes. The Petri-dishes were kept in a climate room at 23±1°C, 60±5% RH and a photoperiod of L:D=16:8. For the predatory mite culture on bean leaves, regular Petri-dishes were used. The predatory mite culture on gerbera leaves was kept in Petri-dishes with an opening of 2.7 cm diameter that was covered with fine-mesh gauze.

Incubation of leaves

The youngest, fully unfolded leaves were picked and placed with their petioles in glass vials containing tap water. To obtain spider mite damage, approximately 75 adult female *T. urticae* were placed on each leaf. Leaves were kept for 7 days at 23±1°C, over 65% RH and a photoperiod of L:D=16:8. Control leaves without spider mites were kept for 7 days under the same conditions as the treated leaves.

Experiment 1: Volatiles produced by undamaged gerbera leaves and leaves with spider mite damage

Collection and analysis of headspace volatiles

Leaves were placed with their petioles in a 200-ml glass vial containing tap water. The vial with leaves was then placed in a 5-liter glass jar with a 10-cm ID opening. The jar was closed with a lid that contained two openings. A glass tube was inserted through one of the openings to be used as air-inlet. The glass tube nearly reached the bottom of the jar. An air stream was generated in the flask at 450 ml/min. Before reaching the inlet, the air was cleaned through silica gel, molecular sieves and activated charcoal, following the procedure described by Takabayashi et al.(1991). Air was passed through the system for 1 hour prior to volatile collection, in order to remove all contaminants from the jar. Subsequently a Pyrex glass tube (160 x 6.0 mm OD, 3 mm ID) containing 90 mg Tenax-TA was connected to the outlet of the jar and air was purged for 15 min (6.75 liter) through this tube in order to collect the volatiles present in the jar.

The number of gerbera leaves used for volatile collection depended on the treatment. For collection of headspace volatiles from leaves that were damaged by spider mites, we used five to six leaves (= ± 30 gram) for each sample. For collection of headspace volatiles from undamaged gerbera leaves we used 10 leaves for each sample. We collected five headspace samples from leaves with spider mite damage and four samples from undamaged leaves. For each sample a different set of leaves was used. The volatiles from the Tenax-tubes were analysed by GC/MS, with the Thermdesorption Cold Trap Unit as inlet on the gas chromatograph as described in Mattiacci et al. (1994). The column used was a Supelcowax 10 fused silica capillary column, 60 m x 0.25 mm ID, 0.25 μm film thickness, with helium as carrier gas at an initial linear velocity of 22 cm/s. The oven temperature was raised from 40 °C to 270 °C with 4 °C/min.

Experiment 2: Response of *P. persimilis* to gerbera volatiles and influence of starvation level and rearing history

Ten undamaged control leaves and 10 leaves with spider mites were placed in separate 2-liter jars that contained a small amount of water, to prevent the leaves from desiccating. The leaves were placed with their stems in the water. The jars were connected to a closed system Y-tube olfactometer, described in more detail by Takabayashi and Dicke (1992). Adult female *P. persimilis* were individually introduced into the olfactometer onto an iron wire running through the centre of the olfactometer glass tube and running parallel to the tube walls. Predators were placed at the base of the Y-tube and had to choose between two olfactometer arms. Air with volatiles from spider mite-damaged leaves was led through one of the arms and air from clean leaves through the other arm. The observation of a predator ended when the animal reached the far end of one of the olfactometer arms. Observations lasted for no more than 5 min per individual predator. Predators that had not reached the end of one of the olfactometer arms within this time were excluded from the statistical procedure. This happened in only 4 cases out of a total number of 297 predators.

Two cultures of predatory mites that originated from the same source (see above) were used, one reared on Lima bean with spider mites and one on gerbera leaves with spider mites. Adult female predators from each culture were divided into two groups. One group was starved for 3 h and the other group was starved for 24 h prior to the start of the olfactometer test. Predators that were starved for 3 h were placed individually in empty Eppendorf vials. Predators that were starved for 24 h were placed individually in Eppendorf vials that contained a small piece of moist cotton wool to prevent the predators from desiccating. When predators of different treatments were tested on the same day (which can be read from Fig. 2 below), we used the same set of gerbera leaves for all treatments. In these cases the predators from the different treatments were introduced alternatingly into the olfactometer. After five predators from each treatment were tested in the Y-tube olfactometer, the connections of the odor sources to the two arms of the olfactometer were interchanged. The number of replicates per treatment are indicated in Fig. 2 below.

Statistical Analysis

To test whether the predators were attracted to one of the volatile sources, for each replicate experiment a χ^2 test was performed on numbers from each predator culture and each starvation level separately. For each of the four treatments the total numbers of predators that went to each volatile source were added and a χ^2 test was performed on these numbers as well. To test whether the response was affected by rearing history, we added the numbers of both starvation levels and performed a 2 x 2 contingency test.

Experiment 3: Influence of previous experience on response of *P. persimilis* to gerbera volatiles

We used the same Y-tube olfactometer set-up as described for experiment 2. From the predatory mite culture on spider mite-infested bean leaves we separated two groups of adult females. One group was kept for six days on spider mite-infested bean leaves in regular Petri-dishes of 9 cm diameter. The other group was kept for a total of six days on spider mite-infested gerbera leaves. They were kept in Petri-dishes of 9 cm diameter with an opening of 2.7 cm diameter that was covered with fine-mesh gauze. Three days after these subcultures were started all adult females were transferred to fresh leaves with spider mites. This was done to make sure that the experiment was performed with the individuals that originated from the stock culture on spider mite-infested bean leaves and not with their offspring.

For each replicate 20 predators from both subcultures were used and were introduced alternatingly into the Y-tube olfactometer. The connections of the odor sources to the two arms of the olfactometer were interchanged after five predators from each subculture were tested. The experiment was repeated three times.

Statistical Analysis

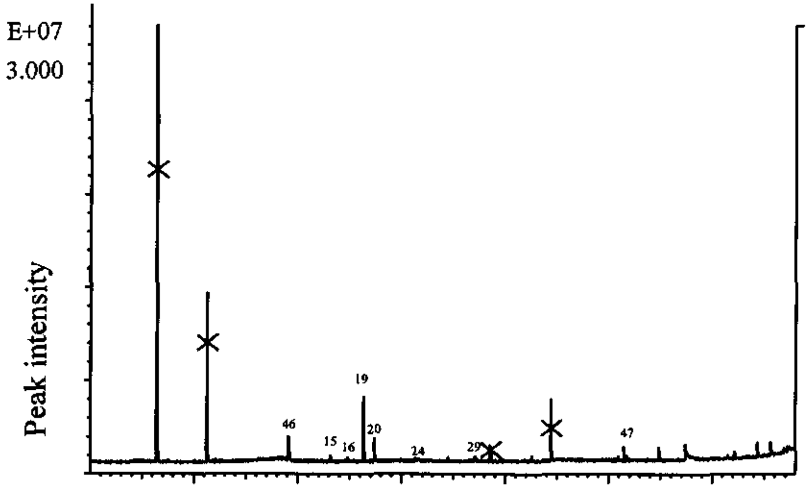
To test whether the predators were attracted to one of the volatile sources, for each replicate experiment a χ^2 test was performed on numbers from each predator subculture separately. To test whether the response from the two predator subcultures differed, we added all numbers for each subculture separately and performed a 2 x 2 contingency test.

RESULTS

Experiment 1: Volatiles produced by undamaged gerbera leaves and leaves with spider mite damage

The gas chromatogram of headspace volatiles of undamaged gerbera leaves (Fig. 1A) shows several peaks. However, the largest peaks are impurities from the collection system, such as siloxanes from silicone rubber seals. Although we used fewer leaves per sample for leaves with spider mite damage, the number of volatiles present in the samples is much larger and the volatiles are produced in higher quantities (Fig. 1B).

1A: Undamaged gerbera leaves



1B: Gerbera leaves with spider mite-damage

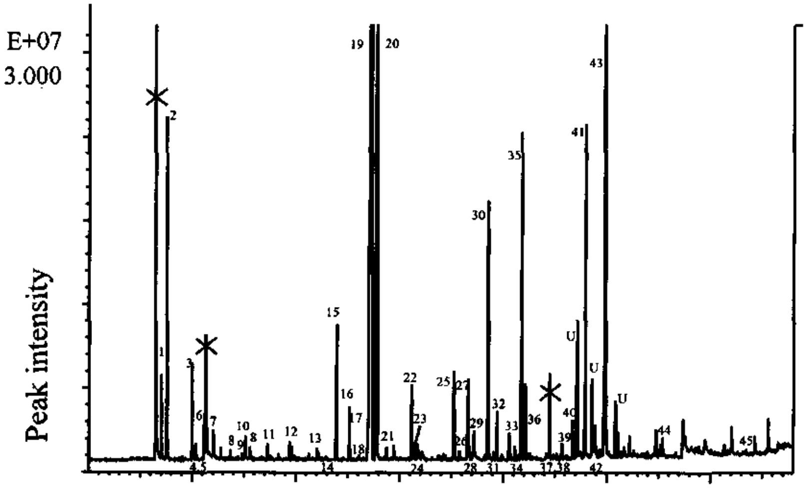


Fig. 1: Representative examples of gas chromatograms of headspace samples taken from undamaged gerbera leaves (A) and gerbera leaves with spider mite damage (B). Peaks with a cross are impurities from the collection system. Both chromatograms are plotted on the same scale. Numbers correspond with numbers from Table 1; only peaks that represent > 0.05% of the total volatile blend are numbered. U=Unidentified compound.

We found considerable variation in total peak area in our gas chromatograms, which is partly due to the fact that not all samples were taken at the same time. Therefore, we calculated for each compound the relative peak area as a percentage of the total volatile production per sample rather than the average absolute peak area. Samples of control leaves and treated leaves were taken over the same time-span.

The compounds that are most prevalent in the headspace of undamaged gerbera leaves are 1-butanol, 4,8-dimethyl-1,3(*E*),7-nonatriene and (*Z*)-3-hexen-1-yl acetate (Table 1). The headspace of spider mite-damaged gerbera leaves is dominated by the compounds (*Z*)-3-hexen-1-yl acetate, 2-butanone and a large number of terpenoids among which, 4,8-dimethyl-1,3(*E*),7-nonatriene, 4,8,12-trimethyl-1,3(*E*),7(*E*),11-tridecatetraene, (*E*)-β-ocimene and (*E*,*E*)-α-farnesene are produced in the largest quantities (Table 1). Furthermore, several nitrogen-containing compounds, such as nitriles and oximes are produced by spider mite-damaged gerbera, but are absent in the headspace of undamaged gerbera leaves.

Table 1: Identification of chemicals in the headspace of undamaged detached gerbera leaves and detached gerbera leaves that have been infested for seven days by the spider mite *T. urticae.* Four headspace samples were analysed for undamaged leaves and five for the leaves with spider mite damage. For each compound, the percentage of the total volatile production is given. Numbers in parentheses represent, for each compound, the absolute minimum and maximum peak area in the individual samples. * = present in detectable levels in one sample only and the peak area for this sample is given. If a compound repesents >0.05% of the total peak area it is indicated with a number in Fig. 1 that corresponds with the numbers in this Table. ~ = compound represents <0.05% of total peak area. n.p. = not present in detectable levels in both of the samples that are shown in Fig. 1.

		Relative percentage (mean ± SE) in headspace of:			
		Undamaged gerbera leaves		**Gerbera leaves with spider mite damage**	
Volatile compound	**No. in figure 1**	**% ± SE**		**% ± SE**	
Aldehydes					
Hexanal	9	0.8 ± 0.78	(6*)	0.17 ± 0.141	(0-9)
(*E*)-2-Hexenal	~			0.006 ± 0.0065	(0.7*)
Octanal	18	0.8 ± 0.78	(6*)	0.07 ± 0.061	(0-16)
Nonanal	24	1.2 ± 0.87	(0-0.5)	0.44 ± 0.240	(0-75)
Decanal	29	4 ± 1.8	(0-7)	0.9 ± 0.31	(0-120)
Dodecanal	n.p.	6 ± 5.8	(23*)		
Phenylacetaldehyde	~			0.019 ± 0.0185	(2*)
Unidentified aldehyde	~			0.05 ± 0.032	(0-2)
Alcohols					
1-Butanol	46	27.8 ± 2.26	(4-63)	0.006 ± 0.0065	(0.7*)
2-Butanol	7			0.83 ± 0.200	(6-103)
1-Hexanol	~			0.030 ±0.0296	(3.2*)
(*Z*)-3-Hexen-1-ol	22	0.25 ± 0.250	(1*)	0.35 ± 0.153	(0-21)
1-Octanol	~			0.025 ± 0.0247	(8*)
1-Nonanol	n.p.			0.10 ± 0.043	(0-13)
1-Dodecanol	n.p.			0.14 ± 0.091	(0-23)
2-Methyl-1-propanol	~			0.0019 ± 0.00185	(0.2*)
2-Phenylethanol	~			0.010 ± 0.0102	(1.1*)
(*E*)-nerolidol	44			0.20 ± 0.099	(0-34)
Esters					
Ethyl acetate	n.p.			0.09 ± 0.056	(0-21)
Butyl acetate	n.p.	2.2 ± 2.20	(17*)		

Table 1: Continued.

		Relative percentage (mean ± SE) in headspace of:			
		Undamaged gerbera leaves		Gerbera leaves with spider mite damage	
Volatile compound	**No. in figure 1**	**% ± SE**		**% ± SE**	
Methyl 2-methylbutanoate	n.p.			0.07 ± 0.041	(0-12)
Methyl 3-methyl-2-butenoate	12			0.33 ± 0.120	(2-62)
Hexyl acetate	16	1.7 ± 1.01	(0-4)	0.08 ± 0.058	(0-6.5)
(*Z*)-3-Hexen-1-yl acetate	20	10 ± 4.0	(0-18)	9.5 ± 1.98	(162-557)
(*Z*)-3-Hexen-1-yl 2-methylbutanoate	26			0.07 ± 0.048	(0-3)
(*Z*)-3-Hexen-1-yl 3-methylbutanoate	27			0.26 ± 0.179	(0-19.7)
Unidentified acetates	25			0.28 ± 0.233	(0-25.9)
Ketones					
2-Butanone	2	3 ± 3.2	(25*)	8.3 ± 2.09	(77-511)
3-Buten-2-one	~			0.005 ± 0.0046	(0.5*)
2-Pentanone	4			0.07 ± 0.045	(0-18)
3-Pentanone	~			0.019 ± 0.0185	(2*)
4-Methyl-2-pentanone	n.p.	1.2 ± 1.17	(9*)		
6-Methyl-5-hepten-2-one	21			0.14 ± 0.063	(0-29)
Geranyl acetone	~			0.04 ± 0.040	(4.3*)
Unidentified ketones	11			0.6 ± 0.40	(0-49)
Nitrogen containing compounds					
2-Methyl-2-propenenitrile	5			0.63 ± 0.209	(3-87)
2-Methylpropanenitrile	6			1.32 ± 0.280	(10-136)
2-Methylbutanenitrile	10			0.17 ± 0.057	(0-10)
Unidentified nitriles	8			0.21 ± 0.068	(2-31)
2-Methyl-1-nitropropane	13			0.23 ± 0.056	(1.4-27)
O-Methyl-2-methylpropanaldoxime	1			0.72 ± 0.235	(7-92)
O-Methyl-2-methylbutanaldoxime	3			1.25 ± 0.294	(0-142)
2-Methylpropanaldoxime	23			0.07 ± 0.032	(0-10)
Aromatic compounds					
Methyl benzoate	~			0.003 ± 0.0032	(0.35*)
Methyl salicylate	n.p.	1.5 ± 1.50	(6*)	0.023 ± 0.0163	(0-2)
2-Methylpropyl-benzoate	~			0.013 ± 0.0130	(1.4*)
***n*-Butyl benzoate**	47	3.9 ± 2.27	(0-3)	0.008 ± 0.0083	(0.9*)
Benzaldehyde	30			3.4 ± 1.44	(0-558)
Terpenoids					
Limonene	n.p.	1.8 ± 1.81	(14*)	0.06 ± 0.038	(0-11)
(*Z*)-β-Ocimene	14			0.12 ± 0.041	(0.2-18)
(*E*)- β-Ocimene	15	0.7 ± 0.69	(1*)	4.9 ± 1.12	(43-519)
4,8-Dimethyl-1,3(*Z*),7-nonatriene	17			0.70 ± 0.197	(3-106)
4,8-Dimethyl-1,3(*E*),7-nonatriene	19	26 ± 13.7	(0-17)	32 ± 4.3	(594-2130)
α-Copaene	28			0.53 ± 0.141	(3.7-85)
β-Cubebene	31			0.32 ± 0.159	(0-75)
Linalool	32			1.8 ± 0.72	(9-143)
(*cis*)-α-Bergamotene	33			1.1 ± 0.76	(0-87)
(*trans*)-α-Bergamotene	34			0.9 ± 0.41	(0.4-170)
β-Elemene	35			2.9 ± 0.77	(8-249)

Table 1: Continued.

		Relative percentage (mean ± SE) in headspace of:			
		Undamaged gerbera leaves		Gerbera leaves with spider mite damage	
Volatile compound	**No. in figure 1**	**% ± SE**		**% ± SE**	
β-Caryophyllene	36			2.1 ± 0.34	(20-287)
(*E*)-β-Farnesene	37			0.38 ± 0.151	(0.3-60)
α-Humulene	38			0.20 ± 0.076	(0.7-30)
Germacrene D	40			1.7 ± 0.48	(8.4-250)
(*E*,*E*)-α-Farnesene,	41			3.5 ± 0.57	(16-344)
δ-Cadinene	~			0.016 ± 0.0155	(4*)
Isomer of next compound	42			0.51 ± 0.150	(0-64)
4,8,12-Trimethyl-1,3(*E*),7(*E*),11-tridecatetraene	43			10.2 ± 1.86	(37-873)
α-Pinene	n.p.	5 ± 5.2	(40*)		
γ-Patchoulene	n.p.	2.5 ± 2.50	(10*)		
Unidentified terpenoids	39			1.0 ± 0.40	(0-184)
Miscellaneous					
Indole	45			0.11 ± 0.090	(0.3-30)
Octanoic acid	~			0.04 ± 0.039	(10*)
Unidentified minor peaks		0%		3.3%	
Total peak area		13.5-193		1225-8379	

Experiment 2: Response of *P. persimilis* to gerbera volatiles and influence of starvation level and rearing history

The predatory mites that were reared on spider mite-infested bean leaves and were starved for 3 h prior to the experiment had only a weak response to spider mite-induced volatiles from gerbera (Fig. 2A). In none of the replicate experiments did we find a significant preference for the volatiles from leaves with spider mite damage (χ^2-test, $\alpha=0.05$). However, when we pooled all data, 63% of the total number of 89 predators made a choice for the leaves with spider mite damage, which is a significant preference (χ^2-test, $0.01<P<0.05$).

Subjecting the predators that were reared on spider mite-infested bean leaves to 24 h of starvation did not increase their response to volatiles from spider mite-infested gerbera leaves (Fig. 2B). From the total group of 68 predators, only 60% made a choice for the leaves with spider mite damage, which is not significant (χ^2-test, $\alpha=0.05$).

The preference for the volatiles from spider mite-damaged gerbera leaves was much stronger for the predators that were reared on gerbera leaves with spider mites (Fig. 2C, 2D). When predators were starved for three h (Fig. 2C), we found a significant preference for leaves with spider mite damage in all six replicates (χ^2-test, $\alpha=0.05$). In total, 90% of the predators chose the spider mite-damaged leaves, which is a strong and significant preference (χ^2-test, $P<0.001$). When predators were starved for 24 h (Fig. 2D), 87% chose the spider mite-infested gerbera leaves (χ^2-test, $P<0.001$). Pooling the data of both starvation levels shows that the predators that were reared on gerbera with spider mites had a stronger response towards the gerbera volatiles than the predators that were reared on bean with spider mites (2 x 2 contingency table, $P<0.001$).

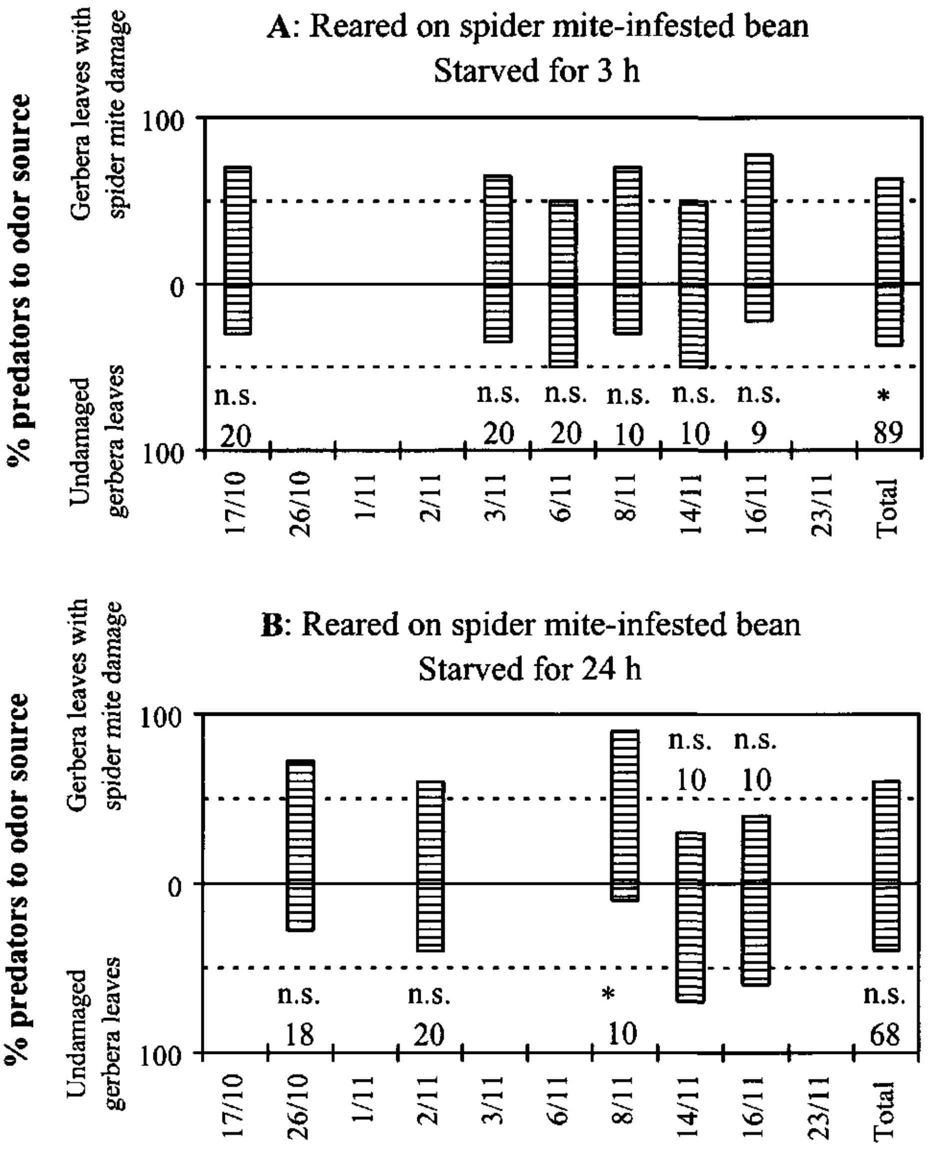
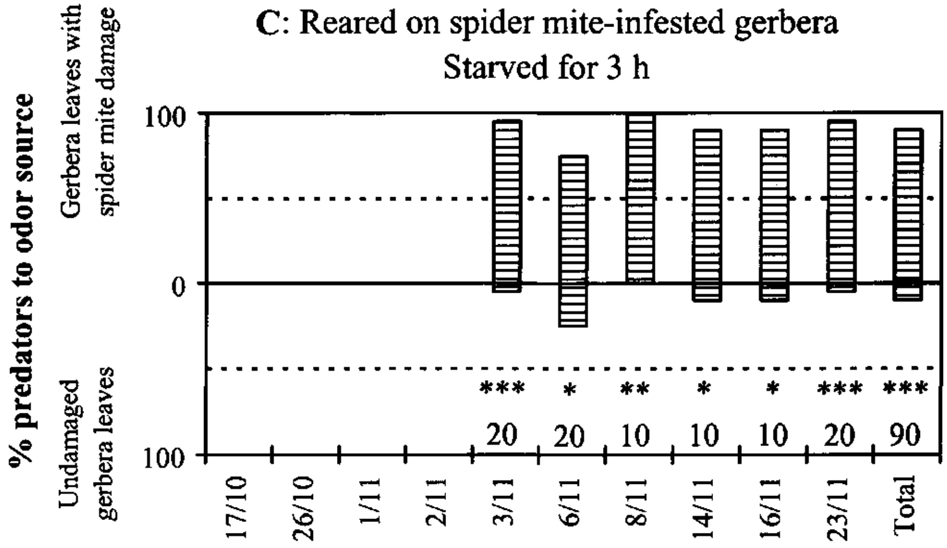
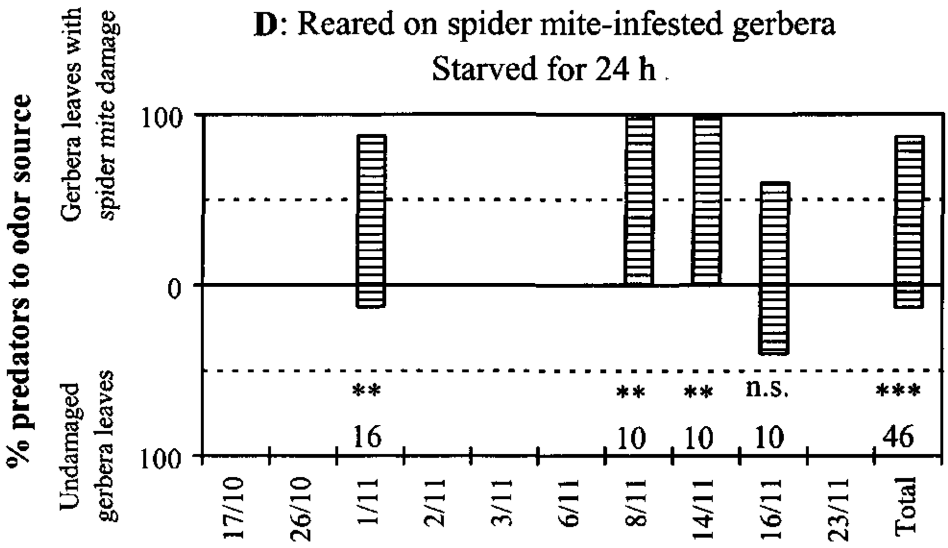


Fig. 2: Response of adult female *P. persimilis* in a Y-tube olfactometer when a choice was given between undamaged gerbera leaves and leaves with spider mite damage. Prior to the experiment *P. persimilis* was either reared on bean with spider mites and starved for 3 h (A) or for 24 h (B), or reared on gerbera with spider mites and starved for 3 h (C) or for 24 hr (D). Dates under the graphs represent dates in 1995 on which the experiments were performed.



C: Reared on spider mite-infested gerbera
Starved for 3 h



D: Reared on spider mite-infested gerbera
Starved for 24 h

Experiments that took place on the same date were performed with the same set of leaves as odor sources. In these cases, predators from each group were tested alternatingly. Data were tested with a χ^2-test; (n.s.= $P>0.05$, *=$0.05>P>0.01$, **=$0.01>P>0.001$, ***= $P<0.001$). Numbers below or above bars indicate the number of replicates.

Experiment 3: Influence of previous experience on response of *P. persimilis* to gerbera volatiles

As in experiment 2, the predators that were kept for six days on spider mite-infested bean leaves did not have a preference for volatiles from gerbera leaves with spider mite damage (Fig. 3). In total, 53% chose the damaged leaves.

Predators that were kept for six days on gerbera, showed a preference for damaged gerbera leaves (Fig. 3). In two out of the three replicates we found a significant preference for the damaged leaves (χ^2-test, α=0.05). In total, 81% chose leaves with spider mite damage. The response towards the spider mite-induced volatiles of gerbera differed between the two groups of predators (2 x 2 contingency table, $P<0.01$).

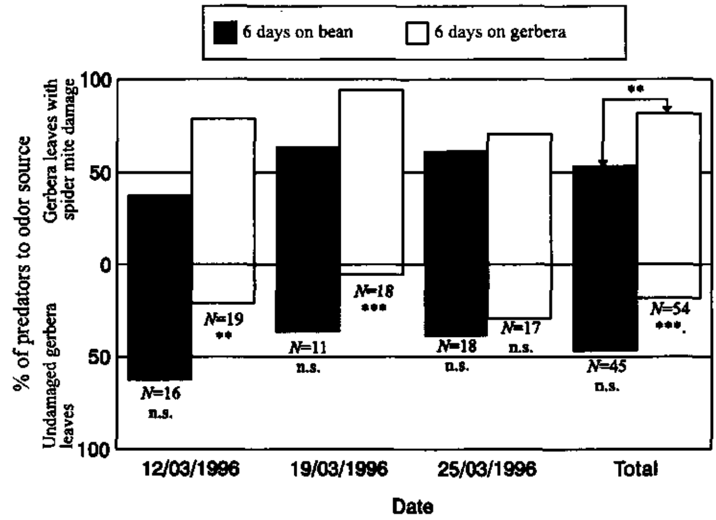


Fig. 3: Response of adult female *P. persimilis* in a Y-tube olfactometer when a choice was given between undamaged gerbera leaves and leaves with spider mite damage. Predators were reared on bean with spider mites and were kept for six days on either spider mite-infested bean leaves or spider mite-infested gerbera leaves. Dates under the graphs represent the dates on which the experiments were performed. Predators from both groups were tested alternatingly while the same set of leaves was used. Data were tested per bar with a χ^2-test. To test whether the response from the groups of predators differed, we added all numbers for each sub culture separately and performed a 2 x 2 contingency test (n.s.= $P>0.05$, *= $0.05>P>0.01$, **=$0.01>P>0.001$, ***= $P<0.001$).

DISCUSSION

It is evident that in gerbera, spider mite damage leads to the production of a large number of volatiles that are not produced by undamaged gerbera leaves. A number of short-chain lipid-derived compounds are present in the headspace of gerbera leaves with spider mite damage. These compounds are usually referred to as green leaf volatiles (Visser *et al.*, 1979) and occur generally in the headspace of artificially damaged leaves or leaves with herbivore damage (see for example: Dicke *et al.*, 1990a; Turlings *et al.*, 1990; Loughrin *et al.*, 1995; Mattiacci *et al.*, 1995). Two of those compounds, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexen-

1-yl acetate, occur in large quantities in the headspace of spider mite damaged Lima-bean leaves. However they do not attract the predatory mite *P. persimilis* when offered as individual compounds in a Y-tube olfactometer (Dicke, *et al.*, 1990a).

Less commonly found are the nitrogen-containing compounds such as the nitriles and oximes that are produced by spider mite-damaged gerbera. These compounds are also produced by cucumber and apple damaged by *T. urticae*. They were not found in the headspace of artificially damaged cucumber, apple with damage by the spider mite species *Panonychus ulmi,* or *Solanum luteum* and Lima bean with *T. urticae*-damage (Dicke *et al.,* 1990a; Takabayashi *et al.,* 1994b). However, techniques for analysis of headspace volatiles have improved over the last decade and recent analyses of the headspace of spider mite-damaged Lima bean did reveal small amounts of these compounds (Dicke *et al.,* 1999).

Approximately 65% of the volatile blend of spider mite-damaged gerbera consists of terpenoids. Among these, three compounds are present that attract *P. persimilis* when offered as individual compounds in a Y-tube olfactometer. These are the monoterpenes (*E*)-β-ocimene, linalool, and the homoterpene 4,8-dimethyl-1,3(*E*),7-nonatriene (Dicke *et al.,* 1990a). Methyl salicylate, which also attracts *P. persimilis,* is present in equally small amounts in the headspace of undamaged leaves and leaves with spider mite damage.

The initial lack of preference of *P. persimilis* for spider mite-damaged gerbera raises the question whether the volatile production is lower than by other plant species. Collection and analysis by Dicke et al. (1999) of an attractive volatile blend of spider mite-damaged bean showed a total volatile production of the same order of magnitude as for gerbera. The production of the attractive compounds (*E*)-β-ocimene and 4,8-dimethyl-1,3(*E*),7-nonatriene are also in the same order of magnitude for bean and gerbera. In contrast, the production of linalool and methyl salicylate is much lower in gerbera, which might explain the initial lack of response of the predators. However, there are many quantitative and qualitative differences between the blends of gerbera and bean, and for most of the gerbera compounds the attractivity to *P. persimilis* is unknown. Hence, it is difficult to know which differences between blends are responsible for the inital difference in effect on *P. persimilis*.

Starvation level obviously does not enhance the response of *P. persimilis* to damaged gerbera leaves, which is in contrast with data for the response to spider mite-damaged pear and apple (Sabelis and Dicke, 1985; Dicke and Sabelis, 1988). Possibly, 24 h of starvation is not long enough to evoke a response to spider mite-induced volatiles from this host plant species, as this was found for the response of the predatory mite *Typhlodromus pyri* to volatiles from apple leaves with *T. urticae*. These predators were only attracted to spider mite-damaged apple leaves after 48 hr of starvation (Dicke, 1988b).

Six days of experience in a spider mite patch on gerbera leaves greatly enhanced the response of *P. persimilis*. This is most likely caused by a change in behavior of individual predators rather than a selection for the best-responding individuals. Dispersal of nonresponding predators was not possible during the experiment and, since there was ample food, mortality was not likely to be affected by response to the volatiles.

Takabayashi et al. (1994b) also showed that the response of *P. persimilis* is affected by experience. The predator's response to infested cucmber leaves increased gradually during seven days that it was reared on this plant. It is not known whether this is the result of learning or of physiological changes. For non-host-feeding parasitoid wasp species, which can be kept on alternative food, it is easier to distinguish between these two mechanisms.

Learning by parasitoid wasps has been well studied and documented (see Vet and Groenewold, 1990; Turlings *et al.,* 1993; and Vet *et al.,* 1995 for reviews). Vet and Dicke (1992) suggest that learning to respond to herbivore-induced plant volatiles is expected for a specialist natural enemy of a polyphagous herbivore. Learning is considered to be adaptive if shifts of host plant species occur frequently over generations, which would prevent the development of a fixed response by selection. However, if shifts of host plant species occur very frequently within generations learning will have no advantage (Papaj and Prokopy, 1989; Stephens, 1990).

Phytoseiulus persimilis is a specialist natural enemy of a very polyphagous herbivore and thus searches for prey on different host-plant species. Shifts of host plants occur, but not frequently within generations since the predators exterminate a prey patch completely before they disperse (Sabelis and van der Meer, 1986), which generally takes longer than one generation. Hence, for this predator the ability to learn to respond to spider mite-induced volatiles is in accordance with the above mentioned hypotheses (Papaj and Prokopy, 1989; Stephens, 1990; Vet and Dicke, 1992).

In the introduction we formulated a hypothesis regarding the foraging behavior of *P. persimilis*. When we include our present data, the hypothesis changes slightly. Most likely, predators that exterminated a prey patch will try to find a new prey patch by responding to a familiar volatile blend that is associated with the presence of spider mites. Arrival on a host plant species with a very different blend of spider mite-induced volatiles is likely to be a matter of chance. After arrival on such a plant, the response to the spider mite-induced volatiles will likely increase due to experience and possibly over generations due to selection. This will arrest the predators in the prey patch on this new host-plant species.

In contrast to parasitoid wasps in which learning can take place within seconds (Turlings *et al.*, 1993; Vet *et al.*, 1995) the increase in response of *P. persimilis* is a gradual process and is a matter of days rather than seconds (Dicke *et al.*, 1990c; Takabayashi *et al.*, 1994b). This may have consequences for the performance of *P. persimilis* as a biological control agent on certain crops. In commercial mass rearings, *P. persimilis* is commonly reared on bean with spider mites. Introduction of these predators into other crops may result in large losses of predators, since many of them may not be able to find their prey. Previous experience with spider mite-induced volatiles from the crop of interest or the introduction of *P. persimilis* directly on the infested leaves may greatly enhance the effectiveness of the predators as biological control agents.

In conclusion, spider mite damage induces gerbera to produce a large number of volatiles in large quantities. When reared on bean with spider mites, *P. persimilis* is poorly attracted by volatiles from spider mite-damaged gerbera and starvation level hardly influences the response. In contrast, experience with spider mite-infested gerbera leaves greatly increases the response of the predators to spider mite-induced gerbera volatiles.

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CHAPTER 7

A COMPARISON OF CULTIVARS OF THE ORNAMENTAL CROP *GERBERA JAMESONII* ON THE PRODUCTION OF SPIDER MITE-INDUCED VOLATILES, AND THEIR ATTRACTIVENESS TO THE PREDATOR *PHYTOSEIULUS PERSIMILIS*.

SUMMARY

We investigated whether volatiles that are produced by spider mite-damaged plants of four gerbera cultivars differ in attractiveness to *Phytoseiulus persimilis*, a specialist predator of spider mites. In addition, the chemical composition of the blends of volatiles of these four cultivars was determined. Since the cultivars used differ in host plant resistance to spider mites, damage by spider mites will not have been equal on all cultivars. To correct for such differences, we developed a method to compare the attractiveness of the blends, based on the assumption that a larger amount of spider mite damage leads to higher attraction of *P. persimilis* .

The predators preferred spider mite-induced volatiles of cultivar Rondena over those of cultivar Sirtaki. Similarly, spider mite-induced volatiles of cultivar Sirtaki were less attractive than those of cultivar Bianca. The spider mite-induced volatiles of cultivars Sirtaki and Fame did not differ in attractiveness to *P. persimilis.*

Sirtaki plants had a lower relative production of terpenes than the other three cultivars, which was mainly the result of a low production of of *cis*-α-bergamotene, *trans*-α-bergamotene, *trans*-β-bergamotene and *(E)*-β-farnesene. Furthermore, the production of *(E)*-β-ocimene and linalool was lower by Sirtaki and Fame leaves than by leaves of Bianca and Rondena. Since these two compounds attract *P. persimilis* when offered in a Y-tube olfactometer, poor attractiveness of the volatiles of Sirtaki and Fame may be partly related to the limited production of these compounds.

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INTRODUCTION

The predatory mite *Phytoseiulus persimilis* Athias-Henriot is a specialist predator of the spider mite *Tetranychus urticae* Koch, which is an important pest in greenhouses. Since the predators feed almost exclusively on spider mite species from the genus *Tetranychus* and are unable to survive on alternative food sources such as pollen or nectar (Sabelis, 1985), they fully rely on their ability to locate areas where prey is present.

Several species of host plants respond to damage by spider mites with the production of volatiles that attract *P. persimilis* (Sabelis and van de Baan, 1983; Sabelis *et al.*, 1984; Dicke and Sabelis, 1988; Dicke *et al.*, 1990a,b 1998; Sabelis and van der Weel, 1994; Sabelis and Afman, 1994). *Phytoseiulus persimilis* uses these odors to find leaves with colonies of its prey. The predators do not leave a prey patch until all prey is exterminated locally. This is most likely the result of arrestment in response to volatiles from damaged plants (Sabelis and van der Meer, 1986; Sabelis and Afman, 1994). Arrestment of the predators in a prey patch has a major influence on the population growth of the predators as well as their prey (van Baalen and Sabelis, 1995). Therefore, the response of the predators to spider mite-induced volatiles is likely to be important for the success of spider mite control with *P. persimilis.*

Different species of plants show qualitative and quantitative differences in the production of spider mite-induced volatiles and these blends differ in attractiveness to *P. persimilis*. Even cultivars of the same plant species may differ in production of volatiles and attractiveness (see Takabayashi and Dicke, 1996 and Dicke *et al.*, 1998 for reviews on variability of spider mite-induced plant volatiles). Cultivar differences have been found as well in composition or attractiveness of blends of volatiles that are produced in response to damage by other herbivores (Loughrin *et al.*, 1995; Loughrin *et al.*, 1996; Souissi, 1999). The success of *P. persimilis* to localise spider mite-infested leaves and the speed at which prey populations are exterminated may therefore depend on the plant species or cultivar.

Gerbera jamesonii Bolus is an ornamental crop of which a large number of cultivars is grown commercially. During the plant breeding process these cultivars are mainly selected for colour and shape of the flowers. However, quite a number of other cultivar differences exist, such as leaf shape and texture, density of trichomes and resistance against the spider mite *Tetranychus urticae* Koch (Sütterlin and van Lenteren,1997; Krips *et al.*, 1998; 1999a). Possibly, gerbera cultivars with spider mite damage differ as well in the production of volatiles and these volatile blends may differ in attractiveness. This may result in cultivar effects on the ability of *P. persimilis* to localise its prey and the speed at which a spider mite population is exterminated.

The aim of our present study was to compare the compostition of the blends of volatiles produced by spider mite-damaged leaves of four gerbera cultivars and to determine the attractiveness of these blends to *P. persimilis.* This way we tried to relate differences in blends of volatiles to differences in attractiveness to *P. persimilis.* Comparing the production of volatiles by spider mite-damaged gerbera cultivars and the attractiveness of these volatiles to *P. persimilis* is, however, not as easy as it may seem. Gerbera cultivars differ largely in resistance to *T. urticae* (Krips *et al.*, 1998), which results in unequal consumption rates by the spider mites. This means that placing a standardised number of spider mites on gerbera leaves for a standardised amount of time does not result in equal damage on all cultivars .

Because of the cultivar differences in host plant resistance, we estimated the amount of spider mite damage on the leaves that were used in our experiments. We counted the number of spider mite eggs on each set of leaves used for analysis of volatile blend or attractiveness to *P. persimilis*. This way we had an indication whether or not cultivar differences in volatile production or attractiveness could be explained by differences in damage by spider mites.

MATERIAL AND METHODS

Plant material

Gerbera (*Gerbera jamesonii*) plants were obtained from the gerbera breeding companies Prego Rijsenhout bv and Terra Nigra bv. They were grown at 20-30°C, 50-70 % RH and at least 16 hours of light per day in a greenhouse at Wageningen University, The Netherlands. Plants used for the experiments were between 6 and 12 months old. The youngest fully unfolded leaves of the cultivars Bianca, Fame, Rondena and Sirtaki were used.

Spider mites

Spider mites (*Tetranychus urticae*) were collected from a commercial gerbera greenhouse at Mijdrecht (The Netherlands) in the spring of 1994 and were subsequently reared on the gerbera cultivar Sirtaki. Plants with spider mites were kept at 20-30°C, 50-70 % RH and at least 16 hours of light per day in a greenhouse at Wageningen University, The Netherlands.

Predatory mites

Predatory mites (*Phytoseiulus persimilis*) were originally obtained from Entocare CV, a commercial mass rearing company for biological control. In our laboratory they were reared on spider mites (*T. urticae*) on gerbera leaves of the cultivar Sirtaki. The predators were kept in Petri-dishes of 9 cm diameter with an opening in the lid of 2.7 cm Ø covered with fine-mesh gauze and were offered pieces of leaves with spider mites three times a week. Once a week, 5 gravid female *P. persimilis* were transferred from each Petri-dish to new ones. The Petri-dishes were kept in a climate room at 23±2°C, 60±5% RH and a photoperiod of L:D=16:8.

Experiment 1: Preference of *P. persimilis* for the volatiles of certain gerbera cultivars with spider mite damage

We compared the attractiveness of the spider mite-induced volatiles of cultivar Sirtaki with those of cultivars Bianca, Fame and Rondena. For each replicate we detached leaves of Sirtaki and one of the other gerbera cultivars and placed these leaves with their stems in vials with tap water. The weight of the leaf samples was between 30 and 70 gram per cultivar, but was equal for both cultivars per replicate. Adult female spider mites were placed on the leaves after which the leaves were incubated for 7 days at 23±1°C, 60±5% RH and a photoperiod of L:D=16:8. After these 7 days we counted the number of spider mite eggs on the leaves, as a measure of the spider mite damage inflicted.

Leaves of both cultivars were placed in separate jars that contained a small amount of water. The jars were connected to a Y-tube olfactometer, described in more detail by Takabayashi and Dicke (1992). Adult female *P. persimilis,* from the culture on gerbera with spider mites, were starved for 3 h and introduced individually into the olfactometer onto an iron wire running through the centre of the olfactometer glass tube and parallel to the tube walls. Predators were placed at the base of the Y-tube and were allowed to choose between two olfactometer arms. Air with volatiles from spider mite-damaged leaves of one cultivar was led through one arm and air from the other cultivar through the other arm. The observation of a predator ended when it reached the far end of one of the olfactometer arms. Observations lasted for five minutes maximally per predator. From the total number of 600 predators, 44 had not reached the end of one of the olfactometer arms within this time. These cases were not used for further analysis.

Statistical analysis

We used a generalized linear model (Crawley, 1993) for the number of predators choosing for Sirtaki per replicate experiment with 20 predators. This model contained a binomial distribution with p for the proportion of predators choosing Sirtaki and a linear relationship between the logit of p and the amount of damage (=number of spider mite eggs) on each of two cultivars. This amount of damage is quantified by l_x, which is the logarithm of x/(1-x) with x the fraction of eggs found on Sirtaki (eggs on Sirtaki/(eggs on Sirtaki + eggs on other cultivar)). If $l_x>0$ more eggs are found on Sirtaki than on the other cultivar, so we expect to find a higher rate of predators choosing for Sirtaki. If $l_x<0$, more eggs are found on the other cultivar and we expect a higher rate of predators to choose for the other cultivar.

We allowed the residual variance to be larger than prescribed by the binomial distribution through a multiplicative scale parameter. The main interest focussed on the probability p_0 of choosing Sirtaki at $l_x=0$, i.e. with equal amount of damage on both gerbera cultivars. We will test the null hypothesis: $p_0 = 0.5$ versus the alternative $p_0 <> 0.5$. In other words: we tested whether the chance the predators chose for Sirtaki was 0.5 at an equal number of spider mite eggs on both cultivars. We furthermore tested whether there is an effect of amount of damage on the predators' choice as follows: H_0: regression coefficient = 0 versus H_1: regression coefficient > 0. The model was fitted using procedure GENMOD from the statistical program SAS version 6.12 (SAS Institute Inc, 1997).

We will present results from analyses for the comparison of Sirtaki with each of the three other cultivars separately, and from an overall analysis, combining results together.

Experiment 2: Chemical composition of the blends of volatiles from leaves of four gerbera cultivars with spider mite damage

We incubated detached leaves of the gerbera cultivars Bianca, Fame, Rondena and Sirtaki, with adult female spider mites following the procedure described for experiment 1. The fresh weight of the leaf samples was 34.6 ± 1.96 (n=5), 29.7 ± 3.30 (n=6), 32.8 ± 1.12 (n=6) and 33.3 ± 3.73 (n=4) gram (mean ± sd) for Bianca, Fame, Rondena and Sirtaki respectively. Two additional leaf samples were taken for Sirtaki with a smaller total weight (15.7 and 23.3 g) because part of the leaves had wilted during incubation and were discarded. After 7 days we counted the spider mite eggs that were present on the leaves to determine the amount of damage on the leaves. Subsequently, the leaves were placed with their petioles in a 200 ml glass vial containing tap water. The vial with leaves was placed in a 5 litre glass jar with a 10 cm ID opening. The jar was closed with a glass lid that contained two openings. Through one of the openings a glass tube was placed, which was used as air-inlet. This glass tube nearly reached the bottom of the jar. An air stream of 450 ml/min was generated in the flask. Before reaching the inlet, the air was cleaned through silica gel, molecular sieves and activated charcoal, following the procedure described by Mattiacci et al. (1994). Air was purged through the system for 1 h prior to collection of volatiles, in order to remove all contaminants from the jar. Subsequently, a Pyrex glass tube (161 x 6.4 mm OD, ID 3 mm) containing 90 mg Tenax-TA was connected to the outlet of the system. The volatiles from the Tenax-tubes were analysed by GC/MS, using the Thermodesorption Cold Trap Unit as inlet on the gas chromatograph (Mattiacci *et al.*, 1994).

The samples were taken in batches between April 1997 and November 1998. For all samples of Bianca, Fame and Sirtaki and one sample of Rondena the column used was a Supelcowax 10 fused silica capillary column, 60 m x 0.25 mm ID, 0.25 μm film thickness, with helium as carrier gas at an initial linear velocity of 22 cm/s. The oven temperature was raised from 40 °C to 270 °C with 4 °C/min. For the other Rondena samples a Restek Rtx200 column was used, 60 m x 0.25 mm ID, 0.25 μm film thickness. The oven temperature was raised from 40 °C to 250 °C with 4 °C/min. Compounds were identified by comparison of the mass spectra with those in the Wiley library and in the Wageningen Mass Spectral Database of Natural Products and by checking the retention index. One additional sample of Sirtaki was analysed on a BP5 column and was only used to verify the identification of some sesquiterpenoids by comparison with the retention indices as described in Adams (1989) and Joulain and Koenig (1998). An approximate measure of the sensitivity of the GC-MS was obtained by regular quantification of the peak areas of a mixture of standard compounds. The sensitivity was variable between batches of samples and was approximately 0.26, 0.10, and 0.05 ng/peak area unit for the three batches.

RESULTS

Experiment 1: Preference of *P. persimilis* for the volatiles of certain gerbera cultivars with spider mite damage

In Fig. 1 three pairwise cultivar comparisons in attractiveness of spider mite-induced volatiles are shown. Each pairwise comparison consists of a number of olfactometer test. Each point in Fig. 1 represents a separate olfactometer test with 20 predators and a separate set of spider mite-infested gerbera leaves. For each set of leaves we have estimated the amount of spider mite damage by counting the number of spider mite eggs that were present on the leaves. The fraction of eggs on Sirtaki (eggs on Sirtaki/(eggs on Sirtaki + eggs on other cultivar)) is plotted on the x-axis and the fraction of predators that chose for the volatiles of Sirtaki (predators to Sirtaki/ (predators to Sirtaki +predators to other cultivar)) is plotted on the y-axis. One would expect that more damage on one of the cultivars would result in more predators that choose for the volatiles of that cultivar. In the absence of preference for one of the two cultivars one would expect the points in the left-lower quart and right-upper quart of the graphs. On the right of each graph the pooled results for each pairwise cultivar comparison is shown. The percentage of all predators to each of the two cultivars is given.

In the comparison between cultivars Sirtaki and Rondena (Fig 1A) most dots fall within the lower-right quart of the graph, which means that although the leaves of Sirtaki contained more spider mite eggs, the predators still chose for Rondena leaves in the Y-tube olfactometer. The null hypothesis of equal attractiveness of the two cultivars could be rejected (P=0.004). In total, 63% of the 179 predators chose for Rondena. The amount of damage on Sirtaki was positively, though not significantly (P=0.07) related to the proportion choosing for this cultivar.

The same trend was found in the comparison between cultivars Sirtaki and Bianca (Fig. 1B). Although more eggs were present on Sirtaki leaves, the predators had a tendency to choose for the volatiles of Bianca leaves. This preference was close to significance (P=0.08). Of all the 245 predators, 60% chose for Bianca. Again, l_x showed a positive though not significant relationship (P=0.27) with the proportion of predators chosing Sirtaki.

When the predators were given the choice between spider mite-induced volatiles from cultivars Sirtaki and Fame, they did not show any preference (P=0.63; Fig. 1C). In total, 54% of the 132 predators chose for

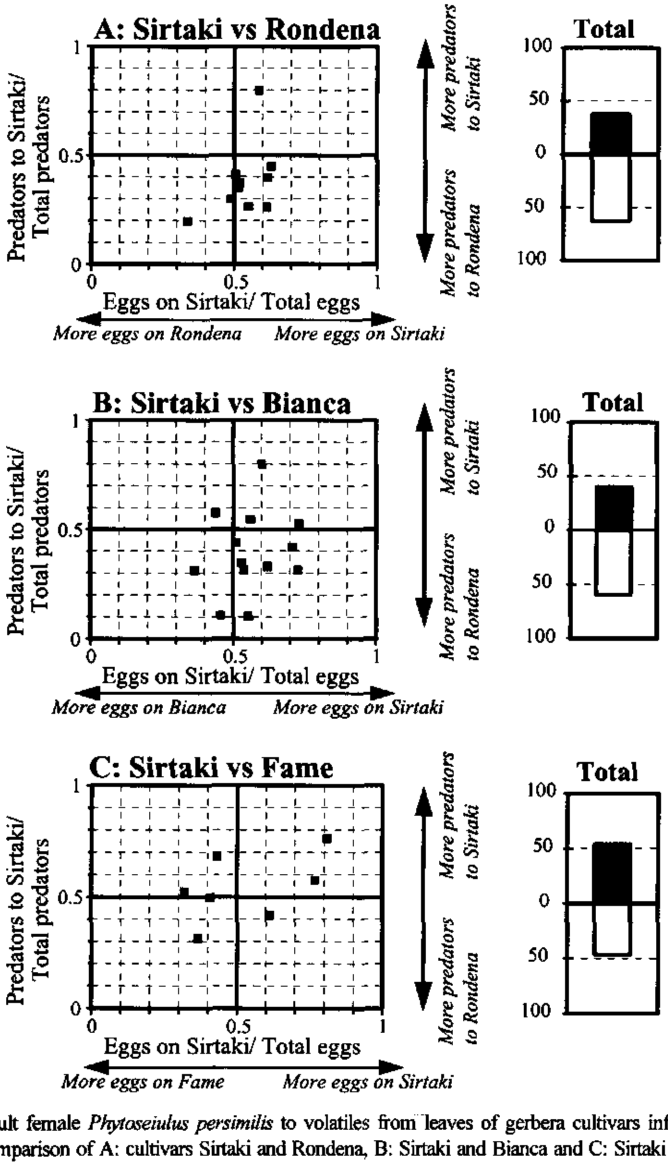

Fig. 1: Response of adult female *Phytoseiulus persimilis* to volatiles from leaves of gerbera cultivars infested by *Tetranychus urticae*. Two choice comparison of A: cultivars Sirtaki and Rondena, B: Sirtaki and Bianca and C: Sirtaki and Fame. Each data point represents a Y-tube olfactometer test in which 20 adult female *P. persimilis* chose between volatiles from damaged leaves of two cultivars. In each olfactometer test we determined the number of *T. urticae* eggs that were present on the leaves and used that as a measure of herbivore damage. Damage on both cultivars was compared with the preference of *P. persimilis*. On the X-axis the number of spider mite eggs (as a measure of damage) on the set of leaves of Sirtaki, divided by the number of spider mite eggs on the leaves of both cultivars is shown. On the Y-axis the number of predators that preferred Sirtaki volatiles, divided by the total number of predators in the Y-tube olfactometer test is shown. On the right of each graph the pooled results for each pairwise cultivar comparison is shown. The percentage of all predators to each of the two cultivars is given.

Sirtaki. Again, l_x had a positive regressioncoefficient, which was not significantly different from zero (P=0.10).

If we analyse all data together, allowing for different success rates for different experimental setups, but with a common effect of amount of damage l_x and a common overdispersion factor, we find the following significant results for Sirtaki versus Rondena (P=0.01) and Sirtaki versus Bianca (P=0.01). No significant preference for Sirtaki versus Fame (P=0.70) was found. The amount of damage l_x showed a significant positive relationship with the rate of predators choosing Sirtaki (P=0.05).

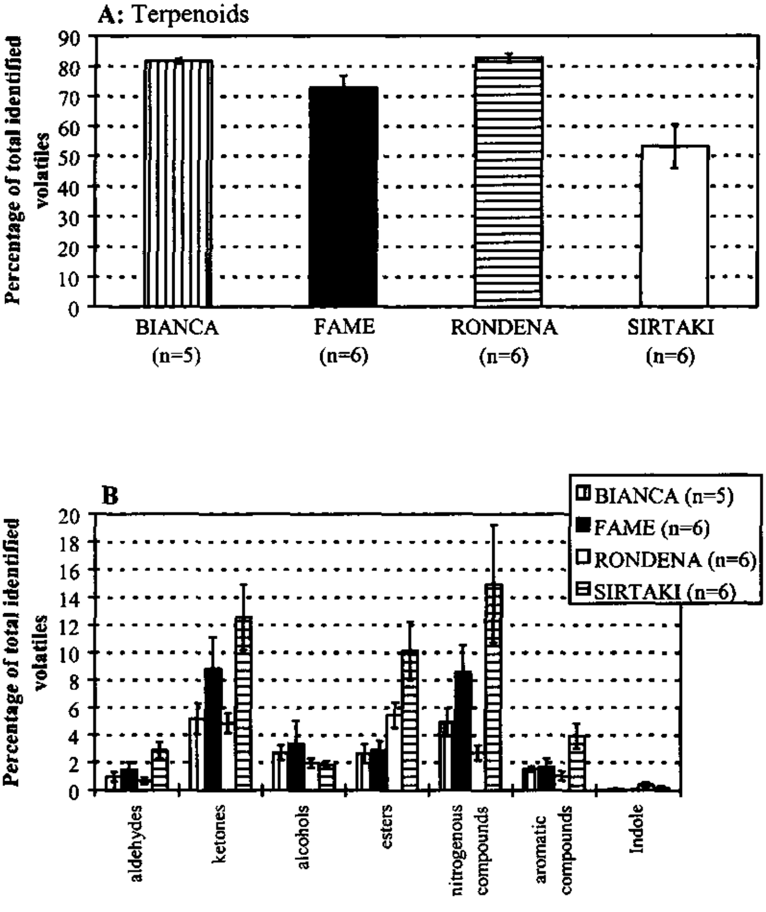

Fig. 2: Volatiles of four gerbera cultivars with *Tetranychus urticae* damage, divided into chemical groups. A: percentage of terpenoids, B: percentages of the remaining groups. Vertical lines represent standard errors.

Experiment 2: Chemical composition of the volatile blend from leaves of four gerbera cultivars with spider mite damage

The volatiles that are emitted by spider mite-damaged leaves of cultivars Bianca, Sirtaki, Fame and Rondena are shown in Table 1. Since the total production of volatiles was variable, we calculated the relative production for each compound, based on the total amount of identified compounds. An approximation of the total production of volatiles by each cultivar is given in Table 1.

The major part of the volatile blends of the four cultivars consists of terpenoids (Fig. 2A). Sirtaki produces the lowest relative amount of terpenoids which is reflected in the highest relative production of aldehydes, ketones, acetates, nitrogenous compounds and aromatic compounds (Fig. 2B). Fame has a higher relative production of ketones and compounds that contain nitrogen than Bianca and Rondena, while Rondena produces more esters than the other two cultivars.

The compounds that represent more than 5% of the average total volatile blend of at least one cultivar are presented in Fig. 3. The major compound is the homoterpene 4,8-dimethyl-1,3 *(E)*, 7-nonatriene with a relative production that varies from 18% on Bianca to 26% on Sirtaki. Another homoterpene, 4,8,12-trimethyl-1,3*(E)*, 7*(E)*, 11-tridecatetraene, has a relative production that varies from 6.7% on Bianca to 14% on Fame.

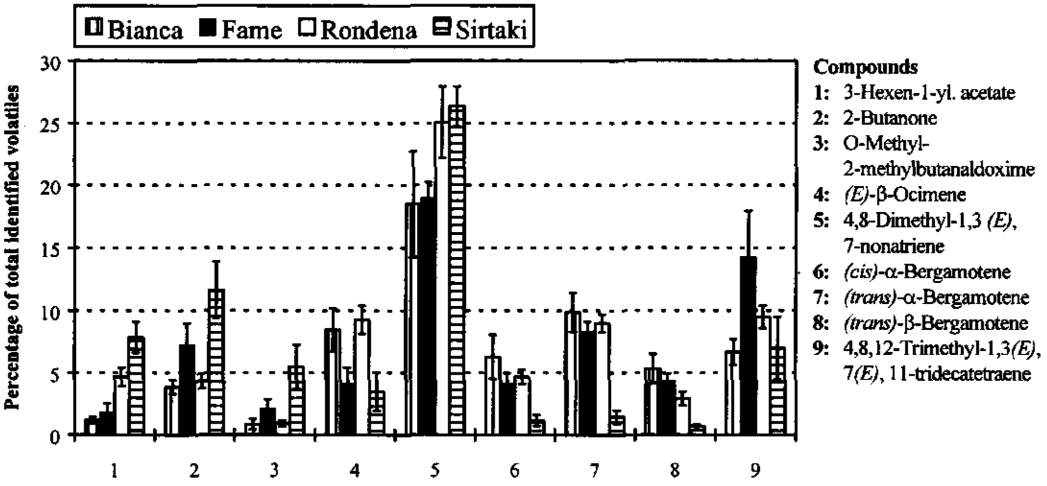

Fig. 3: Volatiles produced by leaves of four gerbera cultivars with damage of *Tetranychus urticae*. For each compound the average peak area divided by the total peak area of identified compounds is shown. Only compounds that represent at least 5% of the total volatile blend of a cultivar are presented. Vertical lines represent standard errors.

The most striking cultivar differences between the blends of the four cultivars are found in the production of *cis*-α-bergamotene, *trans*-α-bergamotene and *trans*-β-bergamotene. These three compounds each make up around 1% of the blend of Sirtaki whereas they represent around 5%, 9% and 4% of the blends of the other cultivars, respectively (Fig 3). The near absence of these compounds accounts to a large extent for the low total production of terpenoids by Sirtaki. The production of the terpenoid *(E)*-β-ocimene is around 4% in Sirtaki and Fame while it represents more than 8% in the blends of the other two cultivars. Furthermore, *(E)*-β-farnesene is almost absent in the Sirtaki blend whereas it represents between 1.4 and 3.3% of the blends of the other cultivars (Table 1).

The relative production of *(Z)*-3-hexen-1-yl acetate, 2-butanone and 2-methylbutanal-O-methyloxime is higher in Sirtaki than in the other three cultivars (Fig.3). Furthermore, Sirtaki has a higher production of 2-methylpropanenitrile and benzaldehyde (Table 1).

The total volatile production by Sirtaki was less than half of the production by the other three cultivars (Table 1). The number of spider mite eggs found on this cultivar was also about half of what was found on Fame and Rondena. Hence, the difference in total volatile production between Sirtaki as compared to Fame and Rondena can be explained by a difference in spider mite damage. However, the number of eggs on Sirtaki and Bianca is in the same order of magnitude while the total production of volatiles of Sirtaki is less than half of the production by Bianca. This difference in total production of volatiles can, therefore, not be explained by a difference in spider mite damage.

Table 1: Identification of chemicals in the headspace of detached gerbera leaves of four cultivars that have been infested for 7 days by the spider mite *T. urticae*. For each compound the percentage (mean ± SE) of the total amount of identified volatiles is given. Only compounds that represent at least 0.1% of the blend of one cultivar are presented. n.d. = not present in detectable levels in any of the samples of this cultivar

	Percentage (mean ± SE) in headspace of:			
Volatile compound	**Bianca (n=5)**	**Fame (n=6)**	**Rondena (n=6)**	**Sirtaki (n=6)**
Aldehydes				
2-Methyl-2-propenal	0.05±0.048	0.6±0.36	n.d.	1.5±0.79
Hexanal	0.007±0.0068	0.06±0.060	0.13±0.045	0.014±0.0136
***(E)*-2-Hexenal**	n.d.	0.17±0.175	0.021±0.0151	n.d.
Nonanal	0.28±0.093	0.15±0.099	0.23±0.094	0.41±0.219
Decanal	0.64±0.274	0.45±0.207	0.22±0.119	0.89±0.299
Alcohols				
Ethanol	0.10±0.062	n.d.	n.d.	n.d.
2-Butanol	0.30±0.040	0.36±0.086	0.30±0.050	1.14±0.194
2-Methyl-1-propanol	n.d.	0.15±0.095	0.013±0.0092	0.20±0.098
1-Butanol	0.09±0.045	0.19±0.129	0.017±0.0166	n.d.
1-Hexanol	0.14±0.113	0.6±0.34	0.10±0.044	n.d.
***(Z)*-3-hexen-1-ol**	0.56±0.226	1.8±1.10	0.72±0.236	0.08±0.056
***(E)*-2-hexen-1-ol**	n.d.	0.11±0.113	n.d.	n.d.
1-Dodecanol	0.07±0.046	n.d.	n.d.	0.12±0.080
Esters				
Ethyl acetate	0.06±0.038	0.7±0.44	0.025±0.0249	0.21±0.124
Methyl 2-methylbutanoate	0.017±0.0167	n.d.	n.d.	0.13±0.042
Methyl 3-methyl-2-butenoate	1.1±0.50	0.15±0.104	n.d.	1.1±0.36
***(Z)*-3-Hexen-1-yl acetate**	1.20±0.246	1.8±0.73	4.7±0.75	7.9±1.24
3-Methylbutyl 3-methylbutanoate	n.d.	n.d.	n.d.	0.15±0.118
***(Z)*-3-Hexen-1-yl 3-methylbutanoate**	0.09±0.039	0.05±0.047	0.47±0.146	0.32±0.227
***(Z)*-3-Hexen-1-yl tiglate**	n.d.	n.d.	0.15±0.038	n.d.
Ketones				
2-Butanone	3.9±0.57	7.3±1.73	4.3±0.58	11.6±2.23
3-Buten-2-one	0.11±0.070	0.13±0.065	n.d.	0.26±0.180

Table 1: Continued.

	Percentage (mean ± SE) in headspace of:			
Volatile compound	**Bianca (n=5)**	**Fame (n=6)**	**Rondena (n=6)**	**Sirtaki (n=6)**
2- or 3-Pentanone	0.008±0.0081	0.14±0.072	0.042±0.0205	0.13±0.072
3-Penten-2-one	0.30±0.202	0.59±0.234	0.10±0.066	0.21±0.105
6-Methyl-5-hepten-2-one	0.114±0.0286	0.08±0.051	0.011±0.0110	0.10±0.066
4-Hydroxy-2-pentanone	0.8±0.51	0.6±0.39	0.39±0.141	n.d.
Unidentified keton 1)	0.021±0.0212	0.012±0.0118	n.d.	0.17±0.098
Nitrogenous compounds				
2-Methyl-2-propenenitrile	0.77±0.130	0.39±0.193	0.27±0.079	1.15±0.253
2-Methylpropanenitrile	1.36±0.119	1.94±0.195	0.43±0.078	4.0±1.16
2-Methylbutanenitrile	0.34±0.077	0.54±0.134	0.14±0.037	1.0±0.42
Unidentified nitriles 1)	0.30±0.065	0.32±0.119	0.16±0.057	0.52±0.195
2-Methyl-1-nitropropane	0.44±0.086	0.60±0.108	0.113±0.0249	0.51±0.178
Isomer of previous compound	0.10±0.084	0.18±0.094	0.004±0.0044	0.37±0.187
O-Methyl-2-methylpropanaldoxime	0.7±0.40	1.3±0.33	0.55±0.180	1.7±0.37
O-Methyl-2-methylbutanaldoxime	0.028±0.0281	0.15±0.072	n.d.	0.31±0.150
Isomer of previous compound	0.9±0.41	2.2±0.72	0.93±0.240	5.5±1.75
2-Methylpropanaldoxime	0.04±0.036	0.6±0.37	0.11±0.049	0.027±0.0271
2-Methylbutanaldoxime	n.d.	0.4±0.39	0.016±0.0155	n.d.
Aromatic compounds				
Benzaldehyde	0.9±0.31	1.0±0.47	0.81±0.272	3.8±0.97
Benzonitrile	n.d.	0.16±0.104	n.d.	0.11±0.070
Methyl benzoate	0.16±0.125	0.11±0.086	0.077±0.0154	0.03±0.035
Methyl salicylate	0.44±0.118	n.d.	0.015±0.0037	0.005±0.0054
2-Methylpropyl-benzoate	n.d.	0.33±0.209	n.d.	n.d.
***(Z)*-3-Hexen-1-ol benzoate**	n.d.	n.d.	0.13±0.075	n.d.
Terpenes				
Sabinene	0.15±0.041	n.d.	n.d.	n.d.
Myrcene	0.136±0.0256	n.d.	0.040±0.0130	n.d.
Limonene	0.256±0.0252	0.21±0.069	0.046±0.0215	0.05±0.034
***(Z)*-β-Ocimene**	0.31±0.056	0.11±0.054	0.137±0.0186	0.09±0.044
***(E)*-β-Ocimene**	8.5±1.71	4.2±1.27	9.3±1.12	3.5±1.54
4,8-Dimethyl-1,3 *(Z)*, 7-nonatriene	0.72±0.165	0.53±0.127	0.24±0.109	1.03±0.215
4,8-Dimethyl-1,3 *(E)*, 7-nonatriene	18±4.2	19.0±1.29	25.1±2.88	26.4±1.61
Linalool	0.9±0.40	0.12±0.119	1.46±0.277	0.6±0.32
***(E)*-Nerolidol**	1.4±0.42	0.08±0.079	0.8±0.35	0.19±0.093
α-Copaene	0.03±0.030	n.d.	0.084±0.0184	1.03±0.244
β-Cubebene	n.d.	0.04±0.037	n.d.	0.9±0.42
***cis*-α-Bergamotene**	6.3±1.77	4.2±0.78	4.7±0.57	1.2±0.43
***trans*-α-Bergamotene**	9.9±1.54	8.3±0.84	9.0±0.73	1.5±0.54
β-Elemene	2.6±1.14	1.7±0.39	2.0±0.45	2.3±0.30
β-Caryophyllene	2.9±0.46	3.9±0.94	3.8±0.44	2.4±0.41
β-Santalene	0.10±0.067	n.d.	0.010±0.0098	n.d.
***(E)*-β-farnesene**	3.3±0.71	1.4±0.31	2.2±0.34	0.05±0.039
α-Humulene	0.11±0.084	0.32±0.229	0.39±0.073	0.16±0.083
***trans*-β-Bergamotene**	5.3±1.16	4.4±0.58	3.0±0.58	0.72±0.082

Volatile compound	Percentage (mean ± SE) in headspace of: Bianca (n=5)	Fame (n=6)	Rondena (n=6)	Sirtaki (n=6)
γ-Curcumene	n.d.	n.d.	0.12±0.43	n.d.
α-Zingiberene	2.7±0.58	0.26±0.260	2.7±0.51	n.d.
Germacrene D	1.0±0.35	1.4±0.72	1.40±0.254	1.3±0.60
β-Bisabolene	1.3±0.51	0.18±0.114	1.5±0.46	n.d
(Z,E)-α-Farnesene	0.06±0.057	n.d.	n.d.	0.20±0.099
(E,E)-α-Farnesene	3.03±0.133	3.1±0.50	2.3±0.39	2.3±0.88
β-Sesquiphellandrene	1.82±0.296	1.1±0.45	0.99±0.147	n.d.
ar-Curcumene	1.6±0.48	1.7±0.51	0.91±0.087	n.d.
Isomer of next compound	0.14±0.141	0.08±0.083	0.25±0.075	0.39±0.175
4,8,12-Trimethyl-1,3*(E)*, 7*(E)*, 11-tridecatetraene	6.7±1.02	14±3.7	9.5±0.89	7.0±2.51
(Z,E,E)-Allofarnesene	0.32±0.116	n.d.	0.008±0.0079	n.d.
Unidentified terpenes [1]	3.0±1.37	2.6±1.06	1.8±0.37	0.41±0.200
Indole	0.09±0.033	0.062±0.0133	0.39±0.170	0.23±0.082
Total	99.6%	99.5%	99.6%	99.5%
Unidentified compounds (% of total peak area)	4.1±1.47	7.4±2.78	1.53±0.208	9.6±2.98
Approximate total volatile production [2] **(ng)**	4456±1137	5645±3339	5057±2282	1672±151 [3]
Average number of spider mite eggs per sample	4379±814	7349±2450	8024±904	3706±410

[1] These unidentifiable compounds were present in many samples and are therefore not excluded from calculation of the total amount of volatiles.

[2] *Based on the approximate sensitivity of the GCMS analysis per batch.*

[3] Two samples with low total leaf weight of Sirtaki excluded.

DISCUSSION

In this study we have presented a method to compare the attractiveness of volatiles produced by spider mite-damaged leaves of four gerbera cultivars without ignoring differences in damage on the leaves as a result of differences in host plant resistance. We based our method on the assumption that attraction of *P. persimilis* is positively related to the amount of spider mite damage on gerbera leaves and found strong indications that such a relation indeed exists. In addition, previous studies showed that the response of *P. persimilis* to volatiles from spider mite-damaged bean leaves increases with the number of damaged leaves offered (Sabelis and van de Baan, 1983). Hence, the total amount of damaged leaf area is important for attraction of the predators. But as the data in our paper demonstrate, the attractiveness of volatiles from spider mite-damaged leaves is also determined by the amount of damage per leaf.

We showed that *P.persimilis* has a preference for spider mite-induced Rondena and Bianca volatiles over those of Sirtaki, while no difference in attractiveness of Sirtaki and Fame volatiles was found. The predators that were used in the experiments had been reared on spider mites on Sirtaki. The response of *P. persimilis* to spider mite-induced volatiles of new a host plant is enhanced by experience (Takabayashi *et al.*, 1994; Krips *et al.*, 1999). However *P. persimilis* does not prefer the volatiles of Sirtaki over those of other cultivars. Hence, the differences in attractiveness of volatiles that were found in this study can not be explained by rearing history or experience of the predators.

A low attractiveness of Sirtaki volatiles as compared with the volatiles of Rondena and Bianca can be the result of a lower production of volatiles by Sirtaki leaves, a different composition of the volatile blend, or a combination of the two. The results of the chemical analyses indicate that at equal damage the total production of volatiles by Sirtaki leaves is lower than by Bianca leaves. However, this does not seem to be the case in the comparison between volatiles of Sirtaki leaves and Rondena leaves. Hence, the composition of blend of volatiles is also important for the attractiveness to *P. persimilis.*

The blend of spider mite-damaged Sirtaki leaves consists of a much lower relative amount of terpenes than the blends of the other three cultivars. Similarly, Loughrin *et al.*, (1995) found that differences in volatile production of cotton cultivars were mainly based on differences in the production of terpenes. The low production of terpenes by gerbera cultivar Sirtaki in our studies is likely responsible for the low attractiveness of the blend to *P. persimilis.*

The low production of terpenes by Sirtaki leaves was mainly the result of a very low production of *cis*-α-bergamotene, *trans*-α-bergamotene, *trans*-β-bergamotene and *(E)*-β-farnesene. Possibly, the low production of these compounds is related to the relative low attractiveness of the blend of Sirtaki leaves. However, Fame has a much higher production of these three compounds, whereas the blend of this cultivar is equally attractive for *P. persimilis* as the blend of Sirtaki. This is an argument against the responsibility of these three compounds for the cultivar differences in attractiveness to *P. persimilis.*

The relative production of *(E)*-β-ocimene is lower in the blends of both Sirtaki and Fame leaves. Interestingly, this compound is known to be attractive for *P.persimilis* when offered as an individual compound in a Y-tube olfactometer (Dicke *et al.*, 1990b) and may therefore be partly responsible for the low attractiveness of Sirtaki volatiles in comparison to those of Rondena and Bianca.

Loughrin et al. (1996) found that cultivar differences of volatiles from undamaged apple leaves in attractiveness for the Japanese beetle *Popillia japonica* were related to the production of linalool. This compound is attractive to *P. persimilis* (Dicke *et al.*, 1990b). In our studies, the relative production of linalool was lowest for the relatively unattractive Fame leaves and highest for the attractive Rondena leaves. The relative production of linalool by Sirtaki and Bianca leaves was in the same order of magnitude. However, at equal damage, the absolute amount of volatiles produced by Bianca was approximately twice as high as the production by Sirtaki. Therefore, the absolute production of linalool was also higher by Bianca leaves than by Sirtaki leaves. This indicates that the cultivar differences in attractiveness of gerbera volatiles may be related to differences in the production of linalool.

Loughrin et al. (1996) also found indications that the homoterpene 4,8-dimethyl-1,3 *(E)*, 7-nonatriene played a role in the differences in attractiveness. This compound is also attractive for *P. persimilis* (Dicke *et al.*, 1990b). The relative and absolute production of this homoterpene was equal by the cultivars Sirtaki and Rondena, although the attractiveness of the blends differed. Furthermore, the attractive cultivar Bianca had a lower relative production of this compound than the unattractive cultivar Sirtaki. Therefore, the cultivar differences in attractiveness can not be explained by differences in the production of this homoterpene.

The relative production of 2-butanone, *(Z)*-3-hexen-1-yl-acetate, 2-methylpropanenitrile and 2-methylpropanal-O-methyloxime is higher in the leaves of Sirtaki than in the leaves of the other three cultivars. *(Z)*-3-hexen-1-yl-acetate is neither attractive nor repellent for *P. persimilis* (Dicke *et al.*, 1990b) and the effect of the other compounds is not known. However, Takabayashi et al. (1994) found two other oximes in the headspace of old cucumber leaves with spider mite damage that were not attractive for *P. persimilis*. Young cucumber leaves did not produce these compounds in reaction to spider mite damage and the volatile blend of these leaves did attract *P. persimilis*. Possibly, oximes play a role in the low attractiveness of the volatile blend of old cucumber leaves with spider mite damage and maybe also with the relatively low attractiveness of the blend of Sirtaki leaves.

In conclusion, interesting differences exist in preference of *P. persimilis* for the blends of spider mite damaged gerbera cultivars and the composition of these blends. For the interpretation of these results it will be necessary to investigate to which extent the differences in preference are related to differences in behaviour of the predators on spider mite infested plants. In the introduction we explained the importance of arrestment of *P.persimilis* in a spider mite patch until all prey are exterminated locally. If only a slight fraction of the predators leaves the prey patch before all prey is exterminated, the prey population would reach much higher numbers and the moment all prey is eliminated by the predators would be postponed (van Baalen and Sabelis, 1995).

It may be that that the less preferred volatile blends of Sirtaki and Fame are related to a lower arrestment response of the predators on these cultivars. If this is the case, the success of biological control on these cultivars will be much lower. On the other hand, it is possible that the production of volatiles by all four cultivars is above a threshold above which *P.persimilis* is arrested until prey extermination. Therefore, it will be necessary to investigate in future greenhouse studies whether the arrestment response of the predators is related to the preference of the predators for the volatile blends of certain cultivars and to the composition of these blends.

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CHAPTER 8

EFFECT OF PLANT CHARACTERISTICS ON BIOLOGICAL CONTROL OF SPIDER MITES ON GERBERA

SUMMARY

Plant cultivars may differ in traits that influence the success of biological control. We investigated which cultivar characteristics of the ornamental crop *Gerbera jamesonii* Bolus (Asteraceae) affect biological control of the spider mite *Tetranychus urticae* (Acarina: Tetranychidae) Koch with the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). We focussed on cultivar differences in resistance to *T. urticae* and trichome density on the undersurface of leaves. In addition, the predation rate and population growth rate of the predators on gerbera were studied. We performed a population experiment with spider mites and their predators on two cultivars, Bianca (218 trichomes/cm^2) and Bourgogne (630 trichomes/cm^2). The results were compared with predictions of a model that describes the population dynamics of spider mites and their predators.

Predators were introduced in a predator-prey ratio of 1: 40. As model input values for population growth rates of *T. urticae* and *P. persimilis* we used r_m values that were found in life-history studies on gerbera (Krips *et al.*, 1998; 1999a). The r_m of *T. urticae* is 0.144/day on cultivar Bourgogne. On cultivar Bianca, the r_m of *T. urticae* varied from 0.088 to 0.168/day and we used both values in model simulations. The r_m of *P. persimilis* is 0.43/day. The predation rate was estimated to be 3.8 prey/predator/day, based on the maximal predation rate on gerbera (Krips *et al.*, 1999b). No dispersal of prey and predators was assumed.

The model predicted spider mite extermination on both cultivars to occur within approximately four days after predator introduction, while this took two weeks in reality. The deviations of predictions from reality were not due to errors in the population growth rates of *T. urticae*, since the measured population growth rates on the plants were close to the r_m values and were 0.139/day on Bourgogne and 0.113/day on Bianca. However, the measured predator population growth rates were much lower than the r_m of 0.43/day. These were 0.326/day on Bianca and 0.285/day on Bourgogne. Using the model, the predation rates were estimated to be 2.2 prey/predator/day on Bianca and 2.1 prey/predator/day on Bourgogne.

The low predator population growth rates may have resulted from low predation rates, since prey density on gerbera may be low. Alternatively, predators may have dispersed from the set-up, possibly because of a low response to volatiles from spider mite-damaged gerbera plants. The difference in predator population growth rates indicates a negative influence of trichome density. An alternative explanation may be a difference in predator dispersal as a result of unequal attraction to volatiles from the cultivars with spider mite damage.

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INTRODUCTION

The spider mite *Tetranychus urticae* Koch is an important pest on many greenhouse crops (van de Vrie, 1985; Hussey and Scopes, 1985). On vegetables it can be successfully controlled with the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Helle andSabelis, 1985). On ornamentals, where aesthetic damage is not tolerated, biological control is not always possible. The ornamental crop gerbera (*Gerbera jamesonii* Bolus) might, however, be a suitable crop for biological control since gerbera cutflowers are sold without leaves, and spider mites usually do not damage the flowers (van de Vrie, 1985).

Many gerbera cultivars are commercially available, which are, at present, mainly selected for colour and shape of the flowers. However, they also differ in a number of other host plant characteristics. Some of these may affect the success of biological control of spider mites with *P.persimilis.* Earlier studies showed that the success of natural enemies in biological control can be influenced strongly by characteristics of the host plant (Bergman and Tingey, 1979, Boethel and Eikenbary, 1986; van Lenteren and de Ponti, 1990; Hare, 1992; Walter, 1996).

One of the host plant characteristics that varies largely among gerbera cultivars is resistance to *T. urticae* (Krips *et al.*, 1998). The intrinsic rate of population increase, r_m, of *T. urticae* varies among gerbera cultivars from 0.088/day to 0.242/day. Laboratory studies showed that the r_m of *P. persimilis* is unaffected by resistance to *T. urticae* in gerbera (Krips *et al.*, 1999a). Because the growth rate of the predators is higher than that of its prey, the difference in population growth rates between prey and predators is largest on resistant cultivars. Biological control of *T. urticae* may therefore be more successful on such cultivars.

Gerbera cultivars also differ in the density of trichomes on the under surface of the leaves. The walking speed of *P. persimilis* is negatively affected by trichome density and a lower rate of encounter with prey is found on cultivars with a high trichome density (Krips *et al.*, 1999b). When prey density is low, this negative effect of trichome density on the encounter rate leads to a lower predation rate (Krips *et al.*, 1999b). However, at high prey density, the predation rate is limited by the rate at which the predator's gut is emptied, rather than by the rate of encounter with prey (Sabelis, 1981, 1986). Therefore, trichomes do not affect the predation rate when prey density is high. Hence, the effect of trichomes on the predation rate is dependent on the prey density that the predators actually experience.

Above we have described two host plant characteristics that may influence the success of biological control of spider mites on gerbera: resistance to spider mites and trichome density. To determine whether these plant characteristics indeed affect the dynamics between spider mites and their predators on gerbera, we conducted a population growth experiment with *T. urticae* and *P. persimilis* on two cultivars. In addition, we focussed on two features of *P. persimilis* that may be influenced by host plant characteristics, which will be explained below.

Spider mites have a patchy distribution and form local colonies on leaves. Since the predators spend much more time searching for prey within colonies than travelling between colonies, the prey density within local prey colonies is more relevant for predator-prey dynamics than for example the average prey density on leaves. On suitable host plants like bean and rose, prey colonies are large and prey density within colonies is high, which makes it reasonable to assume that predator-prey dynamics on these host plants are characterised by high predation rate and maximal population growth rate of the predators (Janssen and Sabelis, 1992; Sabelis, 1992; Sabelis and Janssen, 1994). In contrast, on gerbera plants colonies of *T. urticae* appear to be small and more dispersed. This may lead to lower predation rates and population growth rates of *P.persimilis* on gerbera.

Hence, in the present study we have investigated the effect of the two host plant characteristics resistance to spider mites and trichome density and the two predator features predation rate and population growth rate. To determine the effect of trichome density we used two cultivars that differ largely in this charateristic. For the effect of the other three factors we compared the data with the predictions of a model that was developed to describe the within-patch dynamics between *T. urticae* and *P. persimilis* (Diekmann *et al.*, 1988; Janssen and Sabelis, 1992; Sabelis, 1992; van Baalen and Sabelis, 1995).

All parameters used as input in the model have been estimated in previous studies with *T. urticae* and *P. persimilis* on gerbera. Data on the population growth rates of *T. urticae* and *P. persimilis* were obtained from life-history studies on gerbera cultivars (Krips *et al.*, 1998; 1999a). Data on the predation rate of *P. persimilis* were estimated from the maximal predation rate on gerbera (Krips *et al.*, 1999b). Comparing the data obtained in the population growth experiment with model predictions will give us information on the actual population growth rates of prey and predators on the two gerbera cultivars and the predators' predation rates. Furthermore, we can simulate the effect of resistance to *T. urticae* on the predator-prey dynamics by varying the population growth rate of the spider mites as model input.

METHODS AND MATERIALS

Plant material

Gerbera (*Gerbera jamesonii*) plants were obtained from a commercial gerbera breeding company (Prego Rijsenhout, The Netherlands). They were subsequently grown in a greenhouse at the Laboratory of Entomology (Wageningen University, The Netherlands) at 20-30°C, 50-70% RH and a photoperiod of at least 16 h of light. High pressure mercury lamps were switched on when the light intensity outside dropped below 150 watt/m^2 and switched off when it increased above 250 watt/m^2. Plants used for the experiments were 6 to 12 months old. Cultivars used were Bianca and Bourgogne with a trichome density on the undersurface of the leaves of 218 ± 60 and 630 ± 154 trichomes/cm^2 (mean ± sd) respectively.

Spider mites

For the greenhouse experiment we used a colony of spider mites (*Tetranychus urticae*) that were collected in a commercial gerbera greenhouse in Mijdrecht, The Netherlands in the spring of 1994. These mites were reared in our laboratory on gerbera cultivar Sirtaki. Plants with spider mites were kept in a greenhouse at Wageningen University, The Netherlands under the same conditions as the gerbera plants. Spider mites used as food for the predatory mites were reared on Lima bean and had been kept on this plant species for at least 5 years.

Predatory mites

Predatory mites (*Phytoseiulus persimilis*) were originally obtained from Entocare, a commercial mass rearing company for biological control agents. In our laboratory they were reared on spider mites (*T. urticae*) on Lima bean plants. The predators were kept in closed Petri-dishes of 9 cm diameter and were offered pieces of leaves with spider mites three times a week. Once a week, a new colony was started by transferring 5 gravid female *P. persimilis* from each Petri-dish to new ones. The Petri-dishes were kept in a climate room at 23±1°C, 60±5% RH and a photoperiod of L:D=16:8.

Population growth experiment

Seven groups of four Bianca plants and eight groups of five Bourgogne plants, were placed in climate rooms and kept at 25±1°C, >65% RH, L:D=16:8 and 8000 Lux. This was the highest light intensity possible in our climate rooms. For each cultivar one climate room was used. On each group of plants 200 *T. urticae* individuals were introduced in an age distribution near to the stable age distribution. For Bianca this resulted in 92 eggs, 82 juveniles (larvae, protonymphs and deutonymphs), 20 females and 6 males. For Bourgogne this was 114 eggs, 72 juveniles, 11 females and 3 males. These distributions were calculated using data from life-history studies with *T. urticae* on the same gerbera cultivars (Krips *et al.*, 1998), following the method of Carey (1993). For each group of plants the 200 spider mites were placed on one central leaf which was marked with a label attached with a thread to the leaf petiole.

Every two days, the spider mite population on each group of plants was monitored by counting the number of adult female spider mites only. Exponential lines were fitted through the data for each group of plants separately and with the equation $N_t=N_0e^{(rt)}$ the growth rates of the populations on each group of plants were calculated. N_0 is the initial number of adult female spider mites, N_t is the number at time t, and r is the population growth rate. Part of the spider mites disappeared from the plants immediately after introduction. Therefore, the first three data points were excluded from calculations of the population growth rates.

When the total size of the spider mite population reached an average of approximately 1500 individuals, predatory mites were introduced. The moment of predator introduction differed between the two cultivars because of a difference in host plant resistance to *T. urticae.* For each group of plants, we counted the number of adult female spider mites, estimated the total spider mite population on that group and divided that number by 40 to obtain the number of predators that should be introduced on that plant group. Predatory mites were hence introduced in a predator-prey ratio of 1:40. They were also introduced in a distribution near to the stable age distribution: 58.3% eggs, 34.9% juveniles, 6.7% adults divided into 5.9% adult females en 0.8% males. This was calculated following the method of Carey (1993), using data from life-history studies with *P. persimilis* on gerbera (Krips *et al.*, 1999a). Since adult female *P. persimilis* have to mate for the production of eggs, a minimum number of two males was introduced on each group of plants. Males do not contribute to the growth of the predatory mite population.Therefore the actual number of introduced males is unimportant as long as all females are fertilized. We monitored the predator populations by counting the adult females only and estimated the total size of the populations using the stable age distribution.

Predator-prey model

Desciption of the model

We used a predator-prey interaction model that has been developed to describe the within-patch dynamics of spider mites and predatory mites (Diekmann *et al.*, 1988; Janssen and Sabelis, 1992; Sabelis, 1992; van Baalen and Sabelis, 1995).

$$dN/dt = \alpha N - \beta P$$

$$dP/dt = (\gamma - \mu)\, P$$

N denotes the size of the spider mite population and P denotes the size of the predatory mite population, α is the per capita population growth rate of the spider mites, β is the per capita prey consumption rate of the predators, γ is the per capita population growth rate of the predators and μ is their dispersal rate during the interaction (as long as prey is not extinct). Since dispersal of spider mites is negligible when plants are in good condition (Sabelis and van der Meer, 1986), this parameter is not included in the model.

An important characteristic of this model is that changes in the predator population are independent of the prey population size. This implies that the predators are always in the plateau phase of their functional response and the per capita rate of increase of the predators is maximal. As explained in the introduction, the validity of this assumption will be investigated.

Input in the model

N N_0 is the number of spider mites of all stages at the moment of predator introduction.

P P_0 is the number of predators of all stages at the moment of introduction. We started with an initial predator-prey ratio of 1:40.

α Alpha is the intrinsic rate of population increase (r_m) of the spider mites. We know the r_m of the spider mites on gerbera cultivars Bianca and Bourgogne from previous laboratory studies (Krips *et al.*, 1998). On Bourgogne the r_m was 0.144/day, which was used as input in the model. However, on Bianca the r_m was very variable, it varied from 0.088 to 0.168/day. Therefore, we simulated the predator-prey dynamics on Bianca with both these values as input.

γ If the per capita rate of population increase of the predators is maximal, this parameter represents their r_m. This parameter was previously found to be unaffected by gerbera cultivar and had a value of 0.43/day on average at 25 °C (Krips *et al.*, 1999a). We used this value for γ.

μ Sabelis and van der Meer (1986) showed that the dispersal of a strain of *P. persimilis* that originiated from a commercial mass rearing is negligible until all prey are exterminated. Pels and Sabelis (1999) investigated the dispersal behaviour of several field-collected strains of *P. persimilis* and found some variation between strains in the onset of dispersal. In our experiments we used a strain of *P. persimilis* that originated from a commercial mass rearing. Therefore, we assumed no predator dispersal (μ=0) and will discuss the validity and consequences of this assumption below.

β Beta represents the mean predation rate over all stages of prey and is an average value over all stages of the predator. Publications usually describe the predation rate of young adult females that are reproducing maximally, on one prey stage. To obtain a value for β one has to make several assumptions.

The mean ovipostion rate of adult female *P. persimilis* is 85% of their peak oviposition rate (data from the appendix of Sabelis and Janssen, 1994). Knowing this, one can assume that the mean predation rate of adult females is also 85% of the predation rate of adult females that are reproducing maximally. Under a stable age distribution, the predatory mite population on gerbera consists for 5.9 % of adult females while all other feeding stages represent 35.7 %. The average consumption of prey eggs by the other feeding predator stages, including the adult females in the pre-oviposition phase, is approximately 25% of that of adult females that are reproducing maximally (Sabelis, 1981). We assumed that adult female spider mites are not preyed upon and that predation on each of the other stages was equal to that on eggs. Following these assumptions, the value of β can be obtained from:

$\beta=(0.85*0.059)\beta_f+(0.357*0.25)\beta_f=0.139*\beta_f$

with β_f = average predation rate on prey eggs by adult females that are reproducing maximally. Maximum predation by adult female *P. persimilis* on prey eggs on both the gerbera cultivars Bianca and Bourgogne is 27 eggs/day (Krips *et al.*, 1999b). This gives a value of 3.8 prey/predator/day for β.

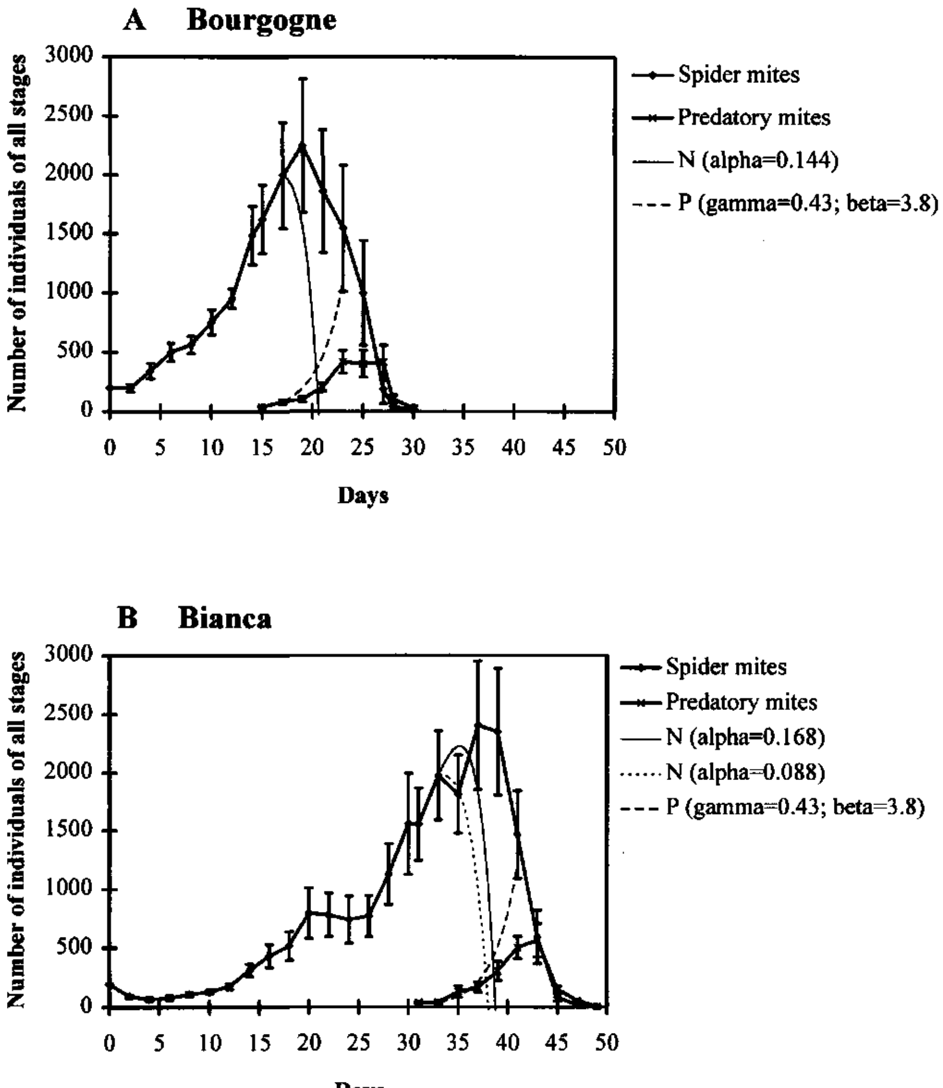

Fig. 1: Dynamics between *Tetranychus urticae* and *Phytoseiulus persimilis* on the gerbera cultivars Bourgogne (630 trichomes/cm^2) and Bianca (218 trichomes/cm^2). Vertical lines represent standard errors; n=8 on Bourgogne and n=7 on Bianca. N and P represent model predictions of population dynamics of *T. urticae* and *P. persimilis* respectively. Input values for prey and predator population growth rates (α and γ) and predation rates (β) were obtained from previous laboratory studies (Krips *et al.*, 1998, 1999a,b).

RESULTS AND DISCUSSION

After introduction of the predators on Bourgogne, the prey population continued to increase for approximately four days (Fig. 1a). Thereafter, the population steeply declined until no prey was left. It took approximately 14 days before the spider mites were exterminated by the predators. The predator population increased for 8 days, then remained constant for six days and decreased when no prey was left.

On Bianca, the prey population continued to increase for six days after introduction of predators and then started decreasing (Fig 1b). All prey was exterminated in approximately 14 days. The predators increased in numbers until the prey population declined steeply, which was after 12 days. Then, the predator population also went extinct.

The model explicitly assumes no predator dispersal. However, on Bianca the predator population decreased slightly within the first two days after introduction, which indicates dispersal. From day 2 after introduction, the predator populations increased on both cultivars. Therefore, we let the model run on both cultivars from day 2 after predator introduction.

On Bourgogne, the prey population was expected to decrease immediately after predator introduction (Fig. 1a). On Bianca, an immediate decrease was also expected when a value of 0.088/day was used for the prey population growth rate (Fig. 1b). If a value of 0.168/day was used, the prey population was expected to increase for approximately two days followed by a rapid decrease (Fig. 1b). Prey extermination on both cultivars was predicted in approximately four days after predator introduction. The large difference in *T. urticae* population growth rates on Bianca had little effect on the predicted moment of prey extermination.

In reality, prey extermination took approximately two weeks on both cultivars. The predator populations were predicted to grow much faster than found in reality.

Analysis of the deviation of predictions from experimental data

Deviations of model predictions from the data can be caused by errors in each of the parameters that were used as model input. Furthermore, the quality of the plants may have been different than in previous studies. Throughout the present experiments the leaves of the plants of both cultivars became slightly yellow, which indicates that the conditions in the climate rooms were not optimal for plant growth. We used a light intensity of 8000 Lux, which was the highest that could be obtained. However, it may not have been high enough for optimal plant growth. But, as we will show below, the population growth rates of the spider mites on the plants were almost identical to those found previously in life-history studies under optimal conditions (Krips *et al.,* 1998). Therefore, we have no reason to believe that plant quality has influenced the predator-prey dynamics in the present study.

We will now give an analysis of possible errors in the parameters that were used as input in the model and of the effect of such errors on the model predictions.

α The observed population growth rates of the *T. urticae* on the plants were 0.113 ± 0.033/day (mean ± sd) on Bianca between day 6 and 15 and 0.139 ± 0.062/day on Bourgogne from day 6 to 31. The population growth rates were not significantly different on the two cultivars (Mann-Whitney U, $P=0.39$). The observed population growth rate was on Bourgogne close to the r_m used as input in the model. On Bianca, the observed population growth rate was in between the two r_m values used as input. Therefore, the deviation of model predictions from reality are not likely to be due to errors in the population growth rates of *T. urticae*.

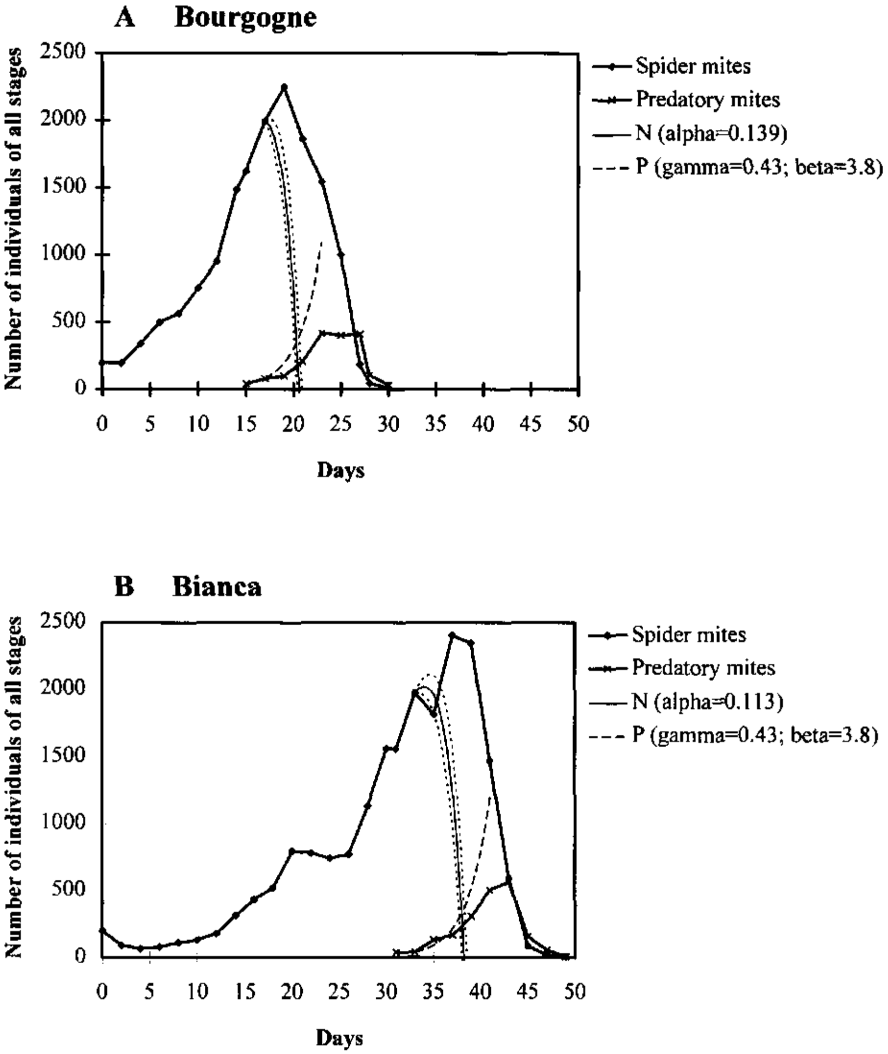

Fig. 2: Dynamics between *Tetranychus urticae* and *Phytoseiulus persimilis* on the gerbera cultivars Bourgogne (630 trichomes/cm^2) and Bianca (218 trichomes/cm^2); n=8 on Bourgogne and n=7 on Bianca. N and P represent model predictions of population dynamics of *T. urticae* and *P. persimilis* respectively Input values for prey population growth rates (α) were estimated from the growth of the prey populations in the experiment. Dotted lines are based on the 95% confidence intervals for α, which are 0.087-0.191/day for Bourgogne and 0.082-0.144/day for Bianca. Values for the predator population growth rates (γ) and predation rates (β) were obtained from previous laboratory studies (Krips *et al.*, 1999a,b).

Recalculating the model predictions with the observed values for the spider mite population growth rates still results in a large deviation of model predictions from reality (Fig. 2a&b). The dotted lines around the model predictions are based on the 95% confidence intervals for α which are 0.087-0.191/day for Bourgogne and 0.082-0.144/day for Bianca. Over such large ranges in prey population growth rates the predicted dynamics are hardly affected, as the dotted lines in both fig 2a and 2b show.

γ The growth of the predator populations was much slower on both cultivars than the r_m of 0.43/day found in previous life-history studies (Krips *et al.*, 1999a). The observed predator population growth rate on Bianca from day 33 to 41 was 0.326 ± 0.061/day (mean ± sd). On Bourgogne the observed predator population growth rate from day 17 to 23 was 0.285 ± 0.053/day. This is substantially lower than on Bianca but not significantly different (Mann-Whitney U, P=0.22). We can recalculate the predictions of the model using the values for γ found in the present study. However, since the population growth rate was not maximal, the predation rate will not have been maximal either which means that we have to adjust β as well.

β Knowing the population growth rates of prey and predators on both cultivars, we can calculate the number of prey that was consumed daily after the predators were introduced, which, when divided by the number of predators, represents an estimate for β. In this way, we find predation rates of 2.1±1.32 prey/predator/day (mean ± sd) on Bourgogne and 2.2±1.25 prey/predator/day on Bianca. This predation rate was not significantly affected by gerbera cultivar (Mann-Whitney U, P=0.85).

Table 1 gives a summary of the values for α, β and γ. We can recalculate the model predictions using the values for α, β and γ that were estimated with data from the population experiment (Fig. 3). The model now gives a good description of the peak of the spider mite population and the moment this population starts declining. This is, however, not surprising since we used the data over this time interval to estimate all input values. The new model predictions begin to deviate from the data from the moment the spider mite populations starts to decline. On both cultivars still earlier prey extermination is predicted than found in reality.

Table 1: Values (mean±sd) for the population growth rates of *Tetranychus urticae* (α) and *Phytoseiulus persimilis* (γ) and of the predation rates (β) on two gerbera cultivars. Values for α and γ were obtained from previous laboratory studies (lab-data) (Krips *et al.*, 1998, 1999a), or were estimated from the growth of the prey and predator populations in the experiment (experimental data). Values for β were obtained from previous laboratory studies (lab-data) (Krips *et al.*, 1999b) or were estimated with the model (estimated data). On Bianca the number of adult female predators initially dropped to zero on one replicate, which therefore could not be used to calculate γ and β.

	α		γ		β	
	lab-data	**experimental data**	**lab-data**	**experimental data**	**lab-data**	**estimated data**
Bianca (218 trichomes/cm^2)	0.088 & 0.168	0.113±0.033 (n=7)	0.43	0.326±0.061 (n=6)	3.8	2.2±1.25 (n=6)
Bourgogne (630 trichomes/cm^2)	0.144	0.139±0.062 (n=8)	0.43	0.285±0.053 (n=8)	3.8	2.1±1.32 (n=8)

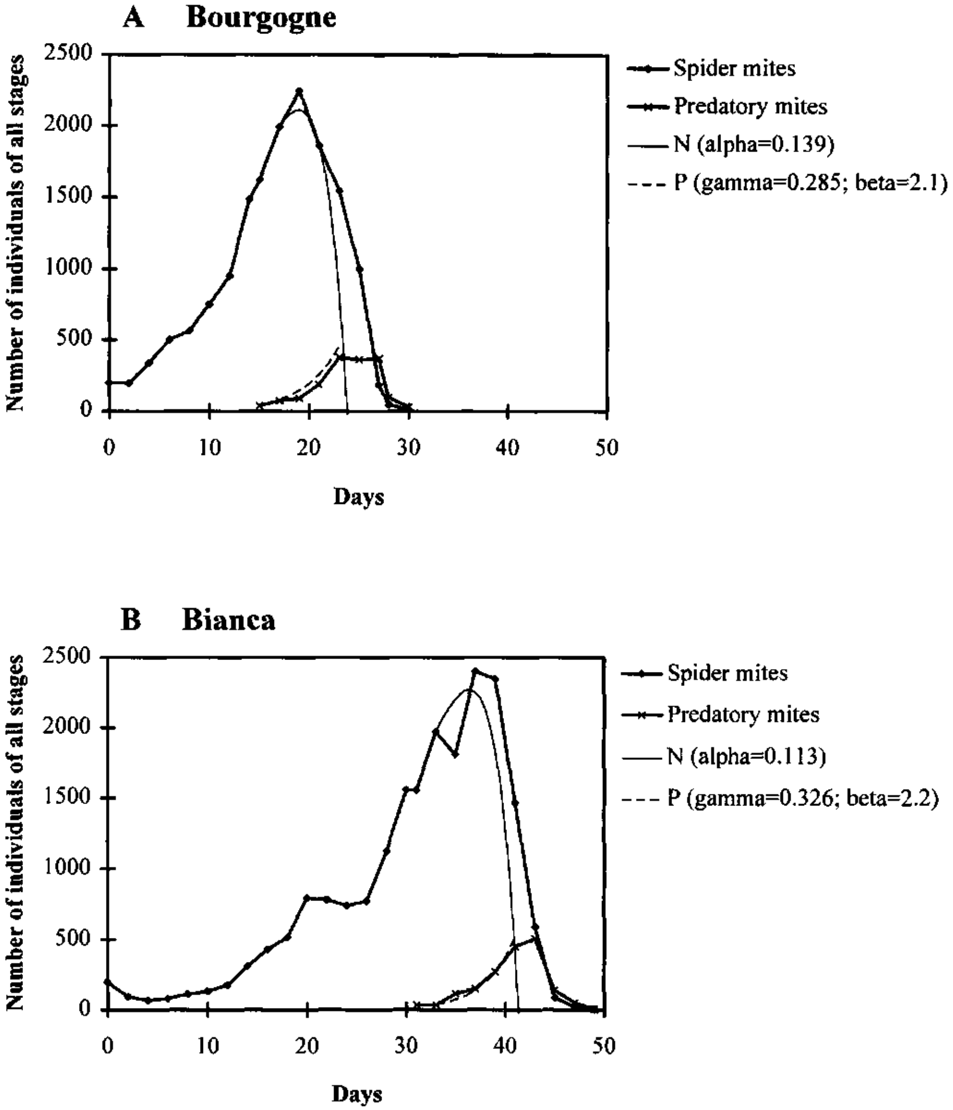

Fig 3: Dynamics between *Tetranychus urticae* and *Phytoseiulus persimilis* on the gerbera cultivars Bourgogne (630 trichomes/cm^2) and Bianca 218 trichomes/cm^2); n=8 on Bourgogne and 7 on Bianca. N and P represent model predictions of population dynamics of *T. urticae* and *P. persimilis* respectively. Input values for the prey and predator population growth rates (α and γ) were estimated from the growth of the populations in the experiment. Values for the predation rates (β) were estimated with the model.

We have not yet discussed the assumptions on the initial prey and predator numbers and the absence of dispersal of predators until all prey is exterminated. Before we give a general discussion on this study we will discuss the probability of errors in these variables and their effect on the model predictions.

N&P The total number of prey and predators were extrapolated from the number of adult females using stable age distributions. Errors in these distributions will have resulted in incorrect assumptions on the initial predator-prey ratios. The distributions were determined in life-history studies in which the population growth rates (r_m) of prey and predators were calculated (Krips *et al.*, 1998; 1999a). The observed population growth rates of the prey were very well comparable with those found in the above-mentioned life-history studies. So it is reasonable to assume that the stable age distributions of the prey in the present study were also similar to the estimated values.

The population growth rates of the predators were much lower than found in the above-mentioned life-history studies. Therefore, it is likely that the stable age distributions differed from those that were found previously. This will have resulted in different initial predator-prey ratios than the ratio of 1:40 that we tried to obtain.

Errors in the stable age distibutions will not have affected the calculated population growth rates of predators and prey, since one of the characteristics of stable age distibutions is that the percentage of females is constant over time. In contrast, the calculated values for β do depend on these distributions since the numbers of prey and predators are used in these calculations. Errors in extrapolation of total number of prey or predators from counted number of females will therefore result in errors in the calculated value of β. For example, if the initial predator-prey ratio in our population growth experiment on Bourgogne would have been 1:80 because the actual number of prey was higher or the number of predators was lower than we estimated, the calculated value for β would have been 4.2 instead of 2.1 prey/predator/day. And similarly, with a ratio of 1:20 the calculated value for β would have become 1.05 prey/predator/day. Hence, errors in estimating the initial number of prey and predators will have led to errors in the calculated value of β.

Large errors in estimating the total number of prey and predators are not unrealistic since the percentages of adult female prey and especially predators were very small. However, the calculated values for β change as a result of changes in initial predator-prey ratio. This makes the model predictions as shown in Fig. 2, independent of the initial total number of prey and predators ratio. Hence, conclusions regarding the population growth rates of prey and predators and the deviations of the model predictions from the data can be considered reliable. But conclusions regarding β have to be taken with caution, also because the calculated values of β depend on the population growth rates of prey and predators and errors in these values will have affected β as well.

μ The adjusted model describes the predator-prey dynamics well until the prey population starts to decline. Thereafter, the predictions start to deviate from the data on both cultivars which is most likely caused by dispersal of predators before all prey is exterminated. Pels and Sabelis (1999) found dispersal of some strains of predators immediately after the prey population began to decline and before total prey extermination. Such dispersal has a large effect on the population growth of prey and predators and the time needed for prey extermination (van Baalen and Sabelis, 1995).

GENERAL DISCUSSION AND CONCLUSIONS

The objectives of this study were to determine the effect of host plant resistance to *T. urticae* and trichome density on the success of biological control of spider mites on gerbera. Furthermore, we investigated whether the predation rate and population growth rate of *P. persimilis* are maximal on gerbera. As shown below, the model has been a useful tool to answer these questions.

Host plant resistance.

On both cultivars the spider mite populations were exterminated in approximately two weeks after introduction of predators. The difference in the prey population growth rates did not affect this interaction time between prey and predators. Even a large change in population growth rate from 0.087/day and 0.191/day, shown as the 95% confidence interval on Bourgogne (Fig. 1a), had little effect on the interaction time . At first sight this seems to be a strong argument against breeding for host plant resistance to spider mites in any crop. However, the prey population growth rate was low on both cultivars, whereas it can be as high as 0.24/day at 25°C on a susceptible gerbera cultivar (Krips *et al.,* 1998) and 0.34/day on other susceptible crops (Sabelis, 1991). In addition, if the same number of predators would be introduced at the same time after spider mite infestation, the predator-prey ratio would be higher on a resistant cultivar than on a susceptible line, which would result in faster extermination of prey. Another advantage of resistant lines lies in the growth of the spider mite population *before* introduction of predators. For example, the prey population on Bianca reached a number of 1550 individuals in 30 days. On the susceptible cultivar Sirtaki, on which the population growth rate of the spider mites is 0.24/day (Krips *et al.*, 1998), this number of prey would already have been exceeded within nine days.

Population growth rates and predation rates.

The predator population growth rates were much lower than previously found in life-history studies (Krips *et al.*, 1999a). In these studies, a high prey density of 15 spider mite eggs/cm^2 was artificially created and dispersal of predators was not possible. In the present population growth experiment the prey density might have been lower than 15 eggs/cm^2 and dispersal of predators may have taken place, which may both have caused the low predator population growth rates. Since it is impossible to monitor individual predators on 68 plants we do not know whether dispersal took place. And as we will explain below the actual prey density on gerbera is difficult to estimate. Therefore we cannot be conclusive on the exact cause of the low population growth rates, but we will evaluate both possible causes.

Prey density. In pilot studies in which we tried to assess prey density on leaves of the gerbera cultivars Bianca and Bourgogne, adult female *T. urticae* were given the opportunity to oviposit for 4 days. We found clusters of prey eggs with an average size of 15 eggs at a density of 12-18 eggs/cm^2 (R. Gols & O.E. Krips, unpublished data). On rose, the predators reach the plateau phase of the functional response at approximately 8 eggs/cm^2. (Sabelis, 1986) and at this density the predation rate on gerbera is as high as on bean (Krips *et al.,* 1999b). Therefore, prey density with egg clusters is not a limiting factor for the predator population growth rate. However, the clusters of prey eggs were very dispersed over the large gerbera leaves and up to 14% of the prey eggs were found singly on the leaves. Similarly, in the present experiments we observed that clusters of spider mites were very dispersed over the plants. Therefore, the predators have to walk large distances searching for clusters of prey.

The time *P. persimilis* needed to localise a standardized prey colony was 6-10 times longer on trimmed gerbera leaves than on equally sized Lima bean leaves (Garms *et al.*, 1998). This may be the result of the presence of a trichome layer on gerbera leaves, while Lima bean leaves have no trichomes. Generally gerbera leaves are much larger than 45 cm^2 and therefore the time that *P. persimilis* spends searching for prey colonies will be large. If this searching time between prey colonies is large compared to the searching time *within* colonies, the average predation rate of the predators will be affected which will result in a lower population growth rate. Considering the characteristics of prey colonies on gerbera this may indeed be the case.

Predator dispersal. Dispersal of predators from plants with spider mites is negligible until the spider mite population declines (Sabelis and van der Meer, 1986; Pels and Sabelis, 1999). The predators are most likely arrested by volatiles that are produced by the host plant in reaction to spider mite damage (Dicke and Sabelis, 1988; Dicke *et al.*, 1990a; Sabelis and Afman, 1994). Gerbera plants also produce spider mite-induced volatiles, but the predators only respond to those volatiles after prior experience with spider mites on gerbera (Krips *et al.*, 1999c).

For practical reasons we were unable to use predators that had been reared on gerbera and used predators from bean with spider mites instead. As a consequence these predators had no experience with volatiles from spider mite-infested gerbera plants. This may have caused the dispersal of predators in the first days after introduction. The initial decrease of the predator populations on both cultivars indicates that such dispersal indeed took place immediately after introduction.

The duration of experience that is necessary for predator response to volatiles from spider mite-damaged plants is 6-7 days for cucumber (Dicke *et al.*, 1990b;Takabayashi *et al.*, 1994) and for gerbera (Krips *et al.*, 1999c). In our experiment the predators will therefore have been responding to gerbera volatiles from day 21 on Bourgogne and day 37 on Bianca. Predator population growth rate on Bourgogne was 0.31 ± 0.057 day^{-1} (mean ± SE) from day 21 tot 23 and was 0.37 ± 0.046 day^{-1} from day 37 to 41 on Bianca. This is still lower than the maximal predator population growth rate of 0.43 day^{-1}. If dispersal was the only factor responsible for the low predator population growth rates, the percentage of predators that disappeared has been as high as 24% from day 33 to 41 on Bianca and 34% from day 17 to 23 on Bourgogne.

Trichome density.

The population growth rate of *P. persimilis* was 0.041/day, or 23%, higher on Bianca (218 trichomes/cm^2) than on Bourgogne (630 trichomes/cm^2). This difference is not significant, which is not surprising regarding the large variation that is common in greenhouse or field studies. But the direction of the difference supports our hypothesis that the leaf hair effects found in previous studies are biologically relevant (Krips *et al.,* 1999b). The predation rate of the predators may have been affected negatively by the dense trichome layer on Bourgogne, which might explain the low predator population growth rate on this cultivar.

An alternative explanation of the observed difference in predator population growth rates may be that prey suitability differs on the two cultivars. The amount of consumed prey that is neccesary for growth of the predator population may be larger on Bourgogne than on Bianca. This is, however, very unlikely, since no cultivar effect on suitability of *T. urticae* was found in previous studies with four gerbera cultivars (Krips *et al.,* 1999a).

The difference in predator population growth rates may also be explained by unequal predator dispersal. The predators'response to spider mite-induced volatiles of Bianca and Bourgogne leaves may not be equal. Preference of predators for volatiles of certain cultivars has been found in a two-choice situation (Krips *et al.,* 1999d). However, it is not known whether these differences in preference are related to differences in arrestment of the predators in a prey patch.

In conclusion, introduction of *P. persimilis* in a predator-prey ratio of 1:40, leads to extermination of *T. urticae* in approximately two weeks. The level of host plant resistance to spider mites has little effect on the predator-prey dynamics. More important are the population growth rate and predation rate of the predators and dispersal of predators before all prey is exterminated. The population growth rate of the predators is not maximal on both cultivars which may be the result of predator dispersal or low predation rates. The population growth rate is lowest on Bourgogne, which may be caused by a negative effect of trichome density. Alternatively, predator dispersal may be not be equal on both cultivars as a result of differences in the production of spider mite-induced volatiles. Which factor is responsible for the low predator population growth rates and the cultivar effects on this variable are interesting subjects for further studies.

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Je zou een boek net zo snel moeten kunnen schrijven
als een ander het lezen kan
C. Buddingh

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CURRICULUM VITAE

Olga Eline Krips werd op 10 april 1967 in Deventer geboren. In 1985 behaalde zij het VWO-diploma aan de Rijks-Scholengemeenschap in Hoorn en begon met haar studie Biologie aan de Universiteit van Amsterdam. Tijdens de doctoraalfase van deze studie deed ze onderzoek bij de sectie Populatiebiologie van de UvA aan de predator-prooi-interactie tussen tripsen en roofmijten op komkommer. Vervolgens deed ze een afstudeeronderwerp bij "the Department of Entomology, Lincoln University" in Nieuw-Zeeland aan gedragseffecten van synthetische pyrethroïden op roofmijten met resistentie voor deze pesticiden. Hierna onderzocht ze het effect van cadmiumverontreiniging in de bodem op pissebedden tijdens een afstudeeronderwerp bij de vakgroep Dieroecologie van de Vrije Universiteit te Amsterdam. In 1991 behaalde ze haar doctoraaldiploma. Hierna werkte ze gedurende een jaar als toegevoegd onderzoeker bij de sectie Aquatische Oecologie en deed onderzoek naar het effect van cadmiumverontreiniging op life-history kenmerken van chydoriden, aan watervlooien verwante zoetwaterorganismen. In 1993 werd ze study director van "MITOX-consultancy for pesticides and beneficial arthropods", een adviesbureau dat gespecialiseerd is in onderzoek naar neveneffecten van pesticiden op biologische bestrijders. In 1994 begon ze als onderzoeker in opleiding aan een promotieonderzoek bij het Laboratorium voor Entomologie van de Wageningen Universiteit. Het onderzoek, beschreven in dit proefschrift, werd gefinancierd door de stichting technische wetenschappen (STW) en richtte zich op effecten van plantkenmerken op het succes van biologische bestrijding van spint in het siergewas gerbera.