# RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* KOCH.

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**ivt**

Dit proefschrift omvat de volgende artikelen:

PONTI, O. M. B. DE, 1977. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 1. The role of plant breeding in integrated control. Euphytica 26:633-640.

PONTI, O. M. B. DE, 1977. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 2. Designing a reliable laboratory test for resistance based on aspects of the host-parasite relationship. Euphytica 26:641-654.

PONTI, O. M. B. DE, 1978. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 3. Search for sources of resistance. Euphytica 27:167-176.

PONTI, O. M. B. DE, 1978. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 4. The genuineness of the resistance. Euphytica 27:435-439.

PONTI, O. M. B. DE, 1979. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 5. Raising the resistance level by exploitation of transgression. Euphytica 28:569-577.

PONTI, O. M. B. DE, 1980. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 6. Comparison of near isogenic bitter and non-bitter varieties for resistance. Euphytica 29:261-265.

PONTI, O. M. B. DE & F. GARRETSEN. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 7. The inheritance of resistance and bitterness and the relation between these characters. Euphytica 29:513–523.

# STELLINGEN

### 1.
Waardplantresistentie is het eenvoudigste en goedkoopste biologische bestrijdingsmiddel.

### 2.
Door het gebruik van pesticiden en herbiciden in selektievelden treedt een geleidelijk verlies aan resistentie en konkurrentievermogen op.

### 3.
Er is in komkommer geen causaal verband tussen resistentie tegen de spintmijt *Tetranychus urticae* Koch en het vóórkomen van de bitterstof cucurbitacine-C.

### 4.
Gewasbescherming en resistentieveredeling zijn nog teveel gericht op een totale bestrijding en te weinig op een regulatie van ziekten en plagen.

### 5.
Door een gericht toelatingsbeleid kan het rassenonderzoek een belangrijke rol spelen bij het verhogen van de duurzaamheid van fysiospecifieke resistenties en daarmee het bindende karakter van een rassenlijst rechtvaardigen.

### 6.
Door gebruik te maken van allelopatische aktiviteiten van een plant is het mogelijk onkruidresistente en mengteeltspecifieke rassen te creëren.

### 7.
Bij de selektie van predatoren en parasieten ten behoeve van de biologische bestrijding wordt onvoldoende rekening gehouden met de genetische variatie binnen een soort.

### 8.
Hybridesuperioriteit: meer schijn dan werkelijkheid.

### 9.
De kleine boer in de ontwikkelingslanden is het meest gebaat bij rassen, die ter plaatse onder marginale omstandigheden zijn ontwikkeld en die door hemzelf in stand gehouden kunnen worden.

10.

Ondanks of juist dankzij de reactie in vakkringen heeft 'Seeds of the Earth',
evenals 'Silent Spring', een bewustwordingsproces in gang gezet.

11.

Smaak en andere intrinsieke kwaliteiten van agrarische produkten zullen alleen
verbeteren als de consument op grond van volledige informatie zijn voorkeur
voor bepaalde rassen en produktiesystemen kenbaar kan maken.

O. M. B. DE PONTI
Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch.

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# SAMENVATTING

RESISTENTIE IN KOMKOMMER, CUCUMIS SATIVUS L., TEGEN DE SPINTMIJT
TETRANYCHUS URTICAE KOCH

1. *Plantenveredeling en geïntegreerde bestrijding.*
In de natuur leveren resistente planten over het algemeen een groter aandeel in de
volgende generatie dan vatbare planten. In de loop van de evolutie resulteert dit in
handhaving van een zeker niveau van natuurlijke resistentie. De natuurlijke selektie is
ook na de domesticatie van planten een positieve rol blijven spelen. Sedert de introduk-
tie van pesticiden werden dierlijke parasieten van kultuurgewassen echter vooral che-
misch bestreden, ook in selektievelden, zodat verschillen in resistentie zich niet meer
manifesteerden. Hierdoor bleef de selektie ten gunste van resistentie achterwege, met
als gevolg een onbedoeld verlies aan resistentie. Door een drastische beperking van het
insekticidengebruik in selektievelden moet aan deze ongunstige ontwikkeling een eind
worden gemaakt, zodat tenminste de van oudsher aanwezige natuurlijke resistentie
gehandhaafd blijft.

Voor de geïntegreerde bestrijding van plagen – een alternatief voor louter chemische
bestrijding, dat steeds meer belangstelling ondervindt – is de beschikbaarheid van
resistente rassen van groot belang. Reeds een geringe vertraging in de populatie-
ontwikkeling van schadelijke insekten en mijten, als gevolg van gedeeltelijke resisten-
tie, heeft onverwacht grote effekten op de bestrijdingsefficiëntie van natuurlijke vijan-
den en andere geïntegreerde bestrijdingstechnieken. Het gebruik van resistente rassen
wordt daarom terecht beschouwd als een belangrijk onderdeel van geïntegreerde
bestrijding en zal de behoefte aan chemische bestrijding sterk verminderen.

In tegenstelling tot de ruime aandacht voor resistentie tegen plantaardige parasieten
heeft de plantenveredeling lange tijd bijzonder weinig aandacht geschonken aan resis-
tentie tegen dierlijke parasieten. Deze studie onderzoekt de perspektieven van een
gerichte selektie op resistentie tegen insekten en mijten. De komkommer-spintmijt
relatie is gekozen als proefobjekt, omdat dit voor Nederland belangrijke kasgewas
zonder afdoende bestrijding ernstig te lijden heeft van deze mijt. Biologische bestrij-
ding met de roofmijt *Phytoseiulus persimilis* A. -H. vormt intussen een goed alternatief
voor de aanvankelijk op grote schaal toegepaste chemische bestrijding. Het telen van
resistente rassen zal deze ontwikkeling bevorderen.

Naast een gedegen kennis van de betreffende waardplant (komkommer, *Cucumis
sativus* L.)-parasiet (spintmijt, *Tetranychus urticae* Koch) relatie en de overerving van
de resistentie is de ontwikkeling van betrouwbare en efficiënte toetsmethoden een
vereiste om enige kans van slagen te hebben. Vandaar de diverse onderdelen van deze
studie.

2. *Het ontwerpen van een betrouwbare laboratoriumtoets voor resistentie gebaseerd
op onderdelen van de waardplant-parasiet relatie.*
Laboratoriumtoetsen kunnen een belangrijke aanvulling vormen op de toetsen onder

1

normale teeltomstandigheden, hierna praktijktoetsen genoemd, vooral indien de resistentie gekompliceerd is in expressie en overerving. De bruikbaarheid van een laboratoriumtoets wordt vooral bepaald door de mate waarin de resultaten overeenstemmen met die van praktijktoetsen. Aan dit verband is veel aandacht gegeven.

In een studie van de waardplant-parasiet relatie met acht rassen, die in een vooronderzoek een uitlopend niveau van resistentie bleken te bezitten, is een aantal reproduktiefaktoren van de spintmijt over één generatie bepaald. Daarbij werden op grond van literatuurgegevens de waardplantkonditie en het milieu gestandaardiseerd. Als inoculum werd gedurende het gehele onderzoek een spintmijtpopulatie gebruikt, die voor Nederland representatief is.

Tussen de rassen werden signifikante verschillen aangetoond voor alle onderzochte reproduktiefaktoren van de spintmijt: levensduur van de volwassenen, ovipositie en preadulte mortaliteit. Deze verschillen stemden goed overeen met de eerder onder kasomstandigheden gevonden verschillen in resistentie. De waarnemingen werden iedere drie dagen gedaan en naarmate de mijten langer op de plant verbleven namen de verschillen in ovipositie en preadulte mortaliteit toe. Korrelatieberekeningen toonden aan, dat de ovipositie over de vierde periode van drie dagen zeer representatief is voor de totale netto reproduktie ( = ovipositie minus preadulte mortaliteit) over één generatie. Dit wijst tevens op een hoge negatieve korrelatie tussen beide reproduktiefaktoren.

Op grond van bovenstaande bevindingen beperkte de laboratoriumtoets zich tot het vaststellen van twee selektiekriteria: 1) *acceptatie*, zijnde het percentage mijten, dat 9 dagen na inoculatie met 20 vrouwelijke mijten nog levend op de plant aanwezig is en 2) *ovipositie* van vijf van deze vrouwtjes over de daaropvolgende drie dagen. De laatste bepaling werd uitgevoerd op bladponsjes. De betrouwbaarheid hiervan bleek uit een goede overeenstemming van relatieve verschillen in ovipositie en preadulte mortaliteit, indien bepaald aan bladponsjes en aan intakte planten van zes rassen met uiteenlopende resistentie. In de loop van het onderzoek is deze laboratoriumtoets nader geëvalueerd, wat leidde tot veranderingen in uitvoering en toepassing.

3. *Het opsporen van resistentiebronnen.*

In een eerste globale beoordeling zijn ongeveer 800 komkommerrassen in praktijktoetsen op resistentie onderzocht. In deze toetsen werden de planten enige dagen na uitpoten in de kas geïnoculeerd met 20 vrouwelijke mijten, waarna gedurende acht weken de populatie-ontwikkeling van de spintmijten werd gevolgd aan beschadigingsbeelden op het blad, uitgedrukt in een schade-index. Vatbare rassen gingen binnen deze termijn volledig ten onder.

Ongeveer 40 rassen, die zich enigszins van de vatbare kontrôle onderscheidden, zijn in herhaalde praktijk- en laboratoriumtoetsen nader bekeken. Slechts 9 rassen bleken in beide toetsen een onderscheidbaar niveau van resistentie te bezitten. Deze gedeeltelijke resistentie manifesteerde zich in de laboratoriumtoets door een aanzienlijke verlaging van zowel acceptatie als ovipositie. In de praktijktoets werd op de meest resistente rassen de ekonomische schadedrempel ( = de omvang van de spintmijtpopulatie, waarbij opbrengstderving gaat optreden) enkele weken later bereikt dan op de vatbare kontrôle, waarbij dit al 4 à 5 weken na inoculatie gebeurde.

Bij een aantal rassen stemden de resultaten van de laboratorium- en praktijktoetsen

2

in het geheel niet overeen. Naast toevallige variatie en de grote milieuverschillen tussen de beide toetsen kunnen tolerantieverschillen, die zich alleen in praktijktoetsen openbaren, hierbij een rol spelen. Gekombineerde selektie op resistentie en tolerantie voegt een extra dimensie toe aan de praktijktoets. Een reden te meer om iedere selektiecyclus met een praktijktoets af te sluiten.

Op kleine schaal zijn ook enkele aan komkommer verwante wilde *Cucumis* soorten onderzocht. Alleen *C. myriocarpus* bleek enige resistentie te bezitten. Vanwege gelijkwaardige of betere resistenties binnen *C. sativus* en te verwachten kruisingsproblemen is het wilde materiaal niet verder bewerkt.

Over de oorzaken van de resistentie valt vooralsnog weinig te zeggen, behalve dat op resistente rassen vele spintmijten helemaal zwart worden. Dit wijst op een verstoring van de spijsvertering, waarvan de werkelijke oorzaak nog onbekend is. Omdat vele van de onderzochte rassen bitter èn vatbaar waren, kan de resistentie niet eenvoudigweg worden toegeschreven aan de bittermakende stof curcurbitacine, zoals in de literatuur is gedaan. Of en in welke mate cucurbitacine een rol speelt, komt nog nader aan de orde in hoofdstuk 5, 6 en 7.

### 4. *De echtheid van de resistentie.*

De reproduktie van spintmijten varieert sterk per waardplant. Indien spintmijten overgaan of overgeplaatst worden van de ene op de andere soort, kan de reproduktie tijdelijk lager zijn dan die welke normaal is voor de betreffende soort. Dit verschijnsel zou zich ook kunnen voordoen tussen rassen van eenzelfde soort en dan tot onjuiste konklusies in termen van waardplantresistentie leiden.

Daarom zijn populaties van spintmijt gedurende enige generaties geïsoleerd voortgekweekt op enkele meer of minder resistente rassen. Daarna werden deze tegelijkertijd getoetst op resistentie tegen hun eigen subpopulatie en de uitgangspopulatie van de spintmijt. De aard van de spintmijtpopulatie bleek geen enkel effect te hebben op de mate van acceptatie, ovipositie en de schade-index, zodat er geen twijfel is aan de echtheid van de resistentie.

Uit deze bevindingen mogen geen konklusies over de duurzaamheid van de resistentie worden getrokken. Door een gekombineerde selektie op diverse resistentiefaktoren wordt echter beoogd een komplexe resistentie te ontwikkelen, in de verwachting aldus de duurzaamheid van de resistentie te bevorderen.

### 5. *Verhoging van de resistentie door het gebruik van transgressie.*

Alvorens de gevonden resistentie in hoogwaardige rassen in te kruisen zijn de eerder geselekteerde, gedeeltelijk resistente rassen onderling gekruist om een verhoging van de resistentie te bewerkstelligen. Na een scherpe selektie in laboratorium- en praktijktoetsen van de $F_2$'s en daaropvolgende generaties van deze kruisingen werden $F_5$ lijnen verkregen, die aanmerkelijk resistenter zijn dan hun ouders. Deze transgressie wijst al op een niet eenvoudige overerving van de resistentie.

Op de meest resistente $F_5$ lijnen waren acceptatie en ovipositie 50 tot 60 % lager dan op de vatbare kontrôle. Voor acceptatie was een dergelijke reduktie ook al op de ouderrassen gekonstateerd, zodat voor deze resistentiefaktor geen vooruitgang is geboekt. In de praktijktoets werd op de meest resistente lijnen gedurende de waarnemingsperiode van 8 weken de ekonomische schadedrempel niet bereikt, terwijl dat op

3

de vatbare kontrôle reeds in de 5e week gebeurde en op de meeste gedeeltelijk resistente rassen aan het eind van de 6e week. Op grond van deze resultaten mag worden verwacht dat met een dergelijk resistentieniveau in geval van chemische bestrijding het aantal bespuitingen minstens tot de helft kan worden teruggebracht. Het voordeel voor de biologische bestrijding is moeilijk te kwantificeren.

Door een relatief grote niet-genetische variatie was de erfelijkheidsgraad van individuele $F_2$ planten voor ovipositie dikwijls bijzonder laag en meestal veel lager dan die van $F_3$, $F_4$ en $F_5$ lijngemiddelden voor ovipositie en schade-index. Laatstgenoemde erfelijkheidsgraden zijn van een orde (30-55 %) dat zowel ovipositie als schade-index redelijk zijn te beselekteren. Wellicht verdient het aanbeveling in de $F_2$ geen selektie uit te voeren en de eerste selektie tot $F_3$ lijnen uit te stellen.

Bij een aantal $F_5$ lijnen bleek een verhoging van de resistentie gepaard te zijn gegaan met een onbedoelde verhoging van het bitterstofgehalte. Deze enigszins alarmerende konstatering behoeft niet noodzakelijkerwijs te duiden op een causale relatie tussen resistentie en bitterstof; koppeling tussen resistentie- en bitterstofgenen geeft ook een sluitende verklaring voor deze en eerdere bevindingen. Deze hypothese zal nog nader aan de orde komen in hoofdstuk 7.

In 1978 zijn de 15 meest resistente lijnen uitgegeven aan Nederlandse veredelingsbedrijven, die daarmee de uitdaging hebben aangenomen veredeling op resistentie tegen insekten en mijten in hun kweekprogramma's op te nemen.

### 6. *Vergelijking van de resistentie van bijna isogene bittere en bittervrije rassen.*

De beschikbaarheid van een aantal paren van bittere en bittervrije komkommerrassen, die bij benadering isogeen zijn, bood een bijzondere mogelijkheid het eventuele effect van de bitterstof op resistentie van komkommer te onderzoeken. Volledige bittervrijheid van alle plantedelen is in de vijftiger jaren op het IVT gevonden in een recessieve mutant van het ras 'Improved Long Green' en is sindsdien de enige bron van bittervrijheid gebleven, die over de gehele wereld is gebruikt. Verwacht mag worden dat dit ras en zijn bittervrije mutant, waarvan nog oorspronkelijk reservezaad aanwezig was, alleen voor het gen *Bi*, het hoofdgen voor bitterheid, van elkaar verschillen.

Met inbegrip van 'Improved Long Green' en zijn bittervrije mutant zijn vijf isogene rassenparen getoetst in laboratorium- en praktijktoetsen. In beide toetsen werden nauwelijks signifikante verschillen in resistentie binnen de rassenparen aangetroffen. 'Improved Long Green' en zijn bittervrije mutant leverden zo goed als identieke resultaten voor ovipositie en schade-index, terwijl de acceptatie op de bittervrije mutant signifikant lager was. Daarom is een causale relatie tussen bitterstof en resistentie, veroorzaakt door pleiotropie, zo goed als uitgesloten.

### 7. *De overerving van resistentie en bitterheid en de onderlinge relatie tussen deze eigenschappen.*

Om de overerving van resistentie tegen de spintmijt en bitterheid in komkommer te bestuderen zijn kruisingen gemaakt tussen drie eerder geselekteerde, bittere resistente $F_5$ lijnen en één bittervrije vatbare lijn. Omdat laatstgenoemde lijn representatief is voor de hedendaagse komkommerrassen, is met deze kruisingen tevens een begin gemaakt met de introduktie van de resistentie in het huidige rassensortiment. Aan de $P_1$, $P_2$, $F_1$, $T_{11}$, $T_{12}$ en $F_2$ generaties van deze kruisingen zijn waarnemingen verricht

4

betreffende acceptatie, ovipositie en bitterheid.

Zowel gemeten aan acceptatie als aan ovipositie bleek de resistentie te worden gereguleerd door een aantal genen die hoofdzakelijk additief overerven. Een zekere overlapping of koppeling van genen, die beide resistentiefaktoren bepalen is niet uitgesloten. In tegenstelling tot de gangbare opvatting, dat bitterheid monogeen dominant overerft, bleek bitterheid weliswaar hoofdzakelijk door het gen *Bi* gereguleerd te worden, maar dit gen erft intermediair over en zijn werking wordt bovendien beïnvloed door één of meer additief overervend intensiteitsgenen.

In de voor bitterheid uitsplitsende generaties $T_{11}$ en $F_2$ was de resistentie in gelijke mate verdeel over bittere en bittervrije planten. Bij de bittere planten was er geen enkele korrelatie tussen de resistentiefaktoren acceptatie en ovipositie enerzijds en de hoeveelheid bitterstof anderzijds. Hetzelfde werd gevonden in geselekteerde $T_{11 \circledR}$ en $F_3$ lijnen, die uitsplitsten voor het gen *Bi*. In praktijktoetsen met laatstgenoemde lijnen werden de bittere planten over het algemeen wèl minder aangetast dan de bittervrije en werd een negatieve korrelatie tussen de hoeveelheid bitterstof en de schade-index aangetoond. Enkele bittervrije planten waren echter zeer resistent. Op grond van deze en eerdere bevindingen wordt aangenomen, (1) dat er geen causale relatie bestaat tussen bitterheid en resistentie, (2) dat de genen die de resistentiefaktoren acceptatie en ovipositie reguleren onafhankelijk overerven van het gen *Bi* en (3) dat de in de praktijktoets gevonden relatie verklaard moet worden door koppeling van het gen *Bi* en bijbehorende intensiteitsgenen met resistentie en/of tolerantiegenen, die alleen in praktijktoetsen tot expressie komen.

In de komkommerveredeling worden nieuwe eigenschappen over het algemeen door herhaald terugkruisen ingekruist. Omdat ook in deze proeven de erfelijkheidsgraden van individuele $F_2$ planten voor acceptatie en ovipositie dikwijls laag waren, moet selektie in de $F_2$, zo al uitgevoerd, worden gevolgd door tenminste één generatie lijnselektie, voordat de volgende terugkruising wordt gemaakt. Het aantal terugkruisingen kan worden beperkt door gedeeltelijk resistente lijnen, die redelijk goed zijn te herkennen, onderling te kruisen.

Tussen de resistentiefaktoren acceptatie en ovipositie werd een redelijke korrelatie gekonstateerd. Daarom werd voorgesteld de laboratoriumtoets tot één van de beide faktoren te beperken. Hierbij werd de voorkeur gegeven aan ovipositie, waarvan de bepaling in vijfvoud geschiedt en minder gevoelig bleek voor toevallige variatie. Omdat de korrelatie tussen laboratorium- en praktijktoetsen nogal eens te wensen overliet, moet iedere selektiecyclus met een praktijktoets worden afgesloten. Dit biedt tevens de mogelijkheid ongewenste koppelingen tussen resistentie en bitterheid te vermijden. Op deze wijze is de selektie van bittervrije spintmijtresistente lijnen voortgezet, met bemoedigende resultaten.

Dit onderzoek heeft aangetoond, dat de veredeling van komkommer op resistentie tegen spintmijten praktisch uitvoerbaar is en tot aanzienlijke verhoging van het resistentieniveau kan leiden. Door uitgifte van resistente lijnen aan partikuliere veredelingsbedrijven zal de Nederlandse tuinbouw te zijner tijd spintmijtresistente komkommerrassen mogen verwachten en aldus van dit onderzoek profiteren.

# RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* Koch. 1. THE ROLE OF PLANT BREEDING IN INTEGRATED CONTROL

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INDEX WORDS

SUMMARY

The role of plant breeding and particularly of host plant resistance in integrated control is discussed. Host plant resistance to insects and mites, especially to *Tetranychus urticae* is reviewed. A standard terminology for disease and pest resistance is recommended.

EVOLUTION OF HOST PLANT RESISTANCE

Considering the influence of natural selection and domestication on host plant resistance there has generally been a selection advantage for resistant plants during the greater part of evolution (VAN EMDEN & WAY, 1973). Before domestication the most susceptible plants in a natural habitat were often destroyed before reproduction took place and this resulted in a gradual increase in the frequency of resistance genes.

During the process of domestication, when man chose those plants as seed parents for his next crop which were most suitable to him, this picture changed. Sometimes domestication may have adversely influenced the degree of resistance (PAINTER, 1941; VAN EMDEN, 1966). But the most resistant plants were automatically selected as long as resistance was compatible with other desired characters, which continued during the first decennia of this century, when plant breeding speeded up selection. In areas with a permanent contact between plants and pests it led in several cases to varieties that were more resistant than those bred in areas where the pest was absent (PAINTER, 1941; CHESNOKOV, 1955).

The situation did not alter essentially until after the introduction of insecticides. when it became common practice to apply chemical control also in selection fields. In this way any difference in resistance was masked and the above mentioned selection for the more resistant individuals became infeasible. This might have led to a gradual decline in the frequency of resistance genes in breeding populations (PAINTER, 1960, 1968; VAN MARREWIJK & DE PONTI, 1975). This trend must be stopped by limiting the use of insecticides in selection fields.

PLANT BREEDING AND INTEGRATED CONTROL

Insect and mite control will no doubt be most efficient, if varieties can be grown that are so resistant that the economic damage threshold (DAVIDSON & NORGAARD, 1973) will not be overstepped. If the resistance is not strong enough and hence cannot give sufficient control it can markedly contribute to the success of integrated control systems, because the use of resistant varieties is compatible with all other control strategies (PAINTER, 1941; SNELLING, 1941a; PATHAK, 1970, 1975; MAXWELL, 1972; BRADER, 1973; DAHMS, 1974; VAN MARREWIJK & DE PONTI, 1975). The effect of only a slight reduction in population increase of an injurious insect – as occurs on a moderately resistant variety – on the control by natural enemies or on integrated control often proved to be unexpectedly large (PAINTER, 1953, 1967, 1968; VAN EMDEN, 1966; STARKS et al., 1972; JENKINS et al., 1973; LOWE, 1975; PATHAK, 1975). In case of virustransmitting insects it appeared that partial resistance can also be very efficacious in limiting the spread of virus diseases (LOWE, 1975; PATHAK, 1975; JONES, 1976), especially if persistent viruses are concerned (KENNEDY, 1976).

Host plant resistance is the most obvious contribution of plant breeding to integrated control. But, especially in cases where an effective degree of resistance is difficult to obtain, one could look for other points of contact (MAXWELL, 1972; VAN EMDEN & WAY, 1973). It has, for example, been demonstrated that varieties may differ in, mainly morphological, characters which influence the predatory or parasiting activities of natural enemies (PIMENTEL, 1961; WAY & MURDIE, 1965; GURNEY & HUSSEY, 1970; ELSEY, 1974; SCHUSTER et al., 1975). It appeared also possible to change the morphology of a plant in such a way that insecticides are better retained (PARROTT et al., 1973), so that rate and frequency of insecticidal applications can be limited. If these specific morphological characters are known, varieties can be bred which promote natural and integrated control in a rather peculiar way.

RESISTANCE TO INSECTS AND MITES: PRESENT STATE OF AFFAIRS

Insect resistance is certainly not neglected by science, but in contrast to disease resistance it is insufficiently operational. This is best illustrated by the enormous discrepancy between several thousand publications on insect resistance (reviewed by SNELLING, 1941b; PAINTER, 1951, 1958; BECK, 1965; MAXWELL et al., 1972) and the number of insect resistant varieties, which hardly exceeds one hundred (MAXWELL, 1972; GALLUN, 1974). Especially in horticulture the situation is rather disappointing (PAINTER, 1967; STONER, 1970a).

It is felt that there are many reasons why insect resistance has not kept up with disease resistance. The main reasons seem to be insufficient involvement of plant breeders and lack of continuity of research (PAINTER, 1968; STONER, 1970a; GALLUN, 1974). The explanation is supported by the fact that real successes were only reached in those cases where specialists of different disciplines cooperated with the firm purpose of breeding resistant varieties.

Cooperation of breeders and entomologists should start from the beginning with studying the relationship of host and parasite. This joint study must provide the scientific basis of test methods to be used in routine breeding programs. Reliable and

efficient test methods for distinghuishing rather small differences in resistance are prerequisites. With such methods resistant varieties can also be bred if differences in the levels of resistance are very gradual and/or if the resistance is not simply inherited. Without good test methods any breeding program for resistance to insects is doomed to fail as, unfortunately, has happened too often.

In the Netherlands breeding for resistance to insects and mites has formed part of the research programs of several Research Institutes since 1967 (SNEEP & DIELEMAN, 1973) and is coordinated by the Working Group on Integrated Control (BRADER, 1974). At present six projects are under study (Annual Report IVT 1976) and commercial breeding companies have already taken some initiatives.

RESISTANCE TO TETRANYCHUS URTICAE KOCH

Resistance to the twospotted spider mite *(Tetranychus urticae* KOCH) has been found in many different crops as is shown in Table 1. Only the generic names are mentioned, because in the search for resistance species related to the cultivated ones are often tested. These apparently promising reports were seldom followed by papers on genetic and breeding research (CHAPLIN et al., 1968, 1970; STONER, 1970b; PARAMESWARAPPA et al., 1974; CHILDRESS et al., 1976), let alone by the introduction of resistant varieties. Therefore the situation with regard to resistance to the twospotted spider mite is generally as explained above.

In *Cucumis sativus* and related species resistance to *T. urticae* KOCH has also been studied (see Table 1). Only KOOISTRA (1971) and TULISALO (1972) have screened

Table 1. Review of the literature on resistance to the twospotted spider mite (*Tetranychus urticae* KOCH).

| Genus | Crop | Literature |
|---|---|---|
| *Beta* | sugarbeet | BUSH & BREWBAKER, 1956. |
| *Chrysanthemum* | chrysanthemum | MARKKULA et al., 1969. |
| *Cucumis* | cucumber | DACOSTA & JONES, 1971; KOOISTRA, 1971; TULISALO, 1972; SOANS et al., 1973b; KNIPPING et al., 1975. |
| *Fragaria* | strawberry | LESKA et al., 1964; CHAPLIN et al., 1968, 1970; DABROWSKI et al., 1971; SHANKS & BARRITT, 1975. |
| *Gossypium* | cotton | KAMEL & ELKASSEBY, 1965; SCHUSTER et al., 1973; CHILDRESS et al., 1976; SCHUSTER & MAXWELL, 1976. |
| *Humulus* | hop | REGEV & COME, 1975. |
| *Lycopersicon* | tomato | STONER & STRINGFELLOW, 1967; GILBERT et al., 1969; STONER, 1970b. |
| *Manihot* | cassava | SARADAMMA & DAS, 1974. |
| *Nicotiana* | tobacco | PATTERSON et al., 1974. |
| *Pelargonium* | geranium | SNETSINGER et al., 1966. |
| *Ricinus* | castorbean | CHANDRASEKHARAN et al., 1964. |
| *Glycine* | soybean | RODRIGUEZ' & FREEMAN, 1959; CARLSON, 1969; PARAMESWARAPPA et al., 1974; BAILEY & FURR, 1975. |
| *Solanum* | eggplant | SOANS et al., 1973a; SCHALK et al., 1975. |
| | potato | GENTILE et al., 1969; MACDONALD et al., 1972. |
| *Vitis* | grape | DUSCHIN, 1967. |

large collections and have both observed differences in resistance. Tulisalo judged the maximum degree of resistance found insufficient and finished the project, Kooistra's work has been continued in the present research.

DESIRABILITY OF BREEDING CUCUMBER VARIETIES RESISTANT TO THE TWOSPOTTED SPIDER MITE

In the Netherland about 1000 ha of cucumbers are grown in glasshouses every year. With a temperature of 20°C at night and at least 23°C during the day and often a rather low humidity this environment is very favourable for the development of the twospotted spider mite. The control of this pest requires permanent attention and efforts of the grower to prevent economic losses by yield reduction. Without control a crop can be totally destroyed within some weeks after it has become infected.

Particularly in the case of the twospotted spider mite it is rather risky to rely exclusively on chemical control, because this mite has proved to posses a large genetic potential for resistance to acaricides (HELLE & VAN DER VRIE, 1974). In addition to an increased awareness of the negative impact of pesticides on human health and the environment this fact stimulated the research and application of integrated control.

After an enthousiastic start in the early seventies the expansion of the biological control of the twospotted spider mite with the predatory mite, *Phytoseiulus persimilis* A.-H., has come to a standstill at about 20% of the cucumber glasshouse area (WOETS, 1976). Although this control system generally functions well, it is sensitive to environmental changes and still requires investments and care on the part of the grower.

On resistant varieties the population of the twospotted spider mite will thrive less. This might promote the biological control and bring it to further expansion. Resistance is also very useful in combination with chemical control as it diminishes the frequency of its application. Subsequently this will probably delay the development of resistance of the mites to acaricides. The ultimate goal of each resistance project is to breed varieties that do not need any control. The present study aims at investigating the possibilities of breeding cucumber varieties that are highly resistant to the twospotted spider mite.

SCOPE OF RESEARCH

A thorough study of different aspects of the development of the resistance precedes the actual breeding work. The research comprises the following items:
1. Study of the host-parasite relationship to develop reliable tests for resistance.
2. Search for sources of resistance.
3. Research on the genuineness of the resistance.
4. The significance of cucurbitacine.
5. The genetics of the resistance.
6. Breeding highly resistant varieties.

636

APPENDIX

*Terminology*

This chapter is not an attempt to unravel the babel of tongues which exists with regard to resistance against diseases and pests. Because of the permanent confusion it is, however, necessary to describe the meaning of the most important terms as used in this study.

In our opinion there are no essential differences in resistance to plant (fungi, bacteria, viruses) and animal (nematodes, insects, mites) parasites and thus for the sake of simplicity the terminology should be equal (VAN MARREWIJK & DE PONTI, 1975). Two basically different mechanisms of a plant to withstand a parasitic attack can be distinguished: resistance (vs. susceptibility) and tolerance (vs. sensitivity). All four terms are in fact relative, but we prefer to use resistance and tolerance as relative terms, susceptibility and sensitivity as absolute terms (Werkgroep Resistentie IVT, 1974).
*Resistance* is the complex of characters of a host that reduces the reproduction of a parasite.
*Tolerance* is the ability of a host to endure the occupation by a parasite.
In resistance to insects much attention is paid to the non-preference phenomenon. This term is causing much confusion and should be defined more precisely than PAINTER (1951) did originally. OWENS (1975) distinghuished between relative and absolute non-preference, but we prefer to use two distinct terms.
*Non-preference* implies that during the orientation phase (SAXENA, 1969) of a host-parasite contact a particular plant is not chosen as a host because of the presence of a preferred one, whereas that particular plant is accepted as a host if there is no choice.
*Non-acceptance* implies that during the orientation phase a plant is rejected as a host regardless of whether it is offered singly or together with other plants. Non-acceptance should not be considered as an essentially distinct phenomenon but as an expression of resistance during the orientation phase. This has already been advocated by KENNEDY (1958), BECK (1965), LOWE & RUSSELL (1969) and DE PONTI et al. (1975).
Thus defined, non-preference has no value for the protection of crops that are grown in monoculture. Non-acceptance is unquestionably of the utmost importance in insect resistance, as it prevents or limits the settlement of an insect population. Often the term non-preference is used, whereas non-acceptance is meant.

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# RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* Koch.
# 2. DESIGNING A RELIABLE LABORATORY TEST FOR RESISTANCE BASED ON ASPECTS OF THE HOST-PARASITE RELATIONSHIP

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INDEX WORDS

*Cucumis sativus*, cucumber, *Tetranychus urticae*, twospotted spider mite, resistance breeding, host-parasite relationship, test methods, leaf disk.

SUMMARY

The relationship between the twospotted spider mite and cucumber has been studied on plants and on leaf disks of a number of varieties with different levels of resistance. Existing laboratory tests are critically discussed and it appears that they are only reliable if many factors are taken into account. A new, more efficient laboratory test for resistance, measuring acceptance and reproduction is described.

INTRODUCTION

Reliable and efficient test methods are prerequisites to any resistance breeding program. Observations on successful field tests after natural attack or artificial inoculation might provide the most reliable data on differences in resistance. But field tests often fail because many factors influencing the development of a pest are uncontrollable in the field as has been demonstrated by VAN MARREWIJK & DE PONTI (1975).

If great differences occur between resistant and susceptible plants and the resistance is simply inherited, field tests may satisfy in spite of their shortcomings. But if the resistance is complicated in its appearance and inheritance, there is an urgent need for more sophisticated test methods. Such artificial tests, which are mostly executed under controlled environmental conditions in the glasshouse or laboratory, allow the detection of small differences in the level of resistance. This enables:
1. separating a complex resistance into its various components;
2. unravelling the inheritance of the resistance;
3. increasing the level of resistance by the accumulation of resistance genes.

Although artificial tests are often preferred, field tests should remain part of each research on resistance to confirm the results of artificial tests.

In developing artificial tests one should give permanent attention to a high correlation with the field situation (GUTHRIE, 1975; KOGAN, 1975; MOREAU & GAHUKAR, 1975; VAN MARREWIJK & DE PONTI, 1975). These tests must also be efficient so that many

plants can be screened in a short period. If too laborious for routine breeding, artificial tests may still prove useful in the research phase of a breeding program by giving a better insight into the different aspects of the resistance.

A close study of the host-parasite relationship and the influence of the environment on it, preferably on susceptible and resistant varieties, should provide the basis of artificial tests. In this study many sources of variation affecting the relationship can be distinguished, such as:
1. the physiological condition of the plant;
2. the environment during the test;
3. the condition of the insect or mite used as inoculum;
4. differences in resistance.

In measuring differences in resistance the sources of variation mentioned under 1 to 3 can act as disturbing factors, the influence of which must be limited by standardization and good experimental design.

ARTIFICIAL TESTS FOR RESISTANCE TO TETRANYCHUS URTICAE KOCH

In the literature on resistance to the twospotted spider mite, *Tetranychus urticae* KOCH (reviewed by VAN DE VRIE et al., 1972 and DE PONTI, 1977), reference is frequently made to the use of artificial tests. There is, however, very little knowledge of the reliability of these tests and therefore it is astonishing to find that they are used on a rather large scale.

The aim of the present study is to reduce systematically possible shortcomings as far as testing for resistance in cucumber is concerned. Hereafter many aspects of a standard laboratory test, developed in our institute and to be outlined at the end of this paper, will be subjected to investigation.

*Conditioning plant material and environment.* Besides its level of resistance, the suitability of a plant as a host for insects and mites depends largely on its physiological condition, as affected by age, nutrition, turgor, treatments with agricultural chemicals and the environment. The environment also directly influences the development of insects and mites. The abundant literature on these subjects has been reviewed by VAN EMDEN (1966a, 1966b), SINGH (1970) and specifically for spider mites by VAN DE VRIE et al. (1972) and SUSKI & BADOWSKA (1975). In most studies on resistance to spider mites plant material and test environment are well described and often standardized.

The cucumber plants used in our experiments are grown in a glasshouse in accordance with common practice. Seeds are sown in trays containing sandy soil and after four days the plants are transplanted in pots filled with peat-soil of a very homogeneous and constant composition. About 12 days later the plants have one to two true leaves and are suitable for use in experiments. Neither pesticides nor fertilizers are used.

The experiments are carried out in rooms conditioned for temperature (26°C), relative air humidity (50–70%), daylength (14 hours) and light intensity (12000 mW/m$^2$). This environment is suitable for the growth of cucumber plants as well as for the development of twospotted spider mites. The pots are placed on benches, on

a cotton blanket saturated with water to ensure an even water dose. In each experiment ten plants of a susceptible variety are included as standards.

*Conditioning the inoculum.* The twospotted spider mites used as inoculum in our experiments are from a population, which is representative for the Netherlands by repeated collecting on different crops throughout the country. This population is maintained in a glasshouse on susceptible cucumber plants, which are regularly replaced by young ones. The use of a definite laboratory strain, as practised by STONER & STRINGFELLOW (1967), CHAPLIN et al. (1968), GENTILE et al. (1969) and MACDONALD et al. (1972), has been rejected because it never contains the wide genetic variation of a natural population. Thus the risk of breeding varieties to only one particular strain is avoided. For similar reasons the mites are not reared in the laboratory, where the genetic composition and physiological quality of the population might change rather soon (GUTHRIE, 1975).

The initial reaction of an insect or mite to a new hostplant is often strongly influenced by the preceding host. This has been demonstrated for spider mites by BRAVENBOER (1959), SNETSINGER et al. (1966) and MATSUTANI (1968) and for insects by KENNEDY (1958), HOVANITZ (1969), YAMAMOTO et al. (1969) and HUBERT-DAHL (1975). Nevertheless, mites are mostly reared on beans no matter what crop one is studying. SCHUSTER et al. (1972), who studied resistance to the twospotted spider mite in cotton, alternate rearing on bean with rearing on cotton to avoid 'bean-specialization'. To be quite on the safe side we prefer to rear the mites continuously on cucumber, although there is no evidence for an influence of the preceding host on the sequence of a group of plants according to their level of resistance.

For most insects and mites reproduction is a function of age. To avoid irrelevant variation it is necessary to uniform the age of the mites to be used as inoculum. Different techniques were tried out.

1. Teleiochrysales are collected from heavily infected cucumber leaves. Although this technique is very accurate, it was rejected, because an unknown percentage of mites were – often invisibly – injured. Besides, the technique is too laborious.

2. Eight days before inoculation early in the morning an abundance of mites are shaken from heavily infected cucumber leaves on young non-infected plants. At the end of the day the mites are blown from the leaves with compressed air. The plants with eggs are placed in a controlled-climate room at 26°C and after 8 days the newly developed deutonymphs are used as inoculum. A disadvantage of this technique is, that individuals in quiescent stages are not removed by the air current. They will further develop and later contaminate the population of deutonymphs.

3. Another technique differs only in one detail from the preceding one. The mites are not removed from the leaves with compressed air, but killed with an acaricide without ovicidal action, like dichlorvos (DDVP). Thus the individuals in quiescent stages are also killed. It still has to be proved, however, that there is no latent influence of the acaricide through the eggs on the later stages.

4. The fourth technique is similar to the second except that it is carried out on large cucumber leaf disks (10 cm $\varnothing$), which float on a 10 ppm solution of benzimidazol in water (DE PONTI & INGGAMER, 1976). A similar technique is used in RODRIGUEZ's laboratory (see DABROWSKI, 1972). But the question remains whether the deviating

Table 1. Acceptance and reproduction of deutonymphs reared according to three techniques. Figures followed by the same letter do not differ significantly from one another at the 5% level.

| Rearing technique | Acceptance (%) | Reproduction (eggs/♀, 3 days) |
|---|---|---|
| on the plant, mites removed with air current | 97ab | 26.0a |
| on the plant, mites killed with DDVP | 98a | 26.2a |
| on leaf disks, mites removed with air current | 91b | 25.4a |

physiological condition of leaf disks in comparison with intact plants influences the developing mites.

The quality of the deutonymphs reared according to the techniques mentioned under 2, 3 and 4 has been investigated. With deutonymphs of these 3 origins a standard acceptance and reproduction test, which will be described in detail at the end of this paper, has been carried out on 3 × 20 plants of a susceptible cucumber line.

The results, which are shown in Table 1, indicate that the quality of deutonymphs is almost identical. Only after rearing the mites on leaf disks is the degree of acceptance significantly lower. Mainly for the sake of efficiency the number two technique is preferred. The females thus reared are not fertilized, but according to BRAVENBOER (1959) this does not affect their reproduction capacity.

*Investigations on measuring differences in reproduction.* For measuring resistance, acceptance and reproduction must both be taken into account (DE PONTI, 1977). First the reproduction factor will be analyzed, while the acceptance factor will be dealt with later.

Until now resistance to the twospotted spider mite has been investigated almost exclusively by measuring reproduction of the mites or by damage ratings of the plants. In artificial tests the suitability of plants for the mites is mostly determined by measuring the reproduction during 24, 48 or 72 hours after inoculation. Sometimes all developmental stages of the progeny of the inoculum are watched, but mostly the observations are restricted to the eggs. In view of the earlier mentioned influence of the preceding host one might wonder how far the differences thus found are caused by the substrate tested.

To discover the most representative sample of the reproduction of this mite on cucumber we followed for one generation the entire reproduction of individual mites on eight varieties with a different level of resistance (as demonstrated by KOOISTRA, 1971). When the plants were in the third leaf stage one day-old female mite was placed in a 10 mm ($\varnothing$) leaf cage on the lower side of the first leaf (Fig. 1). After three days the number of eggs was counted and the mite and cage transferred to the next leaf. This was repeated every three days until natural death of the female. Thus the entire reproductive phase of the mites was subdivided into successive three-day periods. The brood was prevented from leaving the respective leaves by smearing the petioles with 'Tanglefooth' (Fig. 2). Seven days after the counting of the eggs the egg viability and larval mortality, further abbreviated to preadult mortality, were assessed.

The results are shown in Table 2. Analyses of variance for oviposition and net reproduction (= oviposition minus preadult mortality) have been executed followed

644                                                                                   *Euphytica 26 (1977)*
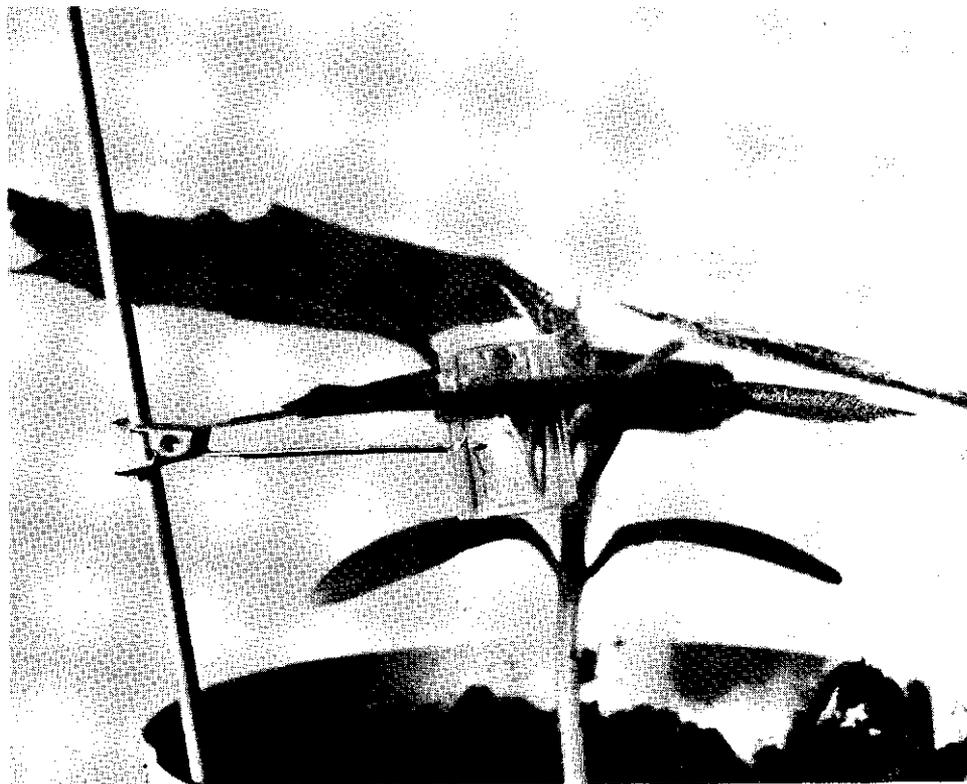
Fig. 1. Cucumber plant with leaf cage on the lower side of the first leaf.

by the multiple range test of KEULS (1952). Differences in preadult mortality, expressed as a percentage of oviposition, have been tested by means of binomial probability paper (FERGUSON, 1956). Only the first four three-day periods have been analyzed, since for practical reasons, later periods are less suitable citeria of resistance.

The results demonstrate that differences in host-suitability are expressed by differences in longevity, oviposition and preadult mortality, resulting in differences in net reproduction of the mite. The sex ratio also influences the rate of reproduction, but whether this ratio is affected by varietal differences has not yet been investigated. Although there are clear differences in longevity on the various varieties, this factor is less suitable as a criterion for resistance, because it requires daily observations for a long period of overlapping mite-generations.

The most complete information on varietal differences is provided by the total net reproduction. To what extent partial observations of oviposition or net reproduction are representative has been examined by comparing results of such observations with the total net reproduction. In most periods analyses of variance revealed significant differences, but the multiple range tests show that the degree to which the varieties can be distinguished from one another, concerning oviposition as well
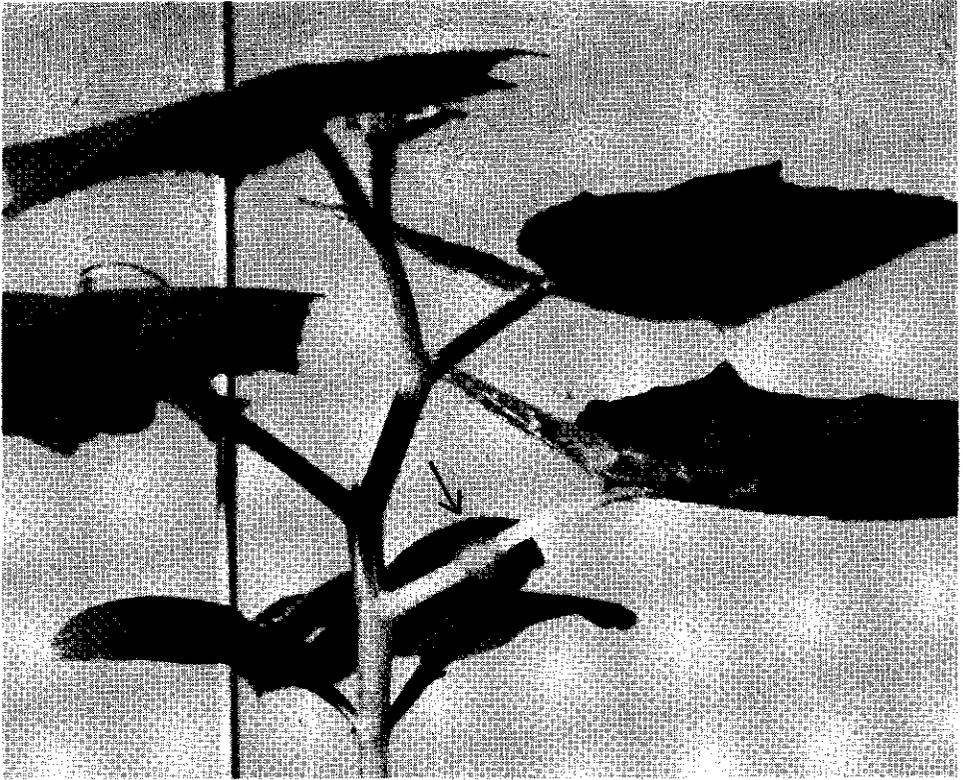
Fig. 2. Cucumber leaf of which the petiole is smeared with 'Tanglefooth'.

as net reproduction of the mites, differs markedly per period. The oviposition during the first few days – a widely used criterion of resistance – hardly gives any information on varietal differences (see periods 1 and 2). This is probably an aftereffect of the preceding host.

If for reasons of efficiency the observations have to be limited, the above analyses indicate that oviposition or net reproduction should be recorded in the third or fourth three-day period. Further information on this point can be obtained from the correlation coefficients between the total net reproduction and the net reproduction and oviposition over the successive periods (Table 3). These correlation coefficients have been calculated between plants, within each variety as well as within all varieties together, exluding the variety effect. Judging from these correlation coefficients the fourth period seems to be the better one.

There appears to be a very high correlation between the total net reproduction and the total oviposition. Although in an absolute sense the contribution of preadult mortality to the reproduction is quite large, as can be seen from Table 2, this high correlation suggests that the preadult mortality hardly affects the relative differences, the key factor for selecting resistant plants.

Although the discrimination between the varieties if based on oviposition is less

646

Table 2. Reproduction factors of females of *Tetranychus urticae* on cucumber varieties with different levels of resistance, recorded per period of three days. Figures followed by the same letter do not differ significantly from one another at the 5% level.

| Variety | Number of plants | Oviposition/♀, 3 days, plant | | | | | | | | | | | Average longevity (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | Period 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1. Granex | 28 | 183a | 33a | 33a | 29ab | 26a | 22 | 16 | 12 | 7 | 3 | 2 | 24 |
| 2. Sporu | 17 | 163ab | 31ab | 31a | 30a | 25a | 18 | 12 | 9 | 5 | 1 | 1 | 21 |
| 3. Varamin | 16 | 157ab | 30ab | 29a | 28ab | 23ab | 17 | 12 | 9 | 5 | 3 | 1 | 23 |
| 4. Német Kigyó | 12 | 142bc | 27ab | 27a | 26ab | 24a | 16 | 12 | 6 | 3 | 1 | 0 | 20 |
| 5. Taipei no 1 | 12 | 136bc | 29ab | 31a | 24ab | 19abc | 16 | 11 | 4 | 1 | 1 | 0 | 20 |
| 6. Nobit | 12 | 126bc | 25b | 32a | 22b | 16c | 12 | 8 | 4 | 4 | 2 | 1 | 19 |
| 7. Hybrid L.G.P. | 12 | 115c | 30ab | 30a | 22b | 16bc | 9 | 6 | 1 | 1 | 0 | 0 | 17 |
| 8. Kecskeméti | 17 | 115c | 28ab | 27a | 27ab | 19abc | 8 | 4 | 2 | 0 | 0 | 0 | 16 |

Preadult mortality/♀, 3 days, plant (%)

| Variety | | | | | | |
|---|---|---|---|---|---|---|
| 1. Granex | 3a | 3a | 3a | 3a | 3a | |
| 2. Sporu | 10b | 6ab | 12cd | 10b | 12b | |
| 3. Varamin | 10b | 7ab | 8bc | 12b | 13b | |
| 4. Német Kigyó | 12c | 3a | 7ab | 14b | 21c | |
| 5. Taipei no 1 | 12bc | 13cd | 15d | 12b | 8ab | |
| 6. Nobit | 9b | 9bc | 8bc | 10b | 7ab | |
| 7. Hybrid L.G.P. | 25d | 21e | 26e | 26c | 29cd | |
| 8. Kecskeméti | 30d | 17de | 26e | 39d | 40d | |

Net reproduction/♀, 3 days, plant

| Variety | Total | Period 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Granex | 176a | 32a | 32a | 28a | 25a | 22 | 15 | 11 | 7 | 3 | 1 |
| 2. Sporu | 145ab | 29ab | 27ab | 27ab | 22ab | 17 | 11 | 8 | 4 | 0 | 0 |
| 3. Varamin | 142ab | 28ab | 27ab | 25abc | 20ab | 16 | 11 | 8 | 4 | 3 | 0 |
| 4. Német Kigyó | 124b | 26ab | 25ab | 22abcd | 19ab | 13 | 9 | 6 | 3 | 1 | 0 |
| 5. Taipei no 1 | 119bc | 25b | 26ab | 21bcd | 17abc | 14 | 10 | 4 | 1 | 1 | 0 |
| 6. Nobit | 116bc | 23b | 29a | 20cd | 15bc | 11 | 7 | 4 | 4 | 2 | 1 |
| 7. Hybrid L.G.P. | 85cd | 23b | 22b | 16d | 11c | 7 | 4 | 1 | 1 | 0 | 0 |
| 8. Kecskeméti | 79d | 23b | 20b | 16d | 11c | 5 | 3 | 1 | 0 | 0 | 0 |

than if based on net reproduction, it appears justified to restrict the observations to oviposition. In that case the fourth three-day period is probably the most effective for measuring differences.

In the above experiment, of each variety a number of plants were tested with one mite per plant. The varieties are genotypically rather homogeneous. The variation within the variety must therefore mainly be due to the variability of the inoculum in addition to random variation. Given this variability one should learn how many plants per variety or how many mites per plant must be used to be able to distinguish differences in resistance of a certain magnitude. This can be determined by means of the nomograph for the power of the F-test of FERGUSON (1962). Table 4 is an abstract of this nomograph. In the above experiment the coefficient of variation in oviposition during the first period was about 20%. The maximum difference between the varieties was 15%. This implies that at least 20 repetitions are necessary to distinguish

Table 3. Correlation coefficients between the total net reproduction and the Net Reproduction (NR) and Oviposition (O) of successive periods of 3 days and the total oviposition.

| Variety | Correlationcoefficients ($\times$ 1000) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | NR. 1 | NR. 2 | NR. 3 | NR. 4 | O.1 | O.2 | O.3 | O.4 | O. total |
| Granex | 326 | 499** | 697** | 717** | 339 | 526** | 716** | 704** | 979** |
| Sporu | 48 | 621** | 618** | 840** | −93 | 553* | 518* | 777** | 973** |
| Varamin | 577* | 634** | 426 | 853** | 492 | 362 | 154 | 790** | 937** |
| Német Kigyó | 308 | 856** | 756** | 682* | 361 | 891** | 812** | 835** | 951** |
| Taipei no 1 | 467 | 720** | 757** | 901** | 173 | 491 | 509 | 869** | 928** |
| Nobit | −124 | 122 | 531 | 652** | −392 | 118 | 386 | 647* | 986** |
| Hybrid L.G.P. | 329 | 775** | 664* | 805** | −29 | 662* | 645* | 848** | 794** |
| Kecskeméti | 702** | 680** | 729** | 855** | 504* | 578* | 502* | 738** | 907** |
| All 126 plants together, variety effect excluded | 306** | 589** | 633** | 776** | 201* | 517** | 538** | 754** | 949** |

* P $\leq$ 0.05; ** P $\leq$ 0.01.

this difference with a probability of 50%. Although in the fourth period the coefficient of variation was larger (about 30%), the maximum difference is 40%, meaning that about eight repetitions are sufficient. This is another indication that testing should preferably take place in the fourth period in stead of in the first.

The importance of the preadult mortality is demonstrated by the fact that the differences between the varieties for net reproduction are much larger than for oviposition. One could also consider using the preadult mortality as an independent criterion for resistance. Whereas the oviposition in the first three-day period, as shown, hardly differs between varieties, the proportional preadult mortality (Table 2) of this brood is very divergent and the differences are in close agreement with the differences in total net reproduction. This confirms the experience of KENNEDY (1958), that the suitability of a hostplant should preferably be measured on the first progeny of an insect on its new host and especially on preadults, which are most sensitive to inadequacies. Although the preadult mortality over the first three days seems to be a reliable criterion for resistance, this has not been further examined, because its determination is too laborious in comparison with other criteria.

Table 4. Power of the F-test for two coefficients of variation (s/m) with several repetitions and at different probability levels ($\beta$) according to FERGUSON (1962) at a significancy level of 5%.

| Number of repetitions | Distinguishable differences (%) | | | | | |
|---|---|---|---|---|---|---|
| | s/m = 20% | | | s/m = 30% | | |
| | $\beta$ = 0.5 | 0.7 | 0.9 | $\beta$ = 0.5 | 0.7 | 0.9 |
| 5 | 32 | 40 | 50 | 49 | 60 | 75 |
| 8 | 24 | 30 | 37 | 37 | 45 | 55 |
| 14 | 19 | 22 | 28 | 28 | 33 | 41 |
| 20 | 15 | 18 | 22 | 23 | 27 | 34 |

The above experiences on the importance of various reproduction factors as parameters for resistance only partly agree with those of DACOSTA (1971) and DACOSTA & JONES (1971). Concerning the preadult mortality the conclusions are similar, although we never found mortality figures up to 99%. This might be due to a difference in technique, because Dacosta transferred young larvae, which were hatched on a susceptible hostplant, to the testplant, whereas in our experiments larvae were born on the testplant. The latter technique seems better adapted to the natural situation, because larvae are not very mobile. The present study does not confirm Dacosta's statement, that differences in resistance are not expressed by differences in oviposition. A disagreement, which might be caused by the difference in varieties tested, but most probably by shorcomings of Dacosta's test, which have already been indicated.

*The reliability of testing on leaf disks.* In testing homogeneous varieties or breeding lines repetitions can be realized by using a number of plants. In a segregating population repeated observations on the reproduction of mites can only be made by using a number of leaf cages or leaf disks per plant. To economize labour the latter technique is to be preferred (DE PONTI & INGGAMER, 1976).

The behaviour and reproduction of insects and mites on excised plant parts, like detached leaves and leaf disks, may differ from the normal pattern on an intact plant (BECK, 1956; MÜLLER, 1958; LE BERRE, 1967; STORMS, 1969; MOREAU, 1971 and VAN EMDEN & BASHFORD, 1976). Nevertheless, leaf disks have been used on a large scale to determine differences in reproduction of the twospotted spider mite (RODRIGUEZ, 1953; DABROWSKI et al., 1971; MACDONALD et al., 1971; DABROWSKI, 1972; TULISALO, 1972; SOANS et al., 1973a, 1973b; SCHALK et al., 1975).

In our opinion the reliability of the leaf disk technique should be investigated thoroughly. For this purpose the reproduction of the twospotted spider mite on intact plants and on leaf disks of four cucumber varieties with a different level of resistance has been compared on 5 to 10 plants per variety. When the plants were in the second leaf stage one day-old female mite was placed in a leaf cage on the lower side of the first leaf and one on a leaf disk from the same leaf. The disks were put on filterpaper in a plastic tray with the lower side upwards according to the technique described by DE PONTI & INGGAMER (1976). After three days egges were counted, the 'plant-mite' and cage were moved to the second leaf and the 'disk-mite' to a disk from this leaf. This was repeated three times. After seven days the preadult mortality was observed.

Figure 3 shows the results of oviposition and preadult mortality, expressed as a percentage of oviposition. Oviposition and preadult mortality of mites on leaf disks do not differ significantly from those on intact plants provided they are not kept there for longer than three days. In case of a continued stay on leaf disks oviposition and preadult mortality become significantly lower. So testing on leaf disks appears to be justified only for short tests. This has been further examined in the following experiment.

On 15 plants of five varieties with a different level of resistance twenty day-old female mites were placed on the first leaf, of which the petiole was smeared with 'Tanglefooth'. After eight days 2 or 3 of these mites were placed individually in leaf cages on separate leaves and the same numbers of mites were placed separately on
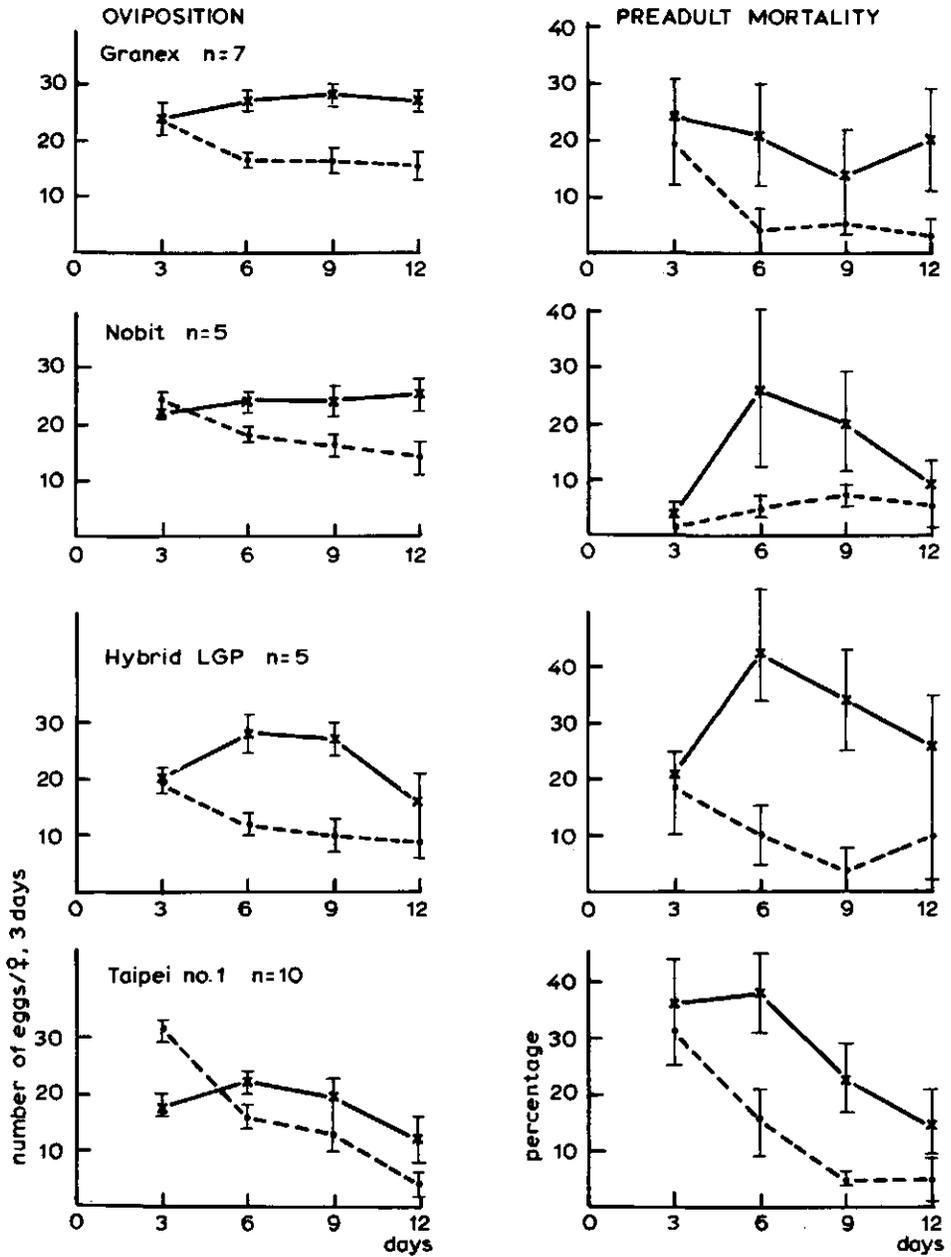
Fig. 3. Comparison of oviposition and preadult mortality per female per period of three days on intact plants ( × ) and on leaf disks (●). The vertical lines mark the standard deviation of the mean.

Table 5. Comparison of oviposition and net reproduction on plants and on leaf disks. Figures followed by the same letter do not distinguish significantly at the 5% level.

| Variety | Oviposition | | Net reproduction | |
|---|---|---|---|---|
| | plant | disk | plant | disk |
| Granex | 27a | 22a | 25a | 19a |
| Taipei no 1 | 20b | 15b | 16b | 12b |
| Varamin | 17bc | 13bc | 12bc | 9bc |
| Hybrid L.G.P. | 13c | 9c | 11c | 7c |
| Kecskeméti | 8d | 11bc | 6d | 8bc |
| Mean | 17.2 | 14.0 | 14.1 | 10.1 |

leaf disks from the same leaves. After three days oviposition and seven days later the preadult mortality were assessed.

The results, which are presented in Table 5 and Fig. 4, show a consistent change in the average level of oviposition and net reproduction. The purpose of a laboratory test can, however, only be to determine relative differences in resistance. Analyses of variance show that testing on leaf disks in this respect is about as informative as testing on intact plants in so far the comparison of varieties is concerned (Table 5). The same conclusion can be drawn from Figure 4, in which individual plants are
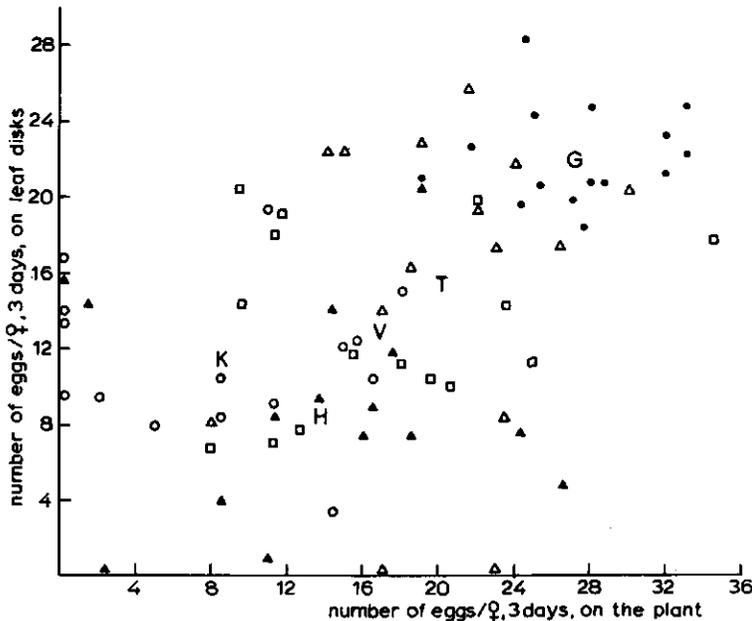


Fig. 4. Correlation between oviposition per female mite during three days on intact plants and on leaf disks from five varieties: ● 'Granex', △ 'Taipei no 1', □ 'Varamin', ○ 'Kecskeméti', ▲ 'Hybrid LGP'. The points represent averages per plant. The averages per variety are indicated by capital letters.

compared. The large variation between plants of one variety must mainly be ascribed to the variability of the inoculum, because the varieties are fairly homogeneous. The variation is extra large due to the fact that each plant was tested with rather few mites. In this connection it is worth noting that the variation on leaf disks is similar to that on intact plants.

It may be concluded from the above experiments that testing on leaf disks is justified provided that the tests are restricted to about three days.

DESIGN OF A LABORATORY TEST FOR RESISTANCE TO THE TWOSPOTTED SPIDER MITE IN CUCUMBER.

Based on the results of the above studies on various aspects of the relationship between cucumber and the twospotted spider mite, the following laboratory test for measuring differences in resistance was designed.
1. The plants are grown in a glasshouse according to common practice.
2. After two to three weeks, when the plants have a full-grown first leaf, they are moved to a controlled-climate room (26°C; 50-70% RAH; 14 hours light of 12000 mW/m²).
3. The same day the petiole of the first leaf is smeared with 'Tanglefooth' and 20 female deutonymphs are placed on the leaf. Rearing the deutonymphs must be started eight days earlier.
4. After 10 days the remaining mites are counted (measure of acceptance).
5. Five disks are punched from the second leaf. On each disk one (nine day old) mite from the first leaf is placed.
6. Three days later the number of eggs is counted (measure of reproduction).

Although this test has been developed for cucumber, it might be adapted for any crop. Besides it might also be used in studies of the influence of non-genetical factors of a plant, like its nutrition, on the mites.

In contrast to glasshouse and field tests the above resistance test distinguishes two essentially different factors, viz. non-acceptance and reproduction, which together are a measure of resistance. This distinction does not mean that both factors are, of necessity, not interrelated. This question will be studied, before separate breeding for both factors is started. Measurement of the preadult mortality has been omitted from this test, because it does not essentially change the relative differences in resistance. With some extension, however, it can easily be assessed.

Testing plants for resistance to the twospotted spider mite by observing the oviposition of any mite on any leaf disk of any plant during some hours or days can hardly give useful information, as can be learned from the present study. This might partly explain, why after optimistic papers on differences in resistance based on such undefined tests, many projects have not been continued.

The research on which the above test is based indicates that also this test must be used with some reserve. The deviations from the natural situation and the large variation of the results may not be underestimated. As a consequence selection in a genetically heterogeneous population may not be too rigorous and the resistance of each selected plant must be confirmed by progeny testing before such a plant can be used for further breeding.

652

Before an ultimate decision about the reliability of this artificial test can be made, the correlation between this test and a field test has to be studied. This will form part of a following paper.

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654

# RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* KOCH. 3. SEARCH FOR SOURCES OF RESISTANCE

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INDEX WORDS

*Cucumis sativus*, cucumber, *Tetranychus urticae*, twospotted spider mite, resistance breeding, tolerance, genetic sources.

SUMMARY

After a first screening of 800 cucumber varieties, a laboratory test and a practical test, the following nine varieties have been selected for their distinguishable level of resistance to the twospotted spider mite: PI 220860, 'Hybrid Long Green Pickle', PI 178885, 'Ohio MR 200', 'Taipei no 1', 'Robin 50', 'Aodai', PI 163222 and PI 218036. Besides resistance tolerance for the twospotted spider mite also appears to occur. Related *Cucumis* species do not seem to possess higher levels of resistance than the most resistant *C. sativus* varieties. The resistance-parameters acceptance and reproduction appear to be positively correlated. On resistant varieties the metabolism of the spider mites is clearly disturbed, but this seems not to be caused mainly by bitter principles.

INTRODUCTION

Reports on resistance in cucumber to the twospotted spider mite, *Tetranychus urticae* KOCH, are scarce. They are based either on incidental observations (EVDOKIMOV, 1954; BOOS, 1971), on screening but a few varieties (LALL & SING, 1968; DACOSTA & JONES, 1971; SOANS et al., 1973) or on screening large germplasm collections (KOOISTRA, 1971; TULISALO, 1972).

After a first screening of 400 varieties of the *Cucumis sativus* collection of the institute, Kooistra selected 12 varieties with a relatively high level of resistance to *Tetranychus urticae*. Screening this collection was continued in the present study and extended to some related *Cucumis* species. According to a procedure of testing decreasing numbers of varieties with increasing intensity and accuracy, this first screening was followed by laboratory tests and practical tests aimed at selecting the most resistant varieties. In addition, the correlation between results of the artificial laboratory test and those of the practical test was studied.

MATERIAL AND METHODS

*First screening.* During the summer of 1975, 395 varieties of the *Cucumis sativus* collection of the institute were planted in a glasshouse with three plants per variety and no replications. The plots were well isolated from one another, so that non-

preference, as defined by DE PONTI (1977a), was excluded. The plants were grown on strings in accordance with common practice at minimum day and night temperatures of 22 and 20°C, respectively. Owing to radiation, temperature occasionally ran up to 35°C at noon. Instead of an overhead watering system irrigation tubes were used. Powdery mildew was controlled by a single treatment with the systemic fungicide pyrazyfos (Curamil), which has no noticeable influence on the twospotted spider mite. In the fourth leaf stage each plant was inoculated with 20 adult female mites. After six weeks, differences in damage were scored on a scale of one to five (1 = slight damage, 5 = heavy damage).

*Laboratory test.* The varieties which were least damaged in the previous test (class 1 and 2), together with the twelve varieties selected by KOOISTRA (1971), were subjected to a laboratory test to determine acceptance and reproduction as parameters of resistance. A technical description of this test is contained in an earlier publication (DE PONTI, 1977b). Ten plants randomized on an individual plant basis in climate rooms, were tested per variety. Because of the large number of varieties consecutive tests were necessary. As a standard each test contained ten plants of a susceptible control: a gynoecious, bitterfree slicing cucumber breeding line, representative of the present Dutch varieties.

*Practical test.* Most of the varieties involved in the previous test were also included in the practical test; only some which appeared clearly susceptible in the laboratory test were omitted. The practical test was similar to the first screening with the following exceptions. The experiment consisted of three replications of four plants per plot, arranged in randomized blocks. Each plant was inoculated with ten adult female mites and the damage caused by the spider mites was scored at weekly intervals on a damage scale designed by HUSSEY & PARR (1963) (Fig. 1). At each observation five leaves per plant (at a level of 60, 100, 140, 180 and 200 cm) were judged and their mean value represented the damage index. Observations were continued until the susceptible control was nearly dead.

As far as seed was available, varieties earlier reported in literature as resistant were screened in the three preceding tests.

*Related species.* In the laboratory and practical tests six related *Cucumis* species were also investigated using experimental designs similar to those above. Because of limited availability only one or two accessions could be tested per species.

RESULTS AND DISCUSSION

The first screening was intentionally carried out in the glasshouse – the 'field' of the Dutch slicing cucumber – to test for resistance under normal growing conditions. Table 1 presents the distribution of the 395 varieties over five damage classes. The identity of the 29 least damaged varieties is given in Table 2. The classification of the other 366 varieties is recorded in the documentation on the *Cucumis* collection.

Some varieties earlier reported as (partly) resistant appeared in this trial not to belong to classes 1 and 2: 'Improved Long Green' (LALL & SING, 1968) and PI 267942
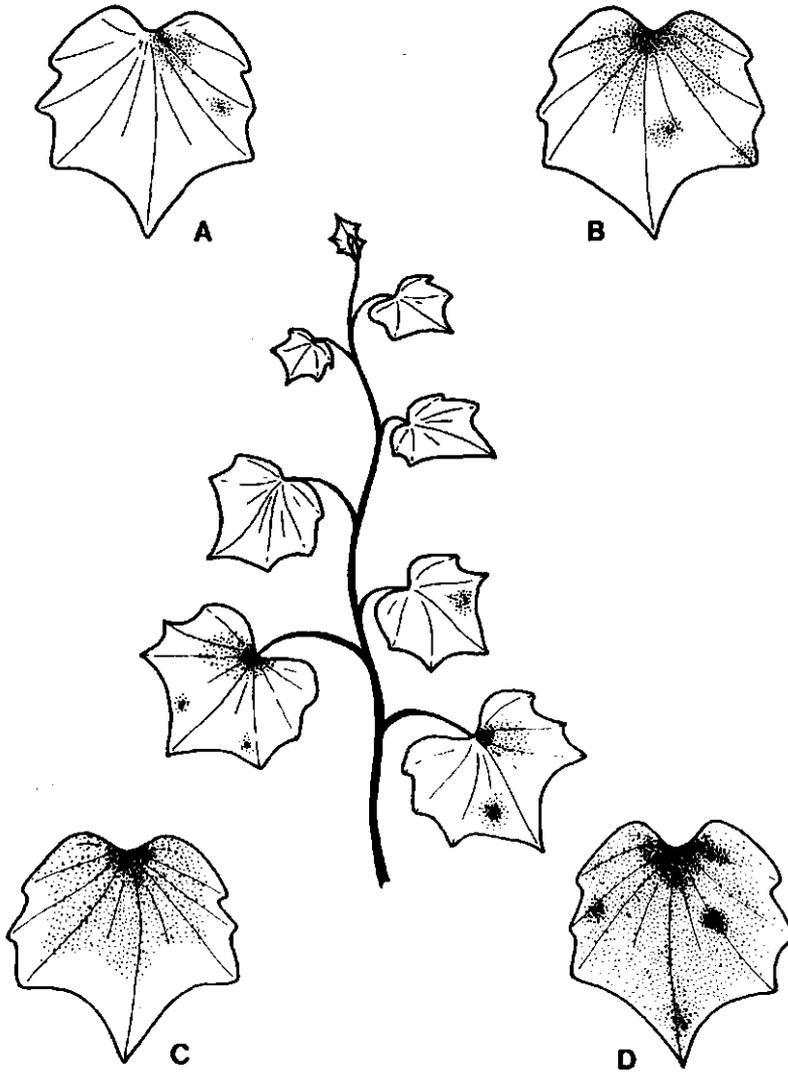
168

Fig. 1. Degrees of leaf damage in cucumber caused by *Tetranychus urticae*. A = 1.0, B = 2.0, C = 3.0, D = 4.0. Damage index 5.0 is the same as D but the leaves are bleached and begin to shrivel. In the centre a plant with an average damage index of 0.4. This figure is a reprint from Growers' Bull. 1 (1972) of the Glasshouse Crops Research Institute, Littlehampton, England.

Table 1. Distribution of 395 cucumber varieties over five damage classes (1 = slight damage; 5 = heavy damage).

| | Damage classes | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Number of varieties | 6 | 23 | 37 | 138 | 191 |

Table 2. Average acceptance, reproduction and damage index (last score) of all varieties tested in the laboratory test and in the practical test for resistance to the twospotted spider mite.

| Variety | Geografic origin | Laboratory test | | Practical test |
|---|---|---|---|---|
| | | acceptance (%) | reproduction (eggs/♀, 3 days) | damage index (0–5) |
| Susceptible control | the Netherlands | 76 | 26.5 | 4.3 |
| PI 220860 | Korea | 24** | 11.6** | 1.2* |
| Hybrid L.G.P. | USA | 36** | 18.5** | 1.5* |
| PI 178885 | Turkey | 35** | 11.5** | 2.6* |
| Ohio M.R. 200 | USA | 37** | 18.3** | 2.8* |
| Taipei no 1 | Taiwan | 39** | 21.1* | 1.9* |
| Robin 50 | USA | 55** | 20.9** | 2.6* |
| Aodai | Japan | 55* | 18.6** | 2.6* |
| PI 163222 | India | 49* | 21.4* | 2.6* |
| PI 218036 | Iran | 58* | 20.4* | 2.7* |
| Kecskeméti | Hungary | 54** | 19.3** | 3.2 |
| Posrednik | USSR | 62** | 12.9** | 3.5 |
| Long day ochiai | Japan | 62 | 13.3** | 2.3* |
| Varamin | Iran | 76 | 17.3** | 2.8* |
| Hybrid P.Y.C.P. | Israel | 66* | 20.4** | 3.3 |
| PI 109483 | Turkey | 48* | 18.5** | 3.6 |
| PI 181756 | Libanon | 46* | 14.9** | 4.1 |
| PI 279469 | Japan | 72 | 17.3* | 1.9* |
| Vladivostokskij 155 | USSR | 74 | 20.7* | 2.8* |
| PI 179263 | Turkey | 56* | 20.0* | 3.4 |
| PI 197085 | India | 46* | 18.7* | 3.9 |
| PI 103049 | China | 58* | 20.3* | 3.9 |
| PI 202801 | Syria | 45* | 17.5* | 4.8 |
| Német Kigyó | Hungary | 58** | 22.9 | 3.9 |
| PI 279466 | Japan | 64 | 23.5 | 1.5* |
| PI 171600 | Turkey | 41** | 21.1 | 3.3 |
| Marketer | USA | 73 | 23.4 | 2.1* |
| NPI | the Netherlands | 70 | 26.3 | 3.9 |
| PI 197087 | India | 61 | 29.6 | |
| PI 167223 | Turkey | 67 | 21.9 | |
| E-c-szan | Vietnam | 65 | 27.9 | |
| PI 113334 | China | 67 | 23.7 | |
| PI 174174 | Turkey | 54* | 22.1 | |
| PI 227210 | Japan | 49* | 25.4 | |
| PI 209068 | USA | 56* | 22.3 | |
| PI 227207 | Japan | 72 | 23.5 | |
| PI 274902 | England | 77 | 25.0 | |
| PI 279463 | Japan | 76 | 24.1 | |
| PI 279467 | Japan | 83 | 24.4 | |
| Tikanari | Japan | 80 | 26.7 | 3.5 |
| Marketmore | USA | –[1] | –[1] | 3.5 |

*,** Significantly different from the susceptible control for $P \leqslant 0.05$ respectively $P \leqslant 0.01$.
[1] No seed available.

(TULISALO, 1972) were scored into class 3; PI 137839 and PI 267741 (TULISALO, 1972) into class 4 and 'Leningradski teplicniji' (EVDOKIMOV, 1954) into class 5.

Although the laboratory tests were carried out in climate rooms, acceptance of the susceptible control varied in the successive tests from 60–93% and reproduction from 22.5–31.5 eggs. Based on the values of the susceptible control the results of the laboratory test, which are presented in Table 2, have been reduced to the same denominator. By analyses of variance the significances of the differences were tested; for the acceptance after an arcsin transformation. The results of the practical test are included in Table 2 as the damage index score in the seventh week after inoculation, when the susceptible control had nearly died.

From the results of the laboratory test and the practical test three groups of varieties can be distinguished. One group includes 9 varieties which appear consistent in their resistance as expressed by the three criteria observed. These varieties will be used for breeding resistant commercial varieties. A second group contains 13 varieties which differ little if at all from the susceptible control. This disagreement with the first screening is probably due to known shortcomings of that test, such as the poor experimental design. PI 279463 and PI 279467, tested by TULISALO (1972), belong to this group. Although incompletely tested, also 'Marketmore', which SOANS et al. (1973) reported to be resistant, does not seem very promising.

Seventeen varieties of an intermediate group containing PI 279466, PI 279469, 'Vladivostokskij 155' (TULISALO, 1972) and 'Marketer' (DACOSTA & JONES, 1971) react quite differently in the two tests. Such inconsistencies also occur in the resistant group, but are then less pronounced. In addition to random variation or the large environmental differences between the laboratory and the practical test, these discrepancies might be caused by an essential difference between the two tests. The former measures aspects of resistance only, whereas the damage index of the latter reflects the resultant of aspects of resistance and tolerance (for definitions see DE PONTI, 1977a). If this explanation is right, PI 181756 is resistant but at the same time very sensitive and reversely PI 279466 is susceptible but tolerant. Similarly, in the resistant group one could call 'Taipei no 1' moderately resistant but very tolerant and PI 178885 very resistant but slightly tolerant. Interactions between resistance and tolerance may be very complicated. If the hypothesis on the occurrence of tolerance can be confirmed by further investigations, it will add an extra dimension to the practical test. Besides acting as a check on the results of the laboratory test, it will also provide information on the tolerance or sensitivity of the preselected material. Thus the finally selected varieties will combine resistance and tolerance, as might be the case in the earlier mentioned group of 9 selected varieties. If it can be proved that in the two tests the opposite results of varieties such as PI 181756 and PI 279466 are indeed caused by an interaction of resistance and tolerance, it should be possible to select from a cross between such varieties lines which are resistant and tolerant. This would throw new light on the value of some of the varieties of the intermediate group, although the above 9 best varieties will keep the highest priority.

The relatively slow increase of the damage index of the five most promising varieties
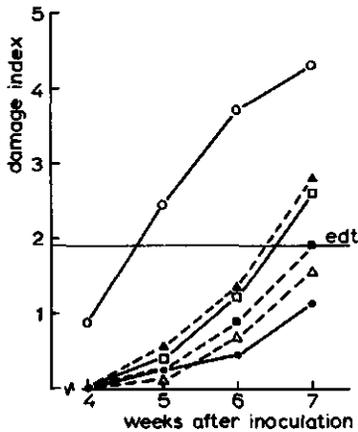
Fig. 2. Increase of the damage index of the five most resistant varieties and the susceptible control. The economic damage threshold (edt) lies at 1.9. ○ = susceptible control, ▲ = 'Ohio MR 200', □ = PI 178885, ■ = 'Taipei no 1', △ = 'Hybrid L.G.P.', ● = PI 220860.

in comparison with the susceptible control is demonstrated in Fig. 2. The line at 1.9 represents the economic damage threshold as calculated by HUSSEY & PARR (1963). For a well growing crop SCHULZE (1975) found a similar value, but according to his experiences the damage threshold can lie markedly lower in a poorly growing crop. This may explain the findings of TULISALO (1970), who disagreed with Hussey and Parr. In any case, with the most promising varieties an economic damage threshold at 1.9 is passed four to five weeks later than with the susceptible control. This means an essential progress in the control of the twospotted spider mite. In case of combined application of resistance with chemical control it will cause a 50% reduction in the chemical control frequency. Biological control will also be favoured by a slower development of the spider mites, although the exact quantitative effect
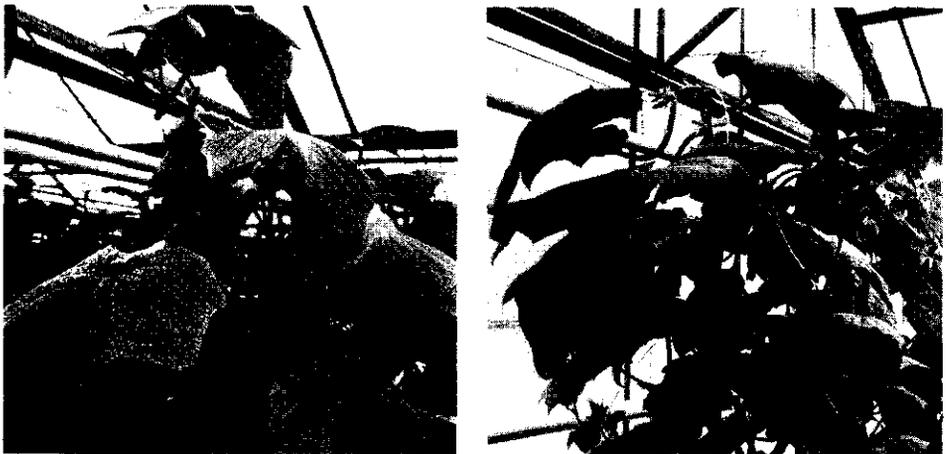


Fig. 3. Left the susceptible control and right the selected variety PI 220860 in the practical test in the seventh week after inoculation.

172

Table 3. Average acceptance, oviposition, net reproduction and damage index of some accessions of *Cucumis* species tested in the laboratory test and in the practical test for resistance to the twospotted spider mite.

| *Cucumis* species | Laboratory test | | | Practical test damage index (0–5) |
|---|---|---|---|---|
| | acceptance (%) | oviposition (per ♀, 3 days) | net reproduc- tion (per ♀, 3 days) | |
| C. africanus L.f. | 38* | 19.9 | 16.2* | 3.9 |
| C. anguria L. | 47* | 25.3 | 17.9* | 3.7 |
| C. anguria L. | 43* | 24.2 | 18.5* | 4.3 |
| C. dipsaceus EHRENB, ex SPACH | 67 | 24.4 | 19.6* | 4.0 |
| C. ficifolius A. RICH | | | | 4.0 |
| C. myriocarpus NAUD. | 55* | 22.9 | 14.3* | |
| C. myriocarpus NAUD. | | | | 2.4* |
| C. zeyheri SOND. | 67 | 20.3 | 14.3* | 4.5 |
| Susceptible control | 76 | 26.5 | 24.2 | 4.3 |

*,** See Table 2

is not easily predictable. Fig. 3 shows the difference in damage of a selected variety and the susceptible control in the seventh week after inoculation as observed in the practical test.

The results of screening some accessions of related *Cucumis* species are presented in Table 3. Because the differences in oviposition were not significant, the laboratory test was extended to measure preadult mortality, so that net reproduction (see DE PONTI, 1977b) was also determined. As far as tested, the species differ from the susceptible control for acceptance and net reproduction, but not for the damage index scored in the practical test. This might indicate that, although these species are (moderately) resistant, they are at the same time rather sensitive. Only *C. myriocarpus* seems to be of general interest as a source of resistance. This species was not tested by KNIPPING et al. (1975), who reported *C. africanus* and *C. anguria* as resistant and moderately resistant respectively. The resistance of these two species agrees with our results. Although the number of *Cucumis* species tested is extremely small in proportion to that of *C. sativus*, these species do not seem to possess higher levels of resistance than the selected *C. sativus* varieties. Besides, the possible resistance of these species is unexploitable as long as interspecific crosses with *C. sativus* do not succeed (DEAKIN et al., 1971).

Breeding for resistance to the twospotted spider mite could be simplified by limiting the laboratory test to only acceptance or by selecting first for low acceptance and only in the selected plants for low reproduction. Whether such changes in the selection procedure are justified, depends on the relationship between the degrees of acceptance and reproduction. This relationship is plotted in Fig. 4, which indicates that acceptance and reproduction are generally interrelated. This relationship, however, seems not strong enough to conclude that the suggested changes in the laboratory test are justified. In the course of this research, this question will be given further attention.
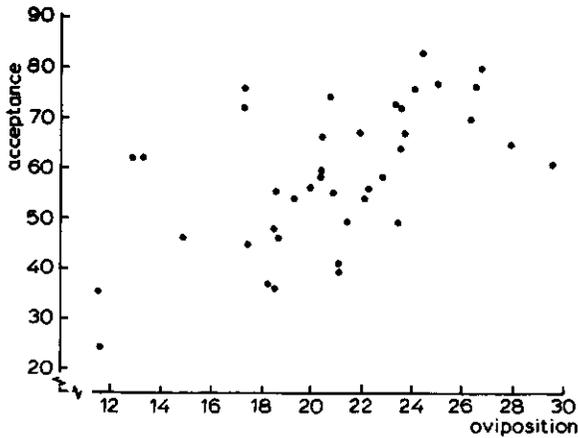
Fig. 4. The relationship between acceptance and oviposition in the laboratory test.

By the laboratory test large numbers of plants can be screened efficiently, a prerequisite for routine breeding. In many aspects, however, this artificial test deviates from the common conditions in a glasshouse, where the crop in question is grown and attacked by the twospotted spider mite. Therefore any relationship between the results of the laboratory and the practical test must be demonstrated before the former test can be used routinely. Fig. 5 showing the scores of 29 varieties in the laboratory and the practical test hardly demonstrates any relationship between the results of the two tests. As discussed earlier, this might be ascribed to the occurrence of differences in tolerance interfering in the practical test with differences in resistance. An insufficient correlation, therefore, does not yet justify the omission of the laboratory test. On the contrary, a tandem application of the two tests is probably indispensable.

Although the mechanisms of resistance have not yet been extensively investigated some preliminary remarks can be made. The majority of the 800 cucumber varieties tested by KOOISTRA (1971) and in this research are bitter by the presence of cucurbitacines. It was demonstrated that many varieties are susceptible to the twospotted
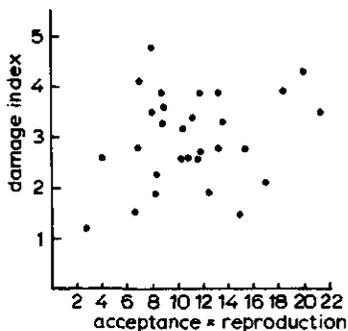


Fig. 5. The relationship between damage index (practical test) and acceptance × oviposition (laboratory test).
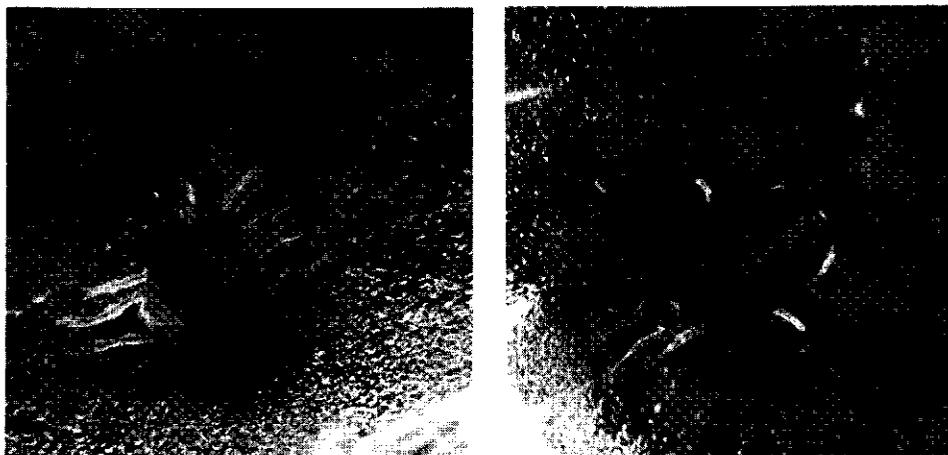
Fig. 6. Left a normal twospotted spider mite on a susceptible variety and right a black one on a resistant variety.

spider mite although they are bitter. The conclusion of DACOSTA (1971) and DACOSTA & JONES (1971) that the bitter principle is the major cause of resistance to the two-spotted spider mite in cucumber can, therefore, not be endorsed. On the other hand, from the experimental data collected so far it is not justified to ignore any influence of cucurbitacines. If and to what extent these principles play a role, will be further investigated.

On many resistant varieties the shape and marking of the mites changed within some days after they were moved to these varieties. They grew thicker, became more or less bloated and turned completely black instead of showing the two common black spots (Fig. 6). Although without an extensive study on the causes of these striking changes any interpretation of them is rather speculative, they seem to indicate that the metabolism of the mites is disturbed. The black mites are completely filled with black pellets containing indigestible food as described by WIESMANN (1968). The abundance of faecal pellets seems to be accompanied by a complete constipation, hampering other functions such as reproduction. This disturbance of the metabolism may be caused by a relatively large number of indigestible substances, such as abundant chlorophyl, in the sap of resistant varieties or by the presence of substances which disturb the normal progress of metabolism. Further investigat ons may clarify this complicated matter. A good knowledge of the mechanisms o. the resistance is important because it may speed up the development of resistant varieties.

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# RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* KOCH.
# 4. THE GENUINENESS OF THE RESISTANCE

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INDEX WORDS

*Cucumis sativus*, cucumber, *Tetranychus urticae*, twospotted spider mite, resistance breeding, precondition, stability of resistance.

SUMMARY

For several generations subpopulations of the twospotted spider mite were reared on cucumber varieties previously selected as partially resistant. Subsequently resistance tests were carried out in the laboratory and the glasshouse with mites from these subpopulations and concurrently with mites from a basic population reared on a susceptible cucumber line. In these tests neither acceptance nor net reproduction or damage index on the partially resistant varieties depended on the kind of mite populations used as inoculum. Therefore it is concluded that the resistance is genuine. The stability of the resistance is discussed.

INTRODUCTION

The reproduction potential of the twospotted spider mite (*Tetranychus urticae* KOCH) varies substantially for its many different host plant species (VAN DE VRIE et al., 1972). When twospotted spider mites move or are moved from one species to another, their reproduction on the second species can markedly deviate from the normal reproduction (DOSSE, 1952; BRAVENBOER, 1959; MATSUTANI, 1968). It has, however, often been observed that this deviation is only temporary and that the reproduction reaches its proper level within but a few generations. JESIOTR & SUSKI (1976), for example, transferred twospotted spider mites from roses to *Phaseolus*-beans. Initially the reproduction was about 75% lower than normal on beans, but this reduction gradually disappeared within four generations of the mite (about two months).

In our studies on resistance in cucumber (*Cucumis sativus* L.) to the twospotted spider mite, mites were reared on a susceptible cucumber line or variety and moved from these to other varieties. In laboratory and practical tests the reproduction in the first generation after transfer and the damage index in the next few generations were used as criteria of resistance. Varieties on which the reproduction of the mite and the damage caused by the mites were significantly lower than on the susceptible variety were classified as resistant (DE PONTI, 1978).

The reduction in reproduction after moving the mites from the susceptible to another variety might also be of a temporary nature. In that case the resistance of the selected varieties would not be genuine. To investigate this, the resistance tests were

repeated after the mites had been reared for a number of generations on the selected varieties.

MATERIAL AND METHODS

For several years now, a population, hereafter called basic population, of the two-spotted spider mite has been maintained on a susceptible cucumber line, which is also used as susceptible control in the resistance tests (DE PONTI, 1977). Subpopulations were built up on thirteen varieties previously selected as resistant (DE PONTI, 1978) by moving fifty female mites to plants of each of these varieties. These plants were grown in a glasshouse, at temperatures between 23 and 28 C, and kept as much isolated as possible from one another. The plants were regularly replaced by fresh ones of the same variety. About two generations of the mite developed per month. Due to mutual contamination or eradication by predatory mites some subpopulations were lost and had to be built up again. Therefore the age of the subpopulations varied from two to eleven months (Table 1) at the time the following resistance tests were carried out.

*Laboratory test.* On seven varieties acceptance and net reproduction ( = oviposition minus preadult mortality) were determined as parameters of resistance. Definitions of these parameters and a technical description of the test were given in an earlier publication (DE PONTI, 1977). Per variety two sets of ten plants were simultaneously tested. One set was inoculated with mites from the basic population and the other with mites from the subpopulation of the variety concerned. The plants were randomized on an individual plant basis in climate rooms. The line on which the basic population is reared was used as susceptible control. This line was only inoculated with mites from the basic population.

*Practical test.* For a detailed description of this test, which was carried out in a glasshouse, reference is made to DE PONTI (1978). Thirteen varieties were tested with two mite populations in three replications. Per replication two plants of each variety were inoculated with 20 adult female mites from the basic population and two with 20 adult female mites from the subpopulation of the variety concerned. According to a split block design the plants were arranged in such a way that one row per replication contained the plants with the basic population mites and the other row the subpopulation mites. The susceptible control was only inoculated with 20 adult female mites from the basic population. The damage caused by the spider mites was scored at weekly intervals on a damage scale of one to five (1 = slight damage; 5 = heavy damage) designed by HUSSEY & PARR (1963). At each observation date five leaves per plant were judged and the mean value for damage represents the damage index (DE PONTI, 1978).

RESULTS AND DISCUSSION

The results of the laboratory test are presented in Table 1. Per variety the differences in acceptance and net reproduction between the two different populations of the

436

Table 1. Average acceptance, net reproduction and damage index of the varieties tested in the laboratory test and the practical test for resistance to the twospotted spider mite. The mites originated either from a basic population (B) or from subpopulations (S) reared on the separate varieties.

| Variety | Laboratory test | | | | | Practical test | | |
|---|---|---|---|---|---|---|---|---|
| | age sub-population (months) | acceptance (%) | | net reproduction (per ♀, 3 days) | | age sub-population (months) | damage index (0–5) | |
| | | B | S | B | S | | B | S |
| Susceptible control | | 84 | | 25.5 | | | 4.4 | |
| Hybrid L.G.P. | 8 | 40 | 32 | 17.7 | 15.7 | 3 | 1.4 | 1.6 |
| Ohio M.R. 200 | 2 | 41 | 40 | 16.9 | 16.2 | 7 | 2.7 | 2.9 |
| Taipei no 1 | 8 | 44 | 36 | 19.3 | 20.9 | 11 | 2.0 | 1.7 |
| Robin 50 | 2 | 61 | 50 | 20.1 | 12.7* | 7 | 2.7 | 2.4 |
| Keckskeméti | 8 | 60 | 54 | 18.5 | 13.8 | 11 | 3.4 | 3.0 |
| Posrednik | 5 | 70 | 73 | 11.7 | 12.4 | 11 | 3.4 | 3.6 |
| Varamin | 8 | 84 | 70* | 17.0 | 18.2 | 4 | 2.8 | 2.8 |
| PI 220860 | | | | | | 3 | 0.9 | 1.4 |
| PI 178885 | | | | | | 4 | 2.5 | 2.7 |
| Aodai | | | | | | 4 | 2.1 | 3.1 |
| PI 163222 | | | | | | 3 | 2.3 | 2.9 |
| PI 218036 | | | | | | 5 | 2.6 | 2.9 |
| PI 279469 | | | | | | 5 | 1.9 | 2.0 |
| Mean | | 57 | 51 | 17.3 | 15.7 | | 2.4 | 2.5 |

* Differences between B and S significant at the 0.05 level.

twospotted spider mite were generally small. After rearing the mites for several generations on resistant varieties acceptance and net reproduction in the laboratory test tended to decrease rather than increase, although according to the Wilcoxon test the decreases are only significant in two cases.

The results of the practical test are included in Table 1 as the damage index score in the seventh week after inoculation, when the susceptible control had nearly died. The differences in damage index between the two populations of the mite were small. According to the analysis of variance (Table 2) the F-value for mite population dif-

Table 2. Analysis of variance for the mean damage index observed on thirteen varieties after inoculation with twospotted spider mites originating from a basic population and from subpopulations reared on the separate varieties.

| Source of variation | Dim. | M.S. | F |
|---|---|---|---|
| Replications (R) | 2 | 1.24 | |
| Mite populations (M) | 1 | 0.63 | 0.41 |
| R × M | 2 | 1.53 | |
| Varieties (V) | 12 | 2.63 | 8.48* |
| R × V | 24 | 0.31 | |
| M × V | 12 | 0.20 | 0.56 |
| R × M × V | 24 | 0.36 | |

* $P < 0.01$.

ferences is not significant. The differences between varieties are very significant, but there is no significant interaction between mite populations and varieties. In the fourth, fifth and sixth weeks after inoculation similar results were obtained. After inoculation with subpopulation mites however, the damage index tended to increase. This is most probably due to the experimental design used. For, unexpectedly, the western rows in the glasshouse were significantly more seriously damaged than the eastern rows and the plants inoculated with subpopulation mites were in two replications situated in western rows and in one replication in eastern rows. For the plants inoculated with basic population mites this was just the reverse.

It appears that the differences in acceptance, net reproduction and damage index between the varieties originally selected as resistant and the susceptible control have persisted after rearing the mites for a number of generations on the selected varieties. It is, therefore, justified to state that the resistance observed in these varieties is genuine. These experiments again demonstrate that testing cucumber for resistance to the twospotted spider mite in the first generation after transfer of the mites from a susceptible line or variety is reliable and effective provided such a test satisfies a number of conditions investigated earlier (DE PONTI, 1977).

It should be noted that the results of these experiments may not at all be interpreted as evidence of the permanence of the resistance. They only indicate that within the population of the twospotted spider mite no genetic changes took place which could affect the resistance of certain varieties. To reduce the chance that the resistance will be overcome by simple mutagenic changes in the mite population breeding is aimed at developing varieties with resistance of a complex nature. This approach results from the modern view that resistances which are complex by nature tend to be more stable (EENINK, 1976; PIMENTEL & BELLOTTI, 1976; ROBINSON, 1976; PARLEVLIET & ZADOKS, 1977; REIMANN-PHILIPP, 1977). Therefore selection for different phenomena of resistance such as non-acceptance, low reproduction and low damage index is carried out, so that the selection will not be focussed on a single factor. Thus it is attempted to make the host-parasite relationship incongruous in many aspects to promote the permanence of the resistance (HOGENBOOM, 1975).

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# RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* KOCH. 5. RAISING THE RESISTANCE LEVEL BY THE EXPLOITATION OF TRANSGRESSION

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SUMMARY

After crossing partially resistant varieties some lines with a markedly higher resistance level were selected. This transgression for resistance indicates a polygenic inheritance of the resistance. On the most resistant $F_5$ lines selected, oviposition was reduced by 50 to 60 % and the economic damage threshold was reached 5 to 8 weeks later than in the susceptible control. The level of acceptance was not influenced by the selection.

The selection of individual $F_2$ plants was hampered by low heritabilities, whereas the heritabilities of $F_3$- and subsequent line means were generally high enough. Selection for higher resistance levels was attended by an unintended increase in cucurbitacin content. This is explained by linkage of genes for resistance and bitterness rather than by identity of these genes.

INTRODUCTION

Although the resistance to the twospotted spider mite (*Tetranychus urticae* KOCH) in some varieties of a collection of *Cucumis sativus* L. was found to be remarkable (DE PONTI, 1978a), an increase in the level of resistance would strengthen the effect of this aspect of integrated control.

Although the pedigree of the partially resistant varieties was not known their diverse geographic origin suggested a large genetic diversity justifying investigations into the potentiality of transgression for resistance. Some of the varieties were, therefore, intercrossed and the offspring checked for plants and lines with an increased level of resistance. In the $F_2$ and following generations the heritability of the resistance was calculated to gather insight into the selectability of this character on individual plants and lines respectively.

Because of some contradictory reports (DACOSTA & JONES, 1971; GOULD, 1978; DE PONTI, 1978a) it is still not clear as to what extent cucurbitacins contribute to the resistance of cucumber to the twospotted spider mite. Therefore, cucurbitacin contents were determined at the end of the above selection process to investiagate whether selection for higher levels of resistance had resulted in higher cucurbitacin contents.

MATERIALS AND METHODS

Ten cross combinations were made between seven previously selected (DE PONTI, 1978a) partially resistant varieties (Table 1), which were genotypically uniform, since they were repeatedly inbred. Some reciprocal crosses were also made, but between the reciprocal $F_1$'s no differences in resistance were noticed. $F_1$ and $F_2$ populations of 10 and 65–160 plants respectively were tested in *laboratory tests* using acceptance and oviposition as parameters of resistance. A technical description of this test was given earlier (DE PONTI, 1977). Per $F_3$, $F_4$, and $F_5$ line 6–16 plants were tested in laboratory tests. As standards each test contained an equal number of plants of a susceptible line and of the partially resistant parental varieties concerned. The plants were always randomized on an individual plant basis in climate rooms.

The $F_3$, $F_4$ and $F_5$ lines that in the laboratory tests appeared to be promising, were also tested in previously described (DE PONTI, 1978a) *practical tests* in a glasshouse. Three replications of three plants per line were arranged in randomized blocks. In the fourth leaf stage each plant was inoculated with ten adult female mites and the damage caused by the spider mites was scored at weekly intervals on a damage scale of one to five (1 = slight damage; 5 = heavy damage). At each observation date five leaves per plant were judged and the mean value for damage represented the damage index. As standards, each test contained a susceptible line and the partially resistant parental varieties concerned also in three replications of three plants. The susceptible standard used in all tests is a gynoecious, bitterfree slicing cucumber breeding line, representative of present Dutch varieties.

*Selection procedure.* The selection in the laboratory test was primarily based on the oviposition, which – inherent to the design of the test – is expressed in a figure averaging five independent scores per plant, whereas acceptance is determined only once. In the $F_2$ only plants on which a lower oviposition occurred than on the most resistant parent were selected. The same criterion was later used for the averages of $F_3$, $F_4$ and $F_5$ lines, but here the average damage index in the practical test was taken into full account as well. Within the lines that in both tests met the selection criterion, the most resistant plants were selected, mostly in the laboratory test, where on oviposition five independent observations were made per plant.

*Determination of cucurbitacin-content.* In a practical test, of the $F_3$ lines per plant two top leaves at a height of 2.50 m were collected. After cutting the two leaves of one plant in small pieces, one sample was taken for a dry weight determination and one for a quantitative determination of cucurbitacin-C, the only detectable cucurbitacin in normal cucumber leaves (REHM, 1960). The latter determination was carried out according to a quantitative method developed in our laboratories by VAN KEULEN (1980) using chloroform extraction, thin layer chromatography and in situ fluorometry after reaction with antimony trichloride.

RESULTS

The results of 32 laboratory tests and 3 practical tests are summarized in Table 1. The

Table 1. Average acceptance, oviposition and damage index (highest score) of the susceptible control (SC) and five successive generations in laboratory and practical tests for resistance to the twospotted spider mite (T = lines tested; S = lines selected). The numbers of plants tested in the F₂, and lines tested respectively selected in the F₃, F₄ and F₅ are in parentheses.

| Cross | SC | P₁ | P₂ | F₁ | F₂ | F₃ T | F₃ S | F₄ T | F₄ S | F₅ T | F₅ S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Laboratory test: average acceptance (%)* | | | | | | | | | | | |
| 1. HLGP × Robin 50 | 86 | 50 | 64 | 53 | 48(70) | 65(9) | 59(3) | 42(7) | 42(7) | 55(13) | 52(7) |
| 2. Robin 50 × Varamin | 83 | 61 | 56 | 61 | 56(65) | 66(6) | 67(3) | 67(6) | 64(3) | 51(7) | 57(5) |
| 3. HLGP × Varamin | 83 | 40 | 68 | 54 | 50(80) | 55(9) | 47(4) | 49(8) | 49(7) | 53(14) | 48(8) |
| 4. Taipei no. 1 × Posrednik | 81 | 74 | 63 | 67 | 59(80) | 66(3) | 65(2) | 54(4) | 52(3) | 66(8) | 66(8) |
| 5. Kecskeméti × Taipei no. 1 | 83 | 59 | 67 | 65 | 69(80) | 63(9) | 66(3) | 56(5) | 56(3) | 66(10) | 68(7) |
| 6. Taipei no. 1 × Robin 50 | 85 | 80 | 73 | 71 | 71(80) | 79(4) | 82(2) | 79(2) | 76(1) | 59(1) | 59(1) |
| 7. PI 220860 × Robin 50 | 66 | 52 | 50 | 49 | 47(160) | 46(12) | 43(5) | | | | |
| 8. HLGP × Kecskeméti | 93 | 75 | 85 | 64 | 68(80) | 69(5) | – | | | | |
| 9. Kecskeméti × Posrednik | 91 | 83 | 64 | 84 | 70(80) | 80(2) | – | | | | |
| 10. Taipei no. 1 × HLGP | 68 | 83 | 30 | 74 | 68(80) | 40(12) | – | | | | |
| Weighted Mean | 82 | | 64 | 64 | 61 | 58 | 58 | 54 | 51 | 58 | 58 |
| *Laboratory test: average oviposition (per ♀♀, 3 days)* | | | | | | | | | | | |
| 1. HLGP × Robin 50 | 21.3 | 16.7 | 17.7 | 19.1 | 17.8(70) | 13.9(9) | 10.1(3) | 13.8(7) | 13.8(7) | 12.2(13) | 10.6(7) |
| 2. Robin 50 × Varamin | 20.5 | 16.7 | 17.2 | 17.6 | 15.7(65) | 16.5(6) | 16.0(3) | 14.1(6) | 12.7(3) | 13.1(7) | 12.3(5) |
| 3. HLGP × Varamin | 21.0 | 16.1 | 16.9 | 19.0 | 16.7(80) | 18.3(9) | 17.1(4) | 12.8(8) | 12.5(7) | 15.1(14) | 14.1(8) |
| 4. Taipei no. 1 × Posrednik | 24.3 | 23.3 | 18.0 | 22.9 | 20.8(80) | 17.8(3) | 16.0(2) | 15.1(4) | 14.2(3) | 12.6(8) | 12.6(8) |
| 5. Kecskeméti × Taipei no. 1 | 20.0 | 17.2 | 19.5 | 16.2 | 16.5(80) | 16.4(9) | 15.0(3) | 12.9(5) | 11.7(3) | 11.8(10) | 11.4(7) |
| 6. Taipei no. 1 × Robin 50 | 22.2 | 21.4 | 17.9 | 21.6 | 23.3(80) | 18.7(4) | 17.8(2) | 16.2(2) | 15.5(1) | 15.7(1) | 15.7(1) |
| 7. PI 220860 × Robin 50 | 23.1 | 17.6 | 20.4 | 19.9 | 18.4(160) | 15.2(12) | 13.8(5) | | | | |
| 8. HLGP × Kecskeméti | 20.2 | 15.9 | 18.2 | 17.6 | 21.9(80) | 19.1(5) | – | | | | |
| 9. Kecskeméti × Posrednik | 20.9 | 17.6 | 15.4 | 20.4 | 21.2(80) | 19.1(2) | – | | | | |
| 10. Taipei no. 1 × HLGP | 22.1 | 23.4 | 14.7 | 20.7 | 19.2(80) | 18.4(12) | – | | | | |
| Weighted Mean | 21.6 | | 18.1 | 19.5 | 19.2 | 16.9 | 14.9 | 13.8 | 13.1 | 13.1 | 12.4 |
| *Practical test: average damage index (0–5)* | | | | | | | | | | | |
| 1. HLGP × Robin 50 | 4.4 | 2.3 | 2.7 | | | 2.5(6) | 2.0(5) | 1.3(4) | 1.3(4) | 0.9(9) | 0.9(9) |
| 2. Robin 50 × Varamin | 4.4 | 2.7 | 3.3 | | | 3.0(4) | 2.3(2) | 3.0(3) | 2.7(2) | 2.4(5) | 2.3(4) |
| 3. HLGP × Varamin | 4.4 | 2.3 | 3.3 | | | 2.7(5) | 2.3(3) | 2.0(3) | 1.9(2) | 1.9(7) | 1.7(6) |
| 4. Taipei no. 1 × Posrednik | 4.4 | 2.4 | 3.4 | | | 3.0(3) | 2.5(1) | 2.7(3) | 2.5(2) | 1.9(6) | 1.9(6) |
| 5. Kecskeméti × Taipei no. 1 | 4.4 | 3.3 | 2.4 | | | 2.2(2) | 2.2(2) | 2.3(4) | 2.1(3) | 1.7(6) | 1.7(6) |
| 6. Taipei no. 1 × Robin 50 | 4.4 | 2.4 | 2.7 | | | 2.7(4) | 2.0(2) | – | | 2.1(1) | 2.1(1) |
| 7. PI 220860 × Robin 50 | 4.4 | 1.7 | 2.7 | | | 1.1(3) | 0.8(2) | 1.5(6) | 1.0(2) | | |
| 8. HLGP × Kecskeméti | 4.4 | 2.3 | 3.3 | | | 2.6(2) | 2.0(1) | – | | | |
| 9. Kecskeméti × Posrednik | 4.4 | 3.3 | 3.4 | | | 3.6(3) | 2.8(1) | – | | | |
| Weighted Mean | 4.4 | | 2.7 | | | 2.6 | 2.0 | 2.0 | 1.8 | 1.7 | 1.6 |

Table 2. Average acceptance, oviposition, damage index (highest score) and cucurbitacin content of the most resistant $F_5$ lines compared with their parent varieties and the susceptible control in laboratory and practical tests for resistance to the twospotted spider mite.

| Material | Laboratory test | | Practical test | |
|---|---|---|---|---|
| | acceptance (%) | oviposition (per ♀, 3 days) | damage index (0–5) | cucurbitacin (mg/10 g dry weight) |
| 1. Susceptible control | 84 | 21.0 | 4.7 | 0 |
| HLGP | 39 | 15.4 | 2.9 | 67 |
| Robin 50 | 49 | 16.1 | 2.7 | 65 |
| $F_5$ 77350 | 51 | 6.6* | 0.9* | 125* |
| $F_5$ 77344 | 32 | 9.8 | 0.8* | 113* |
| $F_5$ 77439 | 45 | 9.8* | 0.8* | 111* |
| 2. Susceptible control | 77 | 21.8 | 4.7 | 0 |
| Robin 50 | 59 | 17.6 | 2.7 | 65 |
| Varamin | 47 | 18.6 | 3.3 | 45 |
| $F_5$ 77356 | 57 | 13.8 | 2.1 | 86 |
| $F_5$ 77355 | 64 | 14.0 | 2.1 | 74 |
| $F_5$ 77359 | 36 | 13.0* | 2.6 | 82 |
| 3. Susceptible control | 84 | 19.1 | 4.7 | 0 |
| HLGP | 34 | 14.7 | 2.9 | 67 |
| Varamin | 62 | 15.5 | 3.3 | 45 |
| $F_5$ 77384 | 32 | 10.6* | 1.8 | 47 |
| $F_5$ 77374 | 41 | 12.1 | 1.9 | 71 |
| 4. Susceptible control | 77 | 24.2 | 4.7 | 0 |
| Taipei no. 1 | 53 | 25.6 | 3.2 | 34 |
| Posrednik | 45 | 14.0 | 3.3 | 23 |
| $F_5$ 77365 | 58 | 10.1* | 1.7* | 76* |
| $F_5$ 77362 | 40 | 12.5 | 1.3* | 56 |
| 5. Susceptible control | 80 | 19.3 | 4.7 | 0 |
| Keckskeméti | 37 | 15.1 | 3.4 | 47 |
| Taipei no. 1 | 67 | 19.9 | 3.2 | 34 |
| $F_5$ 77387 | 53 | 10.2* | 1.6 | 65 |
| $F_5$ 77389 | 62 | 11.2 | 1.0 | 64 |
| 6. Susceptible control | 85 | 19.1 | 4.7 | 0 |
| Taipei no. 1 | 81 | 17.9 | 3.2 | 34 |
| Robin 50 | 68 | 14.5 | 2.7 | 65 |
| $F_5$ 77370 | 59 | 12.4 | 2.2 | 97* |
| 7. Susceptible control | 70 | 21.7 | 4.7 | 0 |
| PI 220860 | 59 | 11.3 | 1.3 | 63 |
| Robin 50 | 69 | 17.3 | 2.7 | 65 |
| $F_3$ 77288 | 53 | 9.7 | 0.6 | 102* |
| $F_3$ 77274 | 59 | 9.0 | 1.2 | 102* |

*Significantly different from the most resistant respectively most bitter parent for p ⩽ 0.05.

original data of the laboratory tests have been corrected per set of generations from one cross to improve the intercomparability. The correction was based on the averages for acceptance and oviposition of the susceptible and the two partially resistant standards. The results of the practical test are included in Table 1 as the damage index score in the eighth week after inoculation, i.e. when the susceptible control had nearly died from spider mite damage. The original data of the three practical tests have been corrected
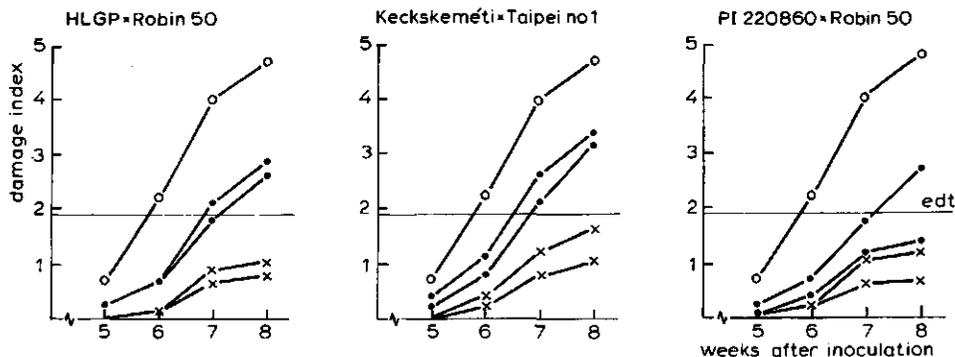
Fig. 1. Increase of the damage index of six $F_5$ lines (x) compared with their parent varieties (●) and the susceptible control (O). The economic damage threshold (edt) lies at 1.9.

on the average damage index of the susceptible and the seven partially resistant standards. In the crosses 8, 9 and 10 the selection was finished after the $F_3$ because no line matched or exceeded the resistance of the most resistant parent as expressed by oviposition and damage index. In cross 7 the selection is continued.

The results of the most resistant $F_5$ lines of the successful crosses 1 to 7 are given in Table 2. The data of the laboratory test are from separate tests, in which per cross the $F_5$ lines were compared with their parent varieties. The data of the practical test come from one test in the spring of 1978 in which all $F_5$ lines were compared among themselves and with their parent varieties. The results of the cucurbitacin analyses are included in this table.

Of three crosses the significantly slower increase in the damage index of the most prominent $F_5$ lines, compared with their parent varieties and the susceptible control, is demonstrated in Fig. 1. The line at 1.9 represents the economic damage threshold as calculated by HUSSEY & PARR (1963). Because the damage index of the $F_5$ lines in the eighth week after inoculation was still very low, it would have been worthwhile to continue the observations. This was, however, hampered by the size of the plants and an increasing mutual contamination of mites between the only spatially isolated plots. Moreover, it appeared very difficult to keep the glasshouse free from the predatory mite *Phytoseiulus persimilis* ATHIAS-HENRIOT for more than six to eight weeks.

In Fig. 2 the progress in selection is shown in full detail using the cross HLGP × Robin 50 as a example. The other crosses behaved analogously. The non-segregating generations demonstrate a large non-genetic variation, which must mainly be ascribed to the variability of the spider mites used as inoculum (DE PONTI, 1977). The impact of this non-genetic variation on the selectability in the subsequent generations is expressed in the heritabilities given in Table 3. After statistical analyses the broad sense heritabilities of the $F_2$ individuals for oviposition were calculated from the variances of $P_1$, $P_2$, $F_1$ and $F_2$. The broad sense heritabilities of the $F_3$, $F_4$ and $F_5$ lines for oviposition and damage index respectively were calculated from the line means. For acceptance heritabilities were not calculated because of the relatively small differences.
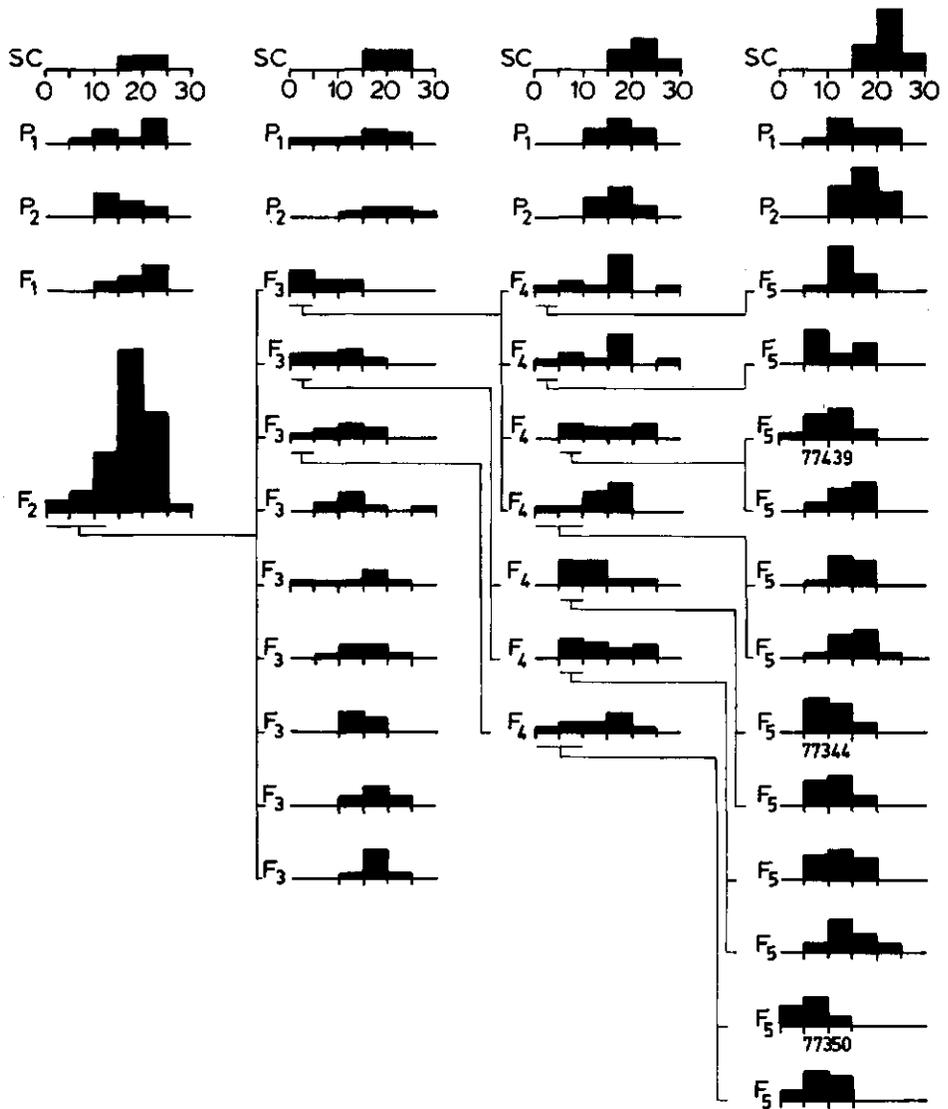
Fig. 2. Distribution of plants of various generations of the cross HLGP ($P_1$) $\times$ Robin 50 ($P_2$) over oviposition classes. The classes are indicated by their upper limit. Solid lines indicate the pedigree of the lines.

Table 3. Broad sense heritabilities for oviposition and damage index of $F_2$ individuals and $F_3$, $F_4$ and $F_5$ line means.

| Cross | Heritabilities | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | oviposition | | | | damage index | | |
| | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_3$ | $F_4$ | $F_5$ |
| 1. HLGP × Robin 50 | 0.11 | 0.76 | 0.00 | 0.82 | 0.52 | 0.46 | 0.11 |
| 2. Robin 50 × Varamin | 0.42 | 0.66 | 0.65 | 0.59 | 0.70 | 0.55 | 0.14 |
| 3. HLGP × Varamin | 0.09 | 0.38 | 0.61 | 0.77 | 0.22 | 0.19 | 0.74 |
| 4. Taipei no. 1 × Posrednik | 0.00 | 0.33 | 0.14 | 0.03 | 0.20 | 0.09 | 0.18 |
| 5. Keckskeméti × Taipei no. 1 | 0.57 | 0.64 | 0.77 | 0.09 | 0.74 | 0.26 | 0.28 |
| 6. 5aipei no. 1 × Robin 50 | 0.25 | 0.00 | 0.00 | – | 0.60 | – | – |
| 7. PI 220860 × Robin 50 | 0.00 | 0.77 | – | – | 0.48 | – | – |
| 8. HLGP × Keckskeméti | 0.34 | 0.33 | – | – | 0.83 | – | – |
| 9. Keckskeméti × Posrednik | 0.19 | 0.00 | – | – | 0.25 | – | – |
| 10. Taipei no. 1 × HLGP | 0.57 | 0.79 | – | – | – | – | – |

DISCUSSION

The above results demonstrate that seven crosses followed by accurate selection for four generations have led to a significant increase in the level of resistance as measured by oviposition and damage index. On the most resistant $F_5$ lines the degree of oviposition is 50 to 60 % lower than on the susceptible control (Table 2), and with these lines the economic damage threshold is – after extrapolation – reached 5 to 8 weeks later than with the susceptible control (Fig. 1). Once varieties with this high level of resistance have been bred, the consequent slow population development of the twospotted spider mite on these varieties will markedly reduce the need for chemical control and will also favour the application of biological control.

Because in most $F_2$'s plants less resistant than their parents also segregated, transgression rather than epistasis is supposed to explain the above results. This transgression for resistance, caused by accumulation of resistance genes, indicates dissimilarities between the resistance genes of the partially resistant varieties. On the other hand the lack of transgression in crosses 8, 9 and 10 points to insufficient differences between the resistance genes of some varieties. This lack of transgression might, however, also be due to the rather small amounts of $F_2$ plants tested or the low heritabilities of $F_2$ individuals (Table 3). Further transgression was found after intercrossing some promising single cross $F_3$ lines. The three double crosses (Taipei no. 1 × Posrednik) × (Robin 50 × Varamin), (Keckskeméti × Taipei no. 1) × (HLGP × Robin 50) and (Taipei no. 1 × Posrednik) × (Keckskeméti × Taipei no. 1) yielded some $F_3$ lines showing a higher resistance level in a practical test than their parental single cross $F_3$ lines.

From the above there is no doubt that the resistance of cucumber to the twospotted spider mite is governed by several genes, probably with additive action. Continuation of the intercrossing procedure, including partially resistant varieties not yet used (DE PONTI, 1978a) might further increase the resistance level.

As shown in Table 3 the heritabilities of the $F_2$ individuals for oviposition were often very low and mostly lower than those of the $F_3$, $F_4$ and $F_5$ line means for oviposition and damage index. These low values are caused by a relatively high non-genetic variation, which is also evident from Fig. 2. This figure also shows that in the course of the selection process the variation within the lines decreases, a general trend in the off-spring of all crosses. This must be ascribed to an increase in the homozygosity of the resistance genes causing a reduced genetic variation within the lines.

Because the heritabilities are only based on single experiments their repeatability is questionable. Moreover, the heritabilities for oviposition can hardly be intercompared, because they all originate from different tests. The heritabilities for damage index are better compared within a generation than between generations, because lines of the same generation were tested in one practical test.

With heritabilities below 0.20 selection becomes useless. This explains why the selection in the $F_2$ caused a rather low response in the $F_3$. Being based on line means, which generally showed a higher heritability, the selection in the $F_3$ and subsequent generations improved, as indicated by the differences between the lines tested and selected (Table 1). In the practical test the differences between both categories of lines were smaller, probably owing to an effective preselection in the laboratory test. The heritabilities also tend to be lower in this test. For reasons mentioned above, selection within lines was not always successful (Table 1, Table 3, Fig. 2).

Based on the present experiences the selection of $F_2$ individuals for resistance to the twospotted spider mite perhaps better be abandoned, thus postponing the first selection to $F_3$ lines. Assuming no unfavourable linkages, selection for a large number of agronomic characters could be carried out in the $F_2$.

It is very remarkable that four generations of selection hardly influenced the level of acceptance, which in the $F_5$ generation equals the mid-parent value. Because the selection was mainly based on the degree of oviposition, the assumed fair correlation between acceptance and oviposition (DE PONTI, 1978a) becomes questionable. It might also be possible that acceptance as a component of resistance reaches its maximum expressivity sooner than oviposition, so that the correlation between both parameters decreases with increasing resistance levels. Assuming that acceptance is mainly governed by olfactory factors and oviposition by both nutrients and secondary plant substances of the host, this might support the experiences of VISSER & DE WILDE (1979) that olfactory factors are less discriminative in host plant selection than the latter factors. Nevertheless, attempts will be made to decrease the level of acceptance by focusing future selection on this character. Marked decrease of both acceptance and oviposition would promote the overall resistance and its diverse composition and so probably favour the stability of the resistance (DE PONTI, 1978b).

At first sight the cucurbitacin contents of the selected $F_3$ and $F_5$ lines (Table 2) are somewhat alarming in view of the aim to breed bitterfree, twospotted spider mite resistant cucumber varieties. That in many cases the selection for resistance was attended by a marked increase in cucurbitacin seems to support the hypothesis that the resistance of cucumber to the twospotted spider mite is mainly based on this principle (DACOSTA & JONES, 1971; GOULD, 1978). The occurrence of many bitter and suscep-

tible varieties (DE PONTI, 1978a) already weakened this hypothesis. Moreover, the production of cucurbitacin is basically governed by one dominant gene Bi (ANDEWEG & DE BRUYN; 1959; ROBINSON et al., 1976), whereas above the polygenic inheritance of the resistance was demonstrated. Because apart from environmental influences, large differences in cucurbitacin concentrations are found between varieties, REHM (1960) suggested that next to the major gene Bi many genes govern in the degree of bitterness. Assuming that at least some of these genes are linked with twospotted spider mite resistance genes, all interrelations between resistance and bitterness reported so far by different research groups can be explained. This hypothesis will be tested on crosses between resistant + bitter and susceptible + bitterfree lines, a research carried out as part of a wider study on the genetics of the resistance.

Until now the typical Dutch glasshouse cucumber has not been included in the crosses. Therefore, no progress was made in the large complex of agronomic characters contained in this special type of cucumber. Recently the fifteen most resistant lines have been released to Dutch breeding firms accepting the challenge to breed the first twospotted spider mite resistant and bitterfree cucumber varieties.

ACKNOWLEDGMENT

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# RESISTANCE OF *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* KOCH. 6. COMPARISON OF NEAR ISOGENIC BITTER AND NON-BITTER VARIETIES FOR RESISTANCE

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SUMMARY

The bitter cucumber variety Improved Long Green did not differ in resistance to the twospotted spider mite from its near isogenic non-bitter mutant, the source of all non-bitter varieties. Four pairs of near isogenic bitter and non-bitter varieties gave similar results. Therefore any causal relation between bitterness and resistance is denied.

INTRODUCTION

Recent reports are not unanimous on the relation between the resistance in cucumber, *Cucumis sativus* L., to the twospotted spider mite, *Tetranychus urticae* KOCH., and the bitter principle cucurbitacin-C.

After screening 400 cucumber varieties for resistance to the twospotted spider mite KOOISTRA (1971) was one of the first to suggest that resistance to the mite is associated with the occurrence of cucurbitacin. Continuing this screening with another 400 varieties, DE PONTI (1978) pointed out that, on the other hand, quite a few varieties were very susceptible despite their bitterness. Earlier SOANS et al. (1973) had a similar experience with the bitter variety Hawaiian. DACOSTA & JONES (1971a), who tested bitter and non-bitter plants of a cross between a bitter and a non-bitter variety backcrossed to the non-bitter variety, claimed the resistance to be due to the presence of cucurbitacin, regulated by the gene Bi. SOANS et al. (1973) and GOULD (1978), who tested two different pairs of bitter and non-bitter varieties, came to the same conclusion. Based on extensive observations during the breeding of highly resistant lines DE PONTI (1979) could not agree to ascribing the resistance simply to bitterness. According to his hypothesis cucurbitacin, if involved at all, would act by linkage rather than by identity of genes for resistance and bitterness.

Mutants often differ in only a single gene, providing near isogenic varieties, which are best suited for analysing the action of that particular gene. We were in the fortunate position that the only known non-bitter mutant (bibi) was found at our institute in the variety Improved Long Green (ANDEWEG & DE BRUYN, 1959; ROBINSON et al., 1976). This mutant is the source of all non-bitter varieties. Germinable seed of the original

sample of this variety and of the first progeny of the mutant was still available. This material, together with four pairs of near isogenic varieties, was tested for resistance to the twospotted spider mite and for bitterness.

MATERIAL AND METHODS

Besides the variety Improved Long Green (ILG) and its non-bitter mutant, four pairs (see Table 1) of near isogenic bitter and non-bitter varieties were tested. Nimbus was bred by crossing Hokus with the non-bitter ILG followed by two backcrosses to Hokus and five generations of selfing. Levo is an $F_1$ hybrid of Femina (bitter) and Hokus. Fablo is an $F_1$ hybrid of Gravina and Nimbus, and Gravina was bred by crossing Femina with the non-bitter ILG followed by two backcrosses to Femina and three generations of selfing. In many morphological and agronomical characters Hokus is similar to Nimbus, and Levo to Fablo. GOULD (1978) reported that Marketmore 70 and 72 are genetically identical with the exception of the gene Bi and those genes closely linked to Bi. The same is supposed for Tablegreen 65 and 72. All pairs of bitter and non-bitter varieties are called near isogenic, although in some cases this is a rather broad interpretation of the term.

After checking the bitterness of the varieties according to the chemical method of ANDEWEG & DE BRUYN (1959), their resistance to the twospotted spider mite was tested. In the *laboratory test* (DE PONTI, 1977) 20 female deutonymphs were placed on the first true leaf. After 10 days the mites that remained were counted as a measure of acceptance. From these mites five were moved to separate leaf discs of the second leaf and after three days oviposition was observed. As standards a susceptible line and the highly resistant line 77344 (DE PONTI, 1979) were used. The numbers of plants tested are given in Table 1. Pairs of one bitter and one non-bitter plant of near isogenic varieties were randomized as split plots in climate rooms. The standards were randomized on an individual plant basis.

Table 1. Average acceptance and oviposition on five pairs of near isogenic varieties compared with those on the susceptible and resistant control.

| Variety | Cucurbitacin | Number of plants | Acceptance (%) | Oviposition (per ♀, 3 days) |
|---|---|---|---|---|
| Improved Long Green (ILG) | + | 48 | 45* | 13.4 |
| Mutant Improved Long Green | − | 45 | 37 | 13.2 |
| Hokus | + | 24 | 49 | 14.9 |
| Nimbus | − | 21 | 48 | 12.6 |
| Levo | + | 23 | 44 | 16.0* |
| Fablo | − | 24 | 57 | 14.9 |
| Marketmore 70 | + | 20 | 50 | 16.3 |
| Marketmore 72 | − | 19 | 43 | 17.2 |
| Tablegreen 65 | + | 20 | 52 | 14.0 |
| Tablegreen 72 | − | 20 | 58 | 13.5 |
| Resistant Control (RC) | + | 7 | 32 | 12.2 |
| Susceptible Control (SC) | − | 8 | 64 | 16.5 |

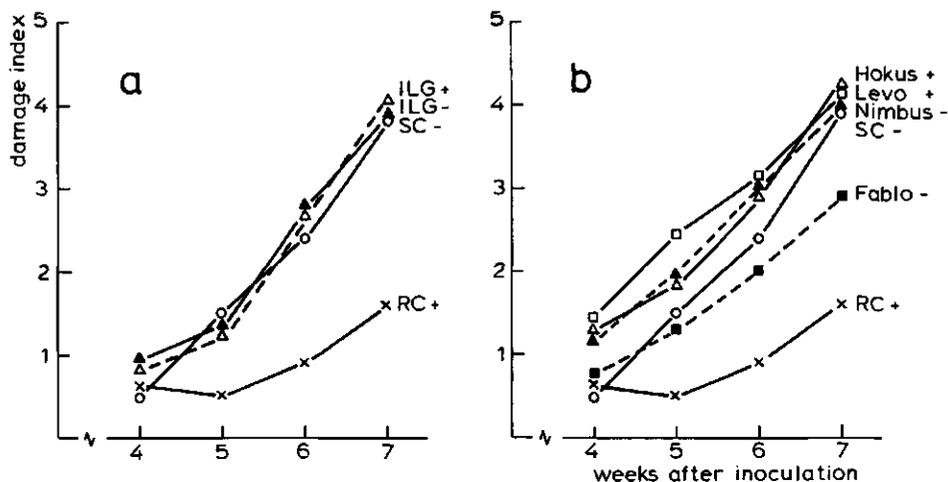*Significantly different from the near isogenic variety for $P \leqslant 0.05$.

262

Fig. 1. Increase in the damage index of three pairs of near isogenic varieties compared with the susceptible (SC) and the resistant control (RC). The presence ( + ) or absence ( – ) of cucurbitacin is indicated. Figure a and b should be considered as a whole.

With the exception of Marketmore 70 and 72 and Tablegreen 65 and 72 the varieties were also included in a *practical test* (DE PONTI, 1978) in a glasshouse. Seven replications of one plant per variety were arranged in a randomized split plot design. Each plot contained one bitter and one near isogenic non-bitter plant. In the fourth leaf stage each plant was inoculated with ten adult female mites. Starting 4 weeks after inoculation, the damage was scored at weekly intervals on a scale of one to five (1 = slight damage; 5 = heavy damage). At each observation date four leaves per plant were judged; the mean value represented the damage index. The same standards as in the laboratory test were used, also in seven replications of one plant.

RESULTS AND DISCUSSION

The results of the laboratory test are presented in Table 1. Our results corroborated the known status of the varieties as bitter or non-bitter. Differences between the near isogenic varieties for acceptance and oviposition were analyzed with the statistical sign test. Figure 1 shows the results of the practical test. In both tests significant differences between near isogenic varieties hardly occurred. Where they were observed they pointed to higher resistance levels in the non-bitter than in the bitter varieties. The conclusion of earlier reports (DACOSTA & JONES, 1971a; KOOISTRA, 1971; GOULD, 1978) that bitterness causes resistance to the twospotted spider mite is, therefore, by no means supported by our results. Especially the absence of a higher resistance level in ILG, compared with its near isogenic non-bitter mutant, strongly denies any causal relation between bitterness and resistance. A genetic relation in terms of linkage of genes for bitterness and resistance is, however, not excluded.

Although this is not confirmed by the practical test, both ILG and its mutant are in the laboratory test more resistant than the susceptible control. The moderate resistance of ILG was earlier observed in the first screening (DE PONTI, 1978), when it scored 3 on

# RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* KOCH.
# 7. THE INHERITANCE OF RESISTANCE AND BITTERNESS AND THE RELATION BETWEEN THESE CHARACTERS

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SUMMARY

The inheritance of resistance to the twospotted spider mite and of bitterness in cucumber has been studied in three sets of $P_1$, $P_2$, $F_1$, $F_2$, $B_{11}$ and $B_{12}$ of crosses between three bitter, resistant lines and one non-bitter, susceptible line. Resistance to the twospotted spider mite as measured by acceptance and oviposition appeared to be determined by several to many genes, which are inherited mainly in an additive fashion. Bitterness is basically governed by the gene Bi, which, contrary to earlier reports, is inherited in an intermediary way, while the expression of Bi is influenced by additively inherited intensifier genes. Whereas Bi and the bitterness intensifier genes are not related to the resistance factors acceptance and oviposition, they are related to resistance or tolerance as measured by the damage index. This relation is explained by linkage rather than by identity of the genes concerned. Changes in the test methods and breeding consequences are discussed.

INTRODUCTION

After lines of cucumber (*Cucumis sativus* L.) highly resistant to the twospotted spider mite, *Tetranychus urticae* KOCH, were selected (DE PONTI, 1979), the development of resistant varieties could start. Knowledge of inheritance of the resistance is a prerequisite to choosing the most effective breeding procedure. Therefore this inheritance was extensively studied.

In the crosses a non-bitter line was used as the susceptible parent, whereas the resistant parental lines were bitter. This enabled us to check earlier findings that bitterness is not causally related to resistance to *T. urticae* (DE PONTI, 1979, 1980). The occurrence of bitterness is regulated by one major gene Bi (ANDEWEG & DE BRUYN, 1959), but the genetic basis of the *degree* of bitterness is still unknown. Therefore the bitter principle cucurbitacin-C was determined quantitatively in part of the material. The inheritance of bitterness and possible linkages between genes for bitterness and resistance were examined.

With the above crosses between resistant, bitter and susceptible, non-bitter lines the breeding of non-bitter twospotted spider mite resistant lines was initiated.

513

MATERIALS AND METHODS

A pure, non-bitter slicing cucumber line, $G_6$, susceptible to the twospotted spider mite and related to current Dutch varieties ($=P_1$), was crossed with three earlier selected (DE PONTI, 1979) bitter cucumber lines ($= P_2$) highly resistant to the twospotted spider mite, viz. $F_5$ (HLGP × Robin 50), $F_5$ (HLGP × Varamin) and $F_5$ (Keckskeméti × Taipei no. 1). Of each $F_1$, one plant was selfed and backcrossed to either parent. The resulting generations, $P_1$, $P_2$, $F_1$, $F_2$, $B_{11}$ and $B_{12}$, in the three sets of crosses were analyzed for resistance and bitterness in 4, 3 and 3 replicates respectively. After the plants were identified in the cotyledon stage as bitter or non-bitter according to the chemical method of ANDEWEG & DE BRUYN (1959), per replicate about 8 $P_1$, 8 $P_2$ and 8 $F_1$, 19 bitter and 19 non-bitter $B_{11}$, 38 $B_{12}$, 75 bitter and 25 non-bitter $F_2$ plants were tested for resistance in the *laboratory test* (DE PONTI, 1977a). The plants were randomized on an individual plant basis in climate rooms and 20 female deutonymphs were placed on the first true leaf. Ten days later the remaining mites were counted as a measure of acceptance. Of these mites five were moved to separate leaf discs of the second leaf and after three days oviposition was observed as a measure of reproduction.

After moving the plants of set 1 to the glasshouse the bitter principle cucurbitacin-C was now quantitatively determined at about the fifth leaf stage. Two top leaves were collected and cut into small pieces. One sample was taken for a dry weight determination and one for a determination of the cucurbitacin-C content, the only detectable cucurbitacin in true leaves of cucumber (REHM, 1960). The latter determination was carried out according to a method developed in our laboratories by VAN KEULEN (1980) using chloroform extraction, thin layer chromatography and in situ fluorometry after reaction with antimony trichloride.

Of the $B_{11}$, the three most resistant bitter and the three most resistant non-bitter plants of each replicate were selected and selfed; of the $F_2$, the seven most resistant bitter and the five most resistant non-bitter plants per replicate. The resulting $B_{11\otimes}$ and $F_3$ lines (about 50 per set of crosses) were tested for resistance in laboratory tests in three or four replicates. Prior to the test the plants were identified as bitter or non-bitter and per replicate two bitter and two non-bitter plants were used per line. Both parental lines were included in these tests and the plants were randomized on an individual plant basis.

Nineteen $B_{11\otimes}$ and $F_3$ lines of set 1 and fourteen of set 3, all segregating for the gene Bi, were also tested in *practical tests* in a glasshouse (DE PONTI, 1978). Six non-bitter $B_{11\otimes}$ and $F_3$ lines of set 1 and nine of set 3, and the parental lines were also included. Of the lines segregating for bitterness, seven replicates of two plants per line were arranged in a randomized split plot design, each plot containing one bitter and one non-bitter plant identified as such in the cotyledon stage. The non-bitter and parental lines were incorporated in this design as single plant plots. At the fourth leaf stage each plant was inoculated with ten adult female mites. Four weeks after inoculation, the damage caused was scored at weekly intervals on a scale of one to five (1 = slight damage; 5 = heavy damage). At each observation date four leaves per plant were judged; the mean value represented the damage index. Bitterness was also de-

termined quantitatively. Of each bitter plant, two top leaves at a height of about 2.50 m were collected and further treated as described above.

RESULTS AND DISCUSSION

*The inheritance of resistance and bitterness.* The results of the laboratory tests of the $P_1$, $P_2$, $F_1$, $F_2$, $B_{11}$ and $B_{12}$ generations of three sets of crosses and of the quantitative cucurbitacin analyses of the same generations of set 1 are summarized in Fig. 1. To normalize distributions, acceptance and cucurbitacin content are presented after an arscin and a log $(x + 1)$ transformation respectively. The mean numbers of plants tested per replicate are given in Table 1. Reciprocal crosses have not been made, because earlier experiments did not reveal differences between reciprocals.

In all tests the frequency distributions of acceptance and oviposition show a continuous variation in the non-segregating as well as in the segregating generations, so that individual genes cannot be identified and quantitative analyses are necessary to elucidate the inheritance of these resistance factors. The discontinuity in the frequency distributions of cucurbitacin content is due to the segregation of the major gene Bi, whose recessive allele causes non-bitterness (ANDEWEG & DE BRUYN, 1959). As observed in the seedling stage the segregations in $B_{11}$ and $F_2$ corroborated the expected 1:1 and 3:1 ratios. The frequency distributions of the bitter plants of $B_{11}$, $B_{12}$ and $F_2$ showed in each replicate about the same shape as for the replicates combined (Fig. 1).

On the base of the means and variances of the $P_1$, $P_2$, $F_1$, $F_2$, $B_{11}$ and $B_{12}$ generations a number of tests and calculations elaborated by VAN DER VEEN (1959) and MATHER & JINKS (1971) were executed to analyse the genetic components of means. Because the results of these tests were consistent among replicates, they were pooled per set (Table 1).

The tests of non-allelic interaction demonstrate that deviations from the zero expectations occur only for oviposition in set 2 (test B and C) and for cucurbitacin in set 1 (test C). Therefore the only two genetical parameters that have been examined are additivity [*d*] and dominance [*h*]. Their estimates were calculated and the validity of the additivity-dominance model was checked in joint scaling tests.

In all three sets the additivity-dominance model appeared to be valid for the resistance factor *acceptance*, additivity being the most important component.

For the resistance factor *oviposition* the additivity-dominance model appeared to be valid in sets 1 and 3, where again additivity is of prime importance. In set 2, however, the additivity-dominance model for oviposition is not valid. This may be due to the unexpected small difference between $P_1$ and $P_2$, resulting in a non-significant and very small additivity component. In view of earlier experiments (DE PONTI, 1979) with the line $F_5$ (HLGP × Varamin) and of its performance in testing $B_{11\otimes}$ and $F_3$ lines (Fig. 2) and also in later experiments, the surprisingly high oviposition of this line in the present test is considered as an irregularity. Therefore no conclusion can be drawn from this set of data.

According to the P-value in the joint scaling test the additivity-dominance model is just acceptable for the inheritance of *bitterness* in set 1, additivity being the main component. On the other hand, this low P-value points to the importance of other
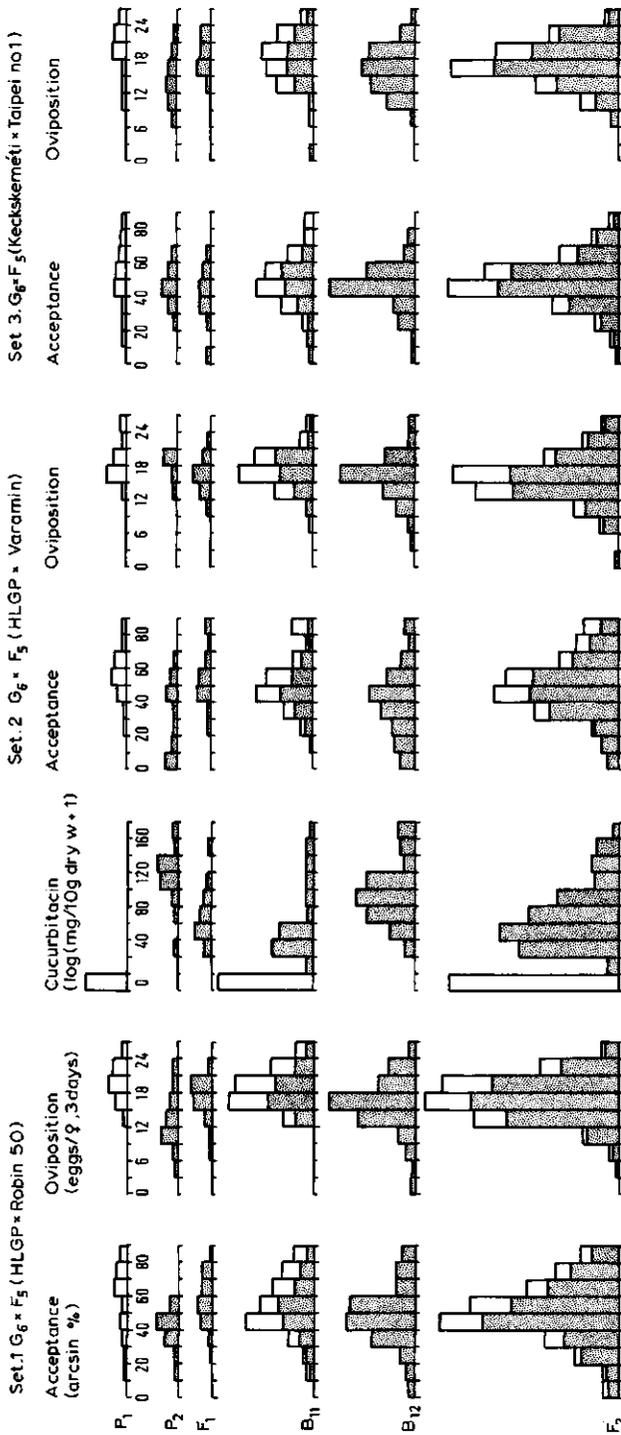
Fig. 1. Distribution of plants of various generations from three crosses between a susceptible non-bitter line and three resistant bitter lines over classes of acceptance, oviposition and cucurbitacin content (only set 1). The classes are indicated by their upper limits. Solid columns represent the bitter plants, open columns the non-bitter plants.

Table 1. Analyses of genetic components of means for the resistance parameters acceptance and oviposition and for the cucurbitacin content. Heritabilities as calculated per replicate are also included.

| | Set 1: $G_6 \times F_5$ (HLGP × Robin 50) | | | Set 2: $G_6 \times F_5$ (HLGP × Varamin) | | Set 3: $G_6 \times F_5$ (Keckskeméti × Taipei) | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | acceptance (arcsin %) | oviposition (eggs/♀, 3 days) | cucurbitacin $(\log \frac{mg}{10\,g\,dry\,w} +1)$ | acceptance | oviposition | acceptance | oviposition |
| | $\bar{n}$  m  $\bar{s}_m$ | $\bar{n}$  m  $\bar{s}_m$ | $\bar{n}$  m  $\bar{s}_m$ | $\bar{n}$  m  $\bar{s}_m$ | $\bar{n}$  m  $\bar{s}_m$ | $\bar{n}$  m  $\bar{s}_m$ | $\bar{n}$  m  $\bar{s}_m$ |
| **1. Generation means and their standard errors (pooled figures)** | | | | | | | |
| $P_1$ | 8  62.1±5.8 | 7  20.0±1.1 | 8  0±0 | 8  58.7±4.7 | 8  19.4±1.2 | 8  55.1±5.8 | 8  20.7±1.2 |
| $P_2$ | 8  45.9±5.8 | 8  13.8±1.5 | 7  1.29±0.11 | 8  31.6±5.9 | 5  18.3±0.9 | 8  47.0±3.8 | 8  14.1±1.4 |
| $F_1$ | 8  57.6±4.4 | 8  17.2±1.0 | 7  0.80±0.11 | 8  57.3±5.7 | 8  17.7±1.1 | 7  47.0±3.6 | 7  18.6±1.1 |
| $B_{11}$ | 38  56.0±2.6 | 37  18.8±0.5 | 35  0.36±0.08 | 38  53.6±2.7 | 38  17.5±0.5 | 38  50.7±2.4 | 38  18.1±0.6 |
| $B_{12}$ | 37  50.4±2.4 | 37  16.0±0.7 | 35  1.04±0.05 | 38  41.7±3.1 | 35  16.0±0.7 | 38  46.6±1.9 | 38  16.2±0.6 |
| $F_2$ | 97  53.4±1.6 | 97  17.2±0.4 | 92  0.55±0.05 | 100  51.7±1.8 | 98  16.0±0.4 | 98  51.4±1.3 | 98  17.1±0.3 |
| **2. Tests of non-allelic interaction** | | | | | | | |
| A | -7.7± 9.0 | 0.4±1.8 | -0.08±0.20 | -8.8± 9.1 | -2.1±1.9 | -0.7± 8.4 | -3.1±2.1 |
| B | -2.7± 8.8 | 1.0±2.3 | -0.00±0.19 | -5.5±10.3 | -4.0±1.9 | -0.8± 6.5 | -0.3±2.2 |
| C | -9.6±13.6 | 0.6±3.1 | -0.67±0.31 | 1.9±15.5 | -9.1±3.1 | 9.5±11.3 | -3.6±3.2 |
| D | 11.2±19.8 | -2.2±4.7 | -0.51±0.49 | 30.5±22.5 | 3.1±4.6 | 12.5±16.7 | 3.2±4.8 |
| **3. Estimates of the genetical parameters** | | | | | | | |
| m | 52.0±3.2 | 17.1±0.7 | 0.63±0.05 | 44.6±3.2 | 18.0±0.6 | 52.1±2.5 | 16.9±0.7 |
| [d] | 6.7±2.7 | 3.0±0.6 | -0.65±0.05 | 12.6±2.8 | 0.8±0.6 | 4.6±2.3 | 2.6±0.6 |
| [h] | 3.3±6.0 | 0.3±1.3 | 0.04±0.11 | 10.9±6.3 | -2.6±1.2 | -4.1±4.9 | 0.8±1.4 |
| **4. Joint scaling test** | | | | | | | |
| $\chi^2_{(3)}$ | 0.802 | 0.231 | 7.738 | 2.513 | 10.449 | 2.070 | 2.710 |
| P | 0.90>P>0.80 | 0.98>P>0.95 | 0.10>P>0.05 | 0.50>P>0.30 | 0.01>P>0.001 | 0.70>P>0.50 | 0.50>P>0.30 |
| **5. Heritabilities of $F_2$ individuals per replicate** | | | | | | | |
| | 0.00; 0.64; 0.16; 0.42 | 0.30; 0.00; 0.00; 0.63 | | 0.43; 0.16; 0.18 | 0.47; 0.00; 0.64 | 0.33; 0.06; 0.00 | 0.03; 0.24; 0.00 |

components such as non-allelic interactions, as was already concluded from test C. Based on these findings and on the comparison of the shapes of the frequency distributions of cucurbitacin in the segregating generations with those of $P_1$, $P_2$ and $F_1$ (Fig. 1), the following hypothesis on the inheritance of bitterness could be made.

Bitterness is basically governed by the major gene Bi, which is inherited in an intermediary way (additivity component). Its action is influenced by one or more additively inherited modifying intensifier genes (additivity component), which are only active in the presence of the allele Bi (non-allelic interaction component).

The large variation in bitterness between cucumber varieties (REHM, 1960; COERTZE, 1977; DE PONTI, 1977b and 1979) corroborates the hypothesis of intensifier genes. A critical evaluation of the results of earlier crossing experiments provides another check on the validity of the hypothesis. Crosses between HLGP and Robin 50, varieties with the same degree of bitterness, yielded some $F_5$ lines with significantly more cucurbitacin than either parent (DE PONTI, 1979). According to the hypothesis both varieties will possess different intensifier genes, which were accumulated in the $F_5$ lines.

*Relations between acceptance and oviposition.* To investigate interdependencies between the resistance factors acceptance and oviposition, the correlation between these factors was calculated on the basis of individual plants of the $F_2$. These correlation coefficients varied markedly per replicate: in set 1 from –0.23 through –0.06 and 0.09 to 0.27, in set 2 from 0.09 through 0.17 to 0.24 and in set 3 from 0.04 through 0.27 to 0.30. For the 100 plants tested per replicate correlation coefficients above 0.19 are significant ($P \leqslant 0.05$). The relations between acceptance and oviposition of the $B_{11\otimes}$ and $F_3$ lines of the three sets are represented graphically in Fig. 2. The correlation coefficients between line means ($r_b$) and the genetic correlation ($r_g$) estimated from analyses of covariance are also included in Fig. 2.
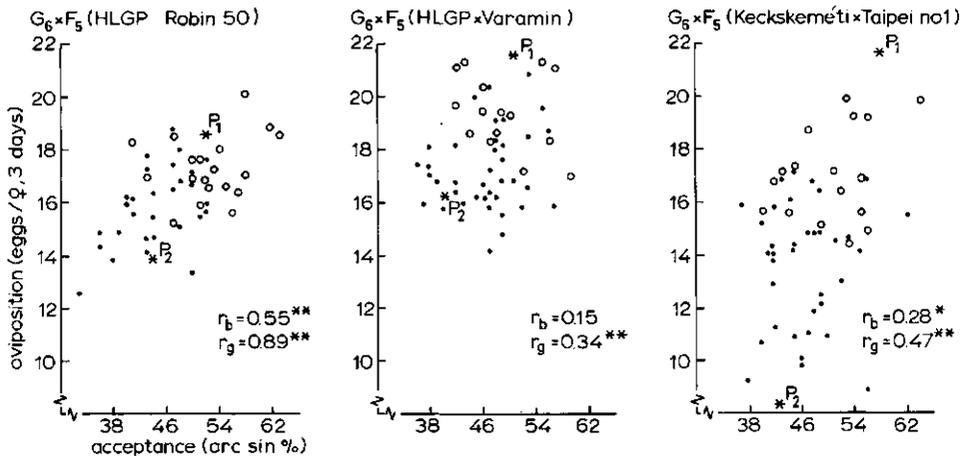


Fig. 2. The relation between the average acceptance and oviposition of $B_{11\otimes}$ ( o ) and $F_3$ lines ( ● ). The correlations between lines ($r_b$) and the genetic correlations ($r_g$) are mentioned; significant values for $P \leqslant 0.05$ and $P \leqslant 0.01$ are starred.

518

In spite of large variations, the correlation coefficients, especially the genetic correlation coefficients, indicate a possitive correlation between the resistance factors acceptance and oviposition. A similar, but phenotypic correlation was found when varieties were examined (DE PONTI, 1978). This correlation might indicate partial identity or linkage of the many genes governing acceptance and oviposition. The present data, however, do not allow further genetical interpretations.

*Relations between bitterness and resistance.* The relation between bitterness and resistance to the twospotted spider mite has yielded quite a few contradictory reports, which have recently been reviewed (DE PONTI, 1980). The present data are wel suited for further analyses of this intriguing question.

The frequency distributions of the $B_{11}$ and $F_2$ generations over acceptance and oviposition classes were divided for bitter and non-bitter plants (Fig. 1). The differences between bitter and non-bitter plants for acceptance and oviposition were t-tested per replicate. The combined probabilities of these t-tests were computed per set of n (3 or 4) replicates according to the $\chi^2_{2n}$-test described by FISCHER (1946). The hypothesis that acceptance and oviposition are lower on bitter than on non-bitter plants was rejected ($P \leqslant 0.05$) in eleven of the twelve one-sided $\chi^2_{2n}$-tests. This hypothesis was also tested on $B_{11\otimes}$ and $F_3$ lines segregating for the gene Bi. The individual plants of these lines are sharing a common gene pool, apart from the gene Bi. The number of lines in which the average acceptance or oviposition was higher, equal or lower on the bitter than on the non-bitter plants is presented in Table 2. Using the one-sided sign test ($P \leqslant 0.05$) the

Table 2. Classification of $B_{11\otimes}$ and $F_3$ lines segregating for the gene Bi according to the average acceptance and oviposition on their bitter (b) and non-bitter (nb) plants.

| | Number of lines total | acceptance on b vs nb plants | | | oviposition on b vs nb plants | | |
|---|---|---|---|---|---|---|---|
| | | higher | equal | lower | higher | equal | lower |
| set 1 | 20 | 7 | 0 | 13 | 12 | 1 | 7 |
| set 2 | 24 | 11 | 0 | 13 | 14 | 0 | 10 |
| set 3 | 27 | 17 | 0 | 10 | 11 | 0 | 16 |
| Σ | 71 | 35 | 0 | 36 | 37 | 1 | 33 |

above hypothesis was again rejected, both for the individual sets and for the summarized data. Both tests allow the conclusion that the gene Bi has no effect on the resistance factors acceptance and oviposition.

In view of the above findings the results of the practical tests are remarkable. Fig. 3 shows that on an average the non-bitter plants of the $B_{11\otimes}$ and $F_3$ lines are significantly ($P \leqslant 0.01$) more damaged than the bitter plants. Moreover the homogeneous non-bitter lines are, relatively, also more damaged. Of some lines, however, the non-bitter plants were equally or even less damaged than the bitter plants, indicating a non-absolute relation.

The above statistical analyses indicate that the genes governing the resistance factors acceptance and oviposition are inherited independently from the gene Bi. The practical
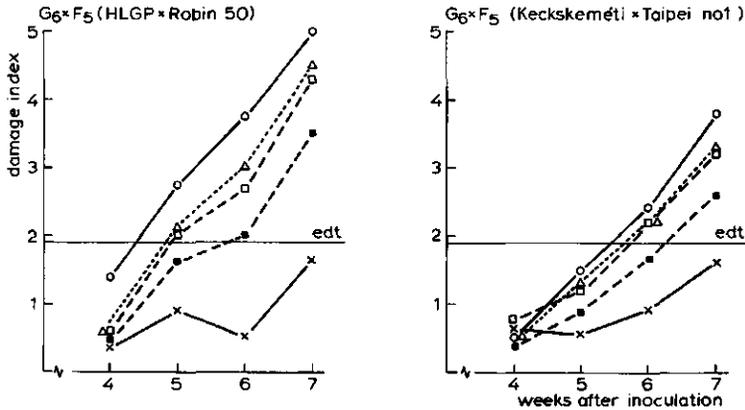
Fig. 3. Increase of damage index of bitter (■) and non-bitter (□) plants of $B_{11\otimes}$ and $F_3$ lines segregating for the gene Bi, compared with their resistant (x) and susceptible (o) parents. Homogeneous non-bitter lines (△) are also represented. The economic damage threshold (edt) lies at 1.9.

tests, however, clearly demonstrate a significant relation between the gene Bi and the damage index. As discussed earlier (DE PONTI, 1978), the damage index reflects the resultant of all aspects of resistance and tolerance. Because no relation was found between the gene Bi and the resistance factors acceptance and oviposition, the evident relation between the gene Bi and the damage index may be reduced to a relation between the gene Bi and additional resistance and/or tolerance factors, which are only expressed in practical tests. Such a relation could be caused by identity or linkage of the genes concerned. In an earlier practical test with near isogenic bitter and non-bitter lines, pleiotropic effects of the gene Bi on bitterness as well as on resistance or tolerance to the twospotted spider mite were ruled out (DE PONTI, 1980). Therefore linkage of the gene Bi with some of these additional resistance genes or with some tolerance genes remains the only plausible explanation for the relation found. The occurrence of such unfavourable linkages, only traceable in practical tests, emphasizes the importance of this way of testing for resistance and tolerance to the twospotted spider mite.

The relation between the *degree* of bitterness and resistance was also investigated. The data of the $P_2$, $F_1$, nor those of the $F_2$, $B_{11}$ and $B_{12}$ generations of set 1 did show any correlation between cucurbitacin content and acceptance or oviposition.

In the practical test of set 3 the correlation between 5 $B_{11\otimes}$ lines of damage index and cucurbitacin content was not significant (–0.33), probably because of a low and non-significant variation in damage index between the 5 lines. Between 11 $F_3$ lines of set 1 and 9 of set 3 a non-significant (–0.48) and a very significant (–0.94) negative correlation was found. This might be explained by linkage between bitterness intensifier genes and resistance or tolerance genes. Such a linkage would also explain an earlier experience (DE PONTI, 1979), that selecting for higher resistance could, unintentionally, lead to a significant increase in cucurbitacin content. The absence of any correlation in the laboratory tests, when correlations were calculated on individual plants, might be ascribed to a large non-genetic variation, as is evident from the homogeneous $P_2$ and $F_1$. This variation is mainly due to large environmental effects on the formation of this

520

compound (REHM, 1960) rather than to inaccuracies of the chemical analyses.

*The selectability of resistance to the twospotted spider mite: preliminary evaluation of selection criteria.* Because of the large variation in the heritabilities of the $F_2$ individuals (Table 1) for acceptance and oviposition , the advantage of early selection in the $F_2$ is questionable. Perhaps it would be better to carry out selection only in $F_3$ lines, as suggested earlier (DE PONTI, 1979). On the other hand Fig. 2 indicates some effect of selection in the $F_2$, because on most $F_3$ lines the acceptance and oviposition are lower than on the midparent.

Because of the desirability to simplify the laboratory test we considered to omit either acceptance or oviposition in routine breeding. This seems justified in view of the positive correlation between these resistance factors. A determination of oviposition is preferred to one of acceptance, because the former is based on five independent observations per plant, whereas the latter is only a single observation. Moreover the variances of acceptance are always much larger than those of oviposition, as was also evident in screening the $F_3$ lines, whose data are presented in Fig. 2. Analyses of variance did not yield significant differences in acceptance between these lines, whereas the differences in oviposition were always very significant ($P \leqslant 0.01$). This justifies the preference of oviposition to acceptance as a prime selection criterion. The synchronisation of the spider mites and their subsequent adaptation to the testplant remain maintained.

The discrimination of the practical test appeared also high. The differences in damage index between the $B_{11\otimes}$ lines were insignificant for both sets, but between the $F_3$ lines they were very significant ($P \leqslant 0.01$), as were the differences between bitter and non-bitter plants of the $B_{11\otimes}$ and $F_3$ lines (Fig. 3). Moreover, because of the unfavourable linkages, which are only detectable in the practical test, this test is an indispensable tool in screening for resistance to the twospotted spider mite. To investigate the effectiveness of preselection based on oviposition, the relation between oviposition and damage index of the $B_{11\otimes}$ and $F_3$ lines segregating for bitterness is presented in Fig. 4.
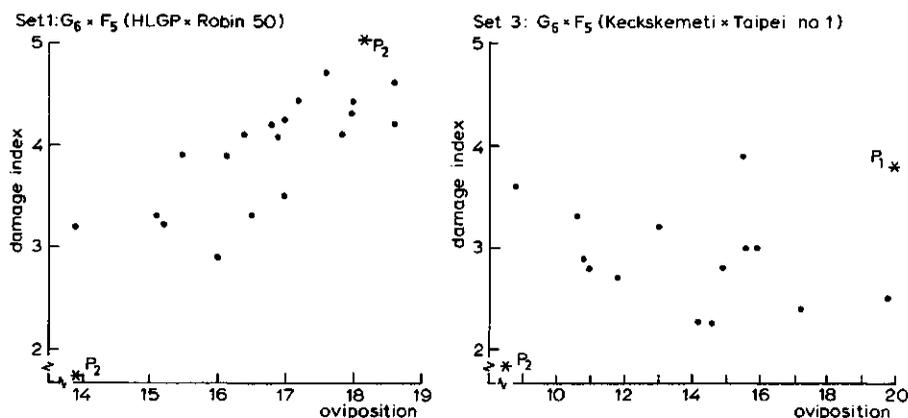


Fig. 4. The relation between oviposition and damage index (in week 7) for $B_{11\otimes}$ and $F_3$ lines of two sets of crosses.

The lines of set 1 show a positive correlation, whereas those of set 3 do not show any correlation at all. This relation should therefore be further studied with a larger number of lines, preferably from an unselected $F_2$ originating from non-bitter parents. Only then will a definite conclusion on the advantage of preselection based on oviposition be justified.

CLOSING REMARKS AND BREEDING CONSEQUENCES

In our experiments with three sets of crosses resistance to the twospotted spider mite, as measured by acceptance and oviposition, appears to be governed by several to many genes, which are mainly inherited in an additive fashion. The positive correlation between acceptance and oviposition indicates that some of the genes governing these factors are identical or linked. As in earlier experiments (DE PONTI, 1978), the results of the laboratory tests and the practical test agree only partially. Probably additional resistance genes, which are only expressed in practical tests, are also involved, but differences in tolerance might also be responsible for this partial lack of agreement. Apparently the genes governing the various resistance factors can hardly be studied separately and therefore should be considered as one complex.

The original hypothesis of ANDEWEG & DE BRUYN (1959), that the gene Bi is inherited in a dominant fashion, was based on a qualitative analysis unsuitable to distinguish degrees of bitterness. Thanks to our quantitative analysis of cucurbitacin this major gene for bitterness appeared to be inherited in an intermediary way, while its action is influenced by a number of also additively acting intensifier genes, which are only active in the presence of the allele Bi.

No relationship was found between the gene Bi and the resistance factors acceptance and oviposition, whereas in the practical test the degree of damage was correlated with the presence of the gene Bi. Because of some exceptions to this relationship and because of earlier experiences with near isogenic lines (DE PONTI, 1980), this relationship is ascribed to linkage between Bi and resistance or tolerance genes rather than to pleiotropy of Bi. The significantly high correlation between the degree of bitterness and damage index suggests linkage between bitterness intensifier and resistance or tolerance genes. Such a linkage does not present difficulties because the intensifier genes are inactive in non-bitter (bibi) genotypes which will preferably be bred.

Our investigations support earlier indications (DE PONTI, 1978, 1979) and evidences (DE PONTI, 1980) that in cucumber no causal relation exists between bitterness and resistance to the twospotted spider mite as claimed by DACOSTA & JONES (1971), KOOISTRA (1971) and GOULD (1978). Therefore cucurbitacin should be omitted from lists of repellents and deterrents as far as the twospotter spider mite is concerned.

In cucumber repeated backcrossing is the common practice to introduce new characters. In view of the relatively low and variable heritabilities of $F_2$ individuals for resistance, selection in segregating generations should be followed by at least one generation of line selection before making backcrosses. The number of backcrosses can be limited by intercrossing partially resistant lines, which can be readily distinguished. Further investigations are required to verify the advantage of the selection of individual plants in segregating populations and of the laboratory test for preselection. The necessity of practical tests at the end of each selection cycle is unquestionable,

522

especially from the viewpoint of tracing undesired linkages between the gene Bi, its intensifiers and resistance genes.

Unless genotypes are found on which the acceptance is more pronounced than the oviposition, the selection in the laboratory test could be limited to a determination of oviposition. This procedure is already being applied in breeding non-bitter lines, with encouraging results.

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## CURRICULUM VITAE

Orlando Marie Bernardo de Ponti, geboren op 12 mei 1945 te Almelo, behaalde in 1963 het diploma Gymnasium-β aan het Katholiek Gelders Lyceum te Arnhem. Daarop aansluitend studeerde hij aan de Landbouwhogeschool te Wageningen. Zijn studie in de plantenveredeling werd op 9 juni afgesloten met het behalen van het doktoraal diploma in deze studierichting met als keuzevakken erfelijkheidsleer en algemene planteziektenkunde.

Sindsdien is hij als onderzoeker werkzaam bij het Instituut voor de Veredeling van Tuinbouwgewassen (IVT) in Wageningen. Als hoofd van de afdeling veredeling kom-kommer en augurk heeft hij eerst gedurende enkele jaren onderzoek verricht aan o.a. parthenocarpie, vrouwelijkheid en resistentie tegen schimmels, bakteriën en virussen bij deze gewassen. Van meet af aan is ook aan het promotieonderzoek gewerkt, als onderdeel van het onderzoekprogramma van het IVT. Als hoofd van de afdeling veredeling op resistentie tegen insekten en mijten wijdt hij zich sedert 1977 volledig aan resistentie tegen dierlijke parasieten in diverse groentegewassen. Tevens vervult hij op dit terrein zowel nationaal als internationaal coördinerende en adviserende funkties.