PREFATORY NOTE

When, in 1949, the "Comité Européen de Zoologie Agricole" organised its first meeting in Amsterdam and Wageningen, on resistance in Solanum species with regard to the Colorado Beetle, it soon became clear that here a field was touched of wide interest. It also was apparent that, in view of the complexity of host plant relations in insects, a considerable amount of fundamental work would have to be done before the plantbreeder could hope to start a breeding program for resistance against insect attack, based on a precise knowledge of the factors involved. A second meeting was held in Brussel in 1950.

The succes of the symposium on "The physiological relations between Insects and their Host Plants", during the IXth Entomological Congress in Amsterdam, gave further testimony of the growing interest in this field and the important achievements made by physiologists and ecologists alike.

Six years later we can again report a remarkable progress. PAINTER's "Insect Resistance in Crop Plants" now provides the plant breeder with a review of high completeness. BECK, FRAENKEL, DE GROOT, HOUSE, SEDEE, and FRIEND have greatly enlarged our knowledge of nutritional requirements of insects in general. DETHIER and his co-workers have added basic information to our knowledge of the releasing mechanisms of feeding reactions. The Heidelberg group has opened new fields with regard to the nature of chemical resistance factors in Solanum species, and painstaking efforts for breeding Colorado Beetle-resistant potato hybrids are now beginning to yield practical results.

When organizing this symposium, we have extended invitations to workers of very different specialization. We did this with the hope to obtain during the discussions a synthetic view of the whole complex of insect-hostplant interactions. The reader may judge for his own in how far our aim has been reached.

In addition, workers on hostplant relations in nematodes were invited, a field much less developed than that in insects, but with many quite similar aspects.

The Organizing Committee is thankful to all readers for sending their typescripts. The Committee furthermore expresses its sincere gratitude to the Landbouwhogeschoolfonds, Wageningen, for sponsoring the Symposium, and to the Editors of "Entomologia Experimentalis et Applicata" for publishing most of the papers, thus reducing our costs considerably.

Wageningen, June 1958
J. DE WILDE

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ÜBER DEN FRASSTEST AN LEPTINOTARSA-RESISTENTEN KARTOFFELN

VON

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Die Testung von Kartoffelhybriden auf Resistenz gegen den Kartoffelkäfer bereitet Schwierigkeiten, da die Ergebnisse schwanken und die Ursache nicht geklärt ist. Trotz ungünstigem Erbgang wurden auf der Basis von *Solanum chacoense* beachtliche züchterische Erfolge erzielt.

Als ich vor 16 Jahren mit der Resistenzzüchtung gegen den Kartoffelkäfer (*Leptinotarsa decemlineata* Say.) begann, hatte ich glücklicherweise keine ausreichende Vorstellung von den Schwierigkeiten, die mich einmal von der Seite der Pflanze, aber nicht minder vom Insekt erwarteten. Sonst hätte ich vielleicht nicht den Mut gehabt, an die mir gestellte Aufgabe heranzugehen. Damals stand die mexikanische Wildkartoffel *Solanum demissum* im Mittelpunkt des züchterischen Interesses. MÜLLER und SELLKE (1941) hatten die Resistenz von Tuberosum-Hybriden dieser Wildart untersucht und in späteren Generationen nur anfällige Bastarde gefunden. Mit diesen Ergebnissen wurde die Resistenzzüchtung auf der Grundlage von *Solanum demissum* problematisch. Sie ist es geblieben bis zum heutigen Tag.

Das war der Grund, daß unser Institut sich nicht lange bei den Demissa aufhielt, sondern der Käferresistenz der Commersoniana zuwandte. Das erste Glied, das uns durch hervorragende Resistenz auffiel, war *Solanum chacoense* (TORKA 1943). Aber auch bei anderen Species dieser Serie ist Käferresistenz weit verbreitet; jedoch treten neben resistenten Pflanzen mehr oder weniger anfällige auf (TORKA 1954). Ihre geographische Verbreitung erstreckt sich auf die atlantischen südamerikanischen Staaten, vor allem Argentinien, Uruguay und Paraguay. Die Resistenz des *Solanum chacoense* läßt sich folgendermaßen charakterisieren: Im Gegensatz zu *Solanum demissum,* dessen Wirksamkeit sich vorwiegend auf die Larven erstreckt (SCHAPER 1939), werden hochresistente Chacoensepflanzen weder von der Larve noch vom Käfer gefressen (TORKA 1949). Bei ausschließlicher Fütterung mit Chacoenselaub müssen beide praktisch verhungern. Die Resistenz wird nach neuesten Untersuchungen von KUHN (1957) durch bisher unbekannte, wasserlösliche Alkaloide hervorgerufen. Diese wirken wahrscheinlich als „repellents" und nicht toxisch.

Der gegenwärtige Stand unserer züchterischen Arbeit ist folgender: Durch Inzucht und strenge Selektion der widerstandsfähigsten Pflanzen wurde *Solanum chacoense* homozygot resistent. Colchicin-Behandlung verdoppelte deren 24 Chromosomen, wonach Kreuzungen mit 48-chromosomigem *Solanum tuberosum* ohne

Schwierigkeit gelangen. Diese Hybriden unterscheiden sich in ihrer Käferresistenz von entsprechenden Kreuzungen mit *Solanum demissum* durch einen anderen Erbgang. Diese Unterschiede sind in Tabelle I gegenübergestellt.

TABELLE I

Käferresistenz der Hybriden von Solanum demissum und Solanum chacoense

1. Solanum demissum-Hybriden			*2. Solanum chacoense-Hybriden*		
Kreuzungs-generation	Eltern	larvenresist. Pflanzen in %	Kreuzungs-generation	Eltern	larven u. käfer-resist. Pfl. in %
F_1	d × t	~ 20 %	F_1	ch × t	0 %
F_2	(d × t) × s	fast 0 %	F_2	(ch × t) × S	1,5—5 %
F'_2	(d × t) × t	2—4 %	F'_2	(ch × t) × t	0 %
	F'_2 × S	fast 0 %		F'_2 × S	~ 2 %
				F'_2 × res. F_2	2—15 %
				res.F_x × res.F_x	> 20 %

Erklärung der Abkürzungen:
d = Solanum demissum; t = Solanum tuberosum; ch = Solanum chacoense; S = Selbstung; F_1 = 1. Kreuzungsgeneration; F_2 = 2. Kreuzungsgeneration; F_2 = Tuberosum-Rückkreuzung.

Bei den Demissum-Hybriden gewinnt man den Eindruck, daß die Wildspecies-resistenz schon in den ersten Generationen immer mehr verdünnt wird und schnell erlischt. Bei Chacoense-Hybriden liegen die Verhältnisse anders. In der F_2 tritt die Resistenz wieder in Erscheinung, nachdem sie in der F_1 fehlte. Durch sorgsam gelenkte Kreuzungen gelingt es langsam, die Leistungsfähigkeit der Zuchtstämme zu verbessern, ohne daß die Resistenz verloren geht (TORKA 1950). Bei dominantem Erbgang wäre die Arbeit wesentlich einfacher. Schon in der F'_4 wären sortenähnliche resistente Kartoffeln zu erwarten. So aber mußten in den zurückliegenden Jahren viele Tausende von Pflanzen einzeln auf Käferfestigkeit geprüft werden, ehe einige resistente Klone übrig blieben. In der F_2 waren beispielsweise von 100 Sämlingen 1—5 Pflanzen resistent. Außerdem bringt nicht jeder Wildkartoffel-Bastard Knollen hervor; manche fallen vor der Ernte Krankheiten zum Opfer. Die zahlenmäßig kleine resistente F_2 mit vielen Wildmerkmalen muß zur Leistungssteigerung mit *Solanum tuberosum* rückgekreuzt werden (TOXOPEUS (1952) nennt diesen Vorgang „tuberisation"). Die so erzielte Leistungssteigerung macht die Pflanzen käferanfällig, und die Resistenz muß durch Selbstung, Geschwisterkreuzung oder Kombination mit einem resistenten Bastard wiedergewonnen werden. Das gibt Riesenzahlen von Sämlingen, die alle einzeln auf Resistenz geprüft werden müssen. Die anfangs geringe Zahl der übrigbleibenden Pflanzen steigt stetig an, und die Basis der Resistenzzüchtung wird immer breiter. Die Chance für die resistente Kartoffelsorte wird durch Leistungsverbesserung immer größer. Soweit sind gegenwärtig die Arbeiten der Käferstation des Max-Planck-Instituts für Züchtungsforschung gediehen; aber bis dahin mußte ein weiter Weg zurückgelegt werden.

Hatte die Übertragung der Resistenzgene in die Kulturkartoffel grosse Schwierigkeiten bereitet, so wurde unsere Arbeit außerdem durch das Verhalten des Kartoffelkäfers erschwert. Wer noch nie derartige Resistenzprüfungen durchgeführt hat, kann sich wahrscheinlich nicht vorstellen, wieviel Zeit und Erfahrung nötig sind, um eindeutig von einer Pflanze sagen zu können: „diese ist resistent".

Wird eine Chacoense-Hybride reichlich gefressen, so ist sie anfällig, und der Fall ist eindeutig. Wird sie n i c h t gefressen, so kann sie resistent sein, aber in vielen Fällen ist sie erfahrungsgemäß anfällig, was sich häufig erst im nächsten Versuchsjahr erweist. Bei manchen Zuchtklonen wird der Irrtum sogar erst im dritten Jahr erkannt. Das liegt an der Unzuverlässigkeit aller Fraßteste. Aber warum eine anfällige Kartoffel manchmal nicht gefressen wird, ist unbekannt. Über die Durchführung der Teste folgendes: Anfangs fütterten wir L_1-Larven im Hygrostaten mit dem Laub unserer Klone bis zur dritten Häutung und bewerteten Fraß und Mortalität. Der Futterwechsel und die Registrierung der überlebenden Larven und ihrer Entwicklung ermöglichten wegen des großen Arbeitsaufwands nur eine relativ kleine Zahl von Prüfungen, die den züchterischen Anforderungen nicht genügten. Darum gingen wir später dazu über, uns auf Fraßbewertungen nach 24- oder 48-stündiger Fütterung zu beschränken. Dabei legen wir großen Wert auf gleichbleibende optimale Bedingungen für die Larven wie hohe Temperatur und Luftfeuchtigkeit und frisches, junges Futterlaub (GRISON 1951). Alle Versuche werden im Brutschrank durchgeführt. Da für jede Wiederholung nur ein Fiederblättchen benötigt wird, können Sämlinge bereits im ersten Jahr und verhältnismäßig jung getestet werden, wodurch sich die Zahl der zu pflegenden Pflanzen schnell verringert, da alle gefressenen sofort ausgeschieden werden. Wir haben die Durchführung derart mechanisiert, daß zwei Personen ohne übermäßige Anstrengungen 800 Teste am Tage durchführen können. In Vergleichsprüfungen fielen diese Fraßversuche im Thermostaten nicht schlechter aus als die Larvenaufzucht im Hygrostaten. Aber beide Ergebnisse lassen zu wünschen übrig.

Einige Beispiele sollen das Gesagte unterbauen. Es gibt in unserem Zuchtmaterial Sämlinge oder Klone, die einen ganzen Sommer lang nicht gefressen werden und auf denen sich die Larven nicht entwickeln. Dabei ist es gleichgültig, ob ich junge oder ältere Blättchen füttere, ob ich eine oder 20 Wiederholungen anstelle. Drei Wochen später wird das gleiche Ergebnis erzielt, alle Wiederholungen sind ohne Fraßspuren, die Larven meiden das Blatt und kriechen suchend umher. Das kann sich im Laufe eines Sommers wiederholen, und der Versuchsansteller gewinnt den Eindruck, daß es sich um eine hochresistente Pflanze handelt. Im kommenden Jahr fressen die Larven reichlich an dem gleichen Klon. Mit der größeren Zahl von Wiederholungen oder aufgesetzten Larven läßt sich dieser Versuchsfehler nicht vermeiden. Ganze Saatnummern, die im ersten Versuchsjahr als besonders hoffnungsreich angesehen wurden, versagten im nächsten Sommer. In einem Jahr narrten uns eine größere Zahl von F_1-Bastarden, die einfach nicht gefressen wurden. Wir glaubten, unsere bisherigen Erfahrungen über die Anfälligkeit der ersten Kreuzungsgeneration korrigieren zu müssen — da war sie im nächsten Sommer ausnahmslos anfällig.

Der geschilderte Tatbestand bringt begreiflicherweise eine ständige Unsicherheit in unsere Arbeit, sodaß wir uns bei dieser Prüfungsmethode nicht wohlfühlen können. Wir besitzen aber vorläufig keine bessere, wenn wir auch noch immer die Hoffnung haben, eines Tages den Resistenzstoff quantitativ bestimmen zu können mit Hilfe einer einfach durchzuführenden chemischen Reaktion.

Eine Erklärung für dieses rätselhafte Verhalten der Larven zu finden, ist bisher nicht gelückt. Dabei erhebt sich die Frage, ob die Larven der Käferpopulationen

unserer Felder sich einheitlich auf der Wildkartoffel verhalten oder Biotypen mit größerer Anpassungsfähigkeit vorhanden sind. Beobachtungen über Unterschiede liegen tatsächlich bei uns vor. Es gibt einzelne Larven, die in bescheidenem Umfang Chacoenselaub zu fressen vermögen, und gelegentlich entwickelt sich daraus ein Käfer. Aber diese Beobachtungen erklären nicht das geschilderte wechselvolle Verhalten. Dann dürften nur einzelne Wiederholungen nicht gefressen werden — es sind aber ganze Serien von Larven, die aus verschiedenen Gelegen stammen und sich doch einheitlich benehmen, d. h. zeitweise fressen sie nicht, zum anderen Zeitpunkt schmeckt ihnen das gleiche Futter offensichtlich. Aus diesem Grunde suchen wir die Erklärung in Veränderungen der Pflanze, beispielsweise modifikativen Unterschieden ihrer Zusammensetzung. Es besteht die Möglichkeit, daß ein Vergällungsstoff nur in der Epidermis lokalisiert ist; denn sobald diese nach langer Hungerzeit von der ersten Larve angenagt ist, beginnen die übrigen Larven an der gleichen Stelle zu fressen.

Wir sind auf dem Rosenhof in der günstigen Lage, dank der starken Verseuchung unseres Gebietes mit *Leptinotarsa*, Freilandprüfungen durchführen zu können. Unter optimalen Bedingungen für das Insekt sind Fraßversuche sehr eindrucksvoll und zur Bestätigung der im Labor gewonnenen Ergebnisse unerläßlich. Aber die optimalen Bedingungen sind in Mitteleuropa erfahrungsgemäß nur in wenigen Sommern vorhanden. Im Frühjahr wird unser Versuchsfeld mit anfälligen und resistenten Hybriden bepflanzt. Schon Ende Mai legen die zufliegenden Käferweibchen an alle jungen Stauden reichlich viele Eigelege. Ist das Frühjahr warm und sonnig, so können die Pflanzen von den sich darauf entwickelnden Larven binnen 20 Tagen kahlgefressen sein, dann sind sie anfällig. Auf resistenten Hybriden gehen die Larven bald zugrunde, und der Fraßschaden ist gering oder fehlt ganz. Mehrfache Wiederholung des Versuchs steigert die Zuverlässigkeit des Ergebnisses — ist doch manchmal der Befall nicht auf allen Teilen des Feldes gleich stark.

Bekanntlich waren die letzten Sommer ungewöhnlich kühl und verregnet, was für unseren Feldversuch üble Folgen hatte. Vielfach gingen die Eigelege zugrunde, bevor die Larven schlüpften, oder diese erstarrten und wurden vom Regen abgespült. So entsprach der Fraßschaden nicht dem gewohnten Bild, und in vielen Fällen war eine Beurteilung der Resistenz nicht möglich. In manchen Sommern beobachteten wir im Juni einen plötzlichen Rückgang der Larven, diese hörten auf zu fressen und sahen krank aus und starben bald. So wird durch Witterungseinflüsse und Krankheiten die Zuverlässigkeit dieser Prüfung stark beeinträchtigt. Zuchtklone, die im einen Jahr keinen nennenswerten Fraßschaden aufwiesen, enttäuschten im folgenden Sommer durch Kahlfraß.

Wenn die 1. Larvengeneration keine befriedigenden Fraßunterschiede zwischen resistenten und anfälligen Hybriden hervorbringt, bleibt die Hoffnung auf günstigere Verhältnisse beim Auftreten der Jungkäfer und 2. Larvengeneration im August. Frühreife Stämme sind zu diesem Zeitpunkt häufig schon welk, aber an späten sind Fraßunterschiede oft besser zu sehen als im Frühsommer. Aber in manchen besonders ungünstigen Jahren sind in beiden Käfergenerationen keine Befallsunterschiede feststellbar, und der Feldversuch ist umsonst angelegt.

Zum Abschluß soll eine Frage gestreift werden, die für die Resistenzzüchtung von außerordentlicher Wichtigkeit ist und auch mit den Larventesten in Ver-

bindung steht. Chacoenselaub verursacht auf der menschlichen Zunge ein starkes Brennen und widerlichen Bittergeschmack. So war zu befürchten, daß der Käfer bzw. die Larve ähnliche Geschmacksempfindungen hat und die Resistenz mit Bitterkeit gekoppelt ist. Die Knollen unserer älteren resistenten Hybriden wiesen tatsächlich einen ebenso widerlichen Geschmack auf wie Chacoenselaub und waren ungenießbar. Darauf hat im Jahre 1953 Prof. KRAUT vom Max-Planck-Institut für Ernährungsphysiologie in Dortmund freundlicherweise mit den gleichen Knollen Fütterungsversuche an Ratten durchgeführt. Erwachsene Ratten starben ausnahmslos nach 1—3 tägiger Fütterung mit Kartoffelbrei dieser resistenten Hybriden, während die Kontrollen gesund blieben.

Inzwischen ergaben planmäßige Geschmacksprüfungen an den Knollen unseres resistenten Zuchtmaterials große Unterschiede. Die resistenten Klone umfassen sowohl neutral als auch bitter schmeckende Stämme, sodaß das Geschmacksproblem verhältnismäßig schnell durch Selektion zu lösen war. Außerdem isolierten KUHN & LÖW (1955) aus *Solanum chacoense* sechs verschiedene nicht larvenwirksame Alkaloide, die wahrscheinlich den widerlichen Geschmack der Wildkartoffel hervorrufen und außerdem giftig sind. An diesen Alkaloiden und nicht am Resistenzstoff dürften die Ratten ums Leben gekommen sein. Eine Wiederholung der Versuche mit solaninarmen, käferresistenten Zuchtstämmen steht noch aus.

RÉSUMÉ

Les *Commersoniana,* Pommes de terre sauvages, sont des géniteurs résistants au Doryphore. Les premières générations de croisement avec *Solanum tuberosum* n'ont que peu d'hybrides résistants, mais la résistance est constante.

Les tests biologiques avec le Doryphore sont satisfaisants pour la sélection des Pommes de terre résistantes. Mais il faut les répéter plusieurs années.

Il y a des différences parmi les insectes. Quelques-uns peuvent consommer un peu de feuillage de *Solanum chacoense,* Pomme de terre sauvage résistante au Doryphore. Mais les hybrides sélectionnés restent résistants en plein air.

Quelques hybrides sensibles ne sont pas toujours mangés par le Doryphore. Peut-être ces plantes contiennent-elles une substance variable répulsive qui n'est pas identique avec le principe de résistance.

Les tubercules de *Solanum chacoense* ont un goût amer résultant de six alcaloïdes toxiques. Nous avons trouvé des hybrides présentant une résistance constante et un goût agréable.

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DISKUSSION

D. HILLE RIS LAMBERS hält die Züchtung toleranter Kartoffelstämme, die nicht in jedem Jahr gleich widerstandsfähig gegen den Kartoffelkäfer sind, für einen beachtlichen Fortschritt.

J. DE WILDE berichtet über eigene Erfahrungen über den Wettereinfluß und physiologisch-chemische Veränderungen des Futterlaubes. Diese lassen sich experimentell während der kurzen Dauer der Fraßteste nicht ergründen.

K. SCHREIBER betont ebenfalls den großen Einfluß des physiologischen Zustandes der Futterpflanzen. Nach seinen toxikologischen Untersuchungen mit weißen Mäusen (perorale Applikation) liegt die Dosis letalis min. für 3 Alkaloide ungefähr in der gleichen Höhe und beträgt für Solanin, Chaconin und Demissin 0,7—0,8 mg Alkaloid/g Maus.

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L'IMMUNITÉ CHEZ LES SOLANACÉES À L'ÉGARD DU DORYPHORE

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RÉSUMÉ

Dans l'étude des forces de la nature pouvant être, au besoin, mises à profit pour réduire les dégâts du Doryphore (*Leptinotarsa decemlineata* Say), trois voies principales de recherches étaient à suivre.

Elles furent abordées en 1927, lorsqu'il apparût que l'on ne pourrait plus éteindre le foyer de l'insecte découvert en 1924 dans le sud-ouest de la France.

Ces trois voies concernaient:

— l'action sur le Doryphore de facteurs a-biotiques (climat et parfois sol);
— l'action sur le Doryphore d'ennemis naturels, entomophages et entomophytes;
— enfin, l'action sur l'insecte de certains facteurs propres d'immunité pouvant être présentés par les végétaux qu'il attaque ou pourrait attaquer.

En suivant la première orientation, un certain nombre d'utilisations en quelque sorte „passives", ont eu lieu. En particulier elles portent sur la mise à profit de l'action freinatrice du climat au printemps découlant du fait que insecte et pommes de terre n'ont pas les mêmes seuils inférieurs d'exigences thermiques, aussi peut-on l'utiliser pour certains déphasages pouvant donner des effets très marqués.

Ils permettent l'établissement de cultures de primeurs pour les pommes de terre, pratiquement sans traitements chimiques, surtout lorsqu'on prend soin de veiller dans le secteur où on les établit, à l'absence de cultures de saison de pommes de terre et de plantations d'aubergines (*Solanum melongena* L.).

Dans la voie de la lutte biologique au sens restreint habituel du terme, les premières études — études de base — se sont déroulées surtout de 1928 à 1939. Les travaux vont être repris par la Commission Internationale de lutte biologique, Commission récemment crée et qui comporte un groupe de travail sur les ennemis naturels du Doryphore présidé par le Dr. FRANZ de Darmstadt. Ce groupe a tenu sa première réunion cet hiver, à Gembloux, et a fixé deux orientations principales pour ses efforts: avoir le minimum d'action fâcheuse des traitements insecticides sur le jeu de la lutte biologique naturelle; si possible, avoir à l'avenir en Europe en appoint de nos entomophages indigènes, d'autres espèces venant, elles, du pays d'origine du Doryphore.

La troisième voie comprenait les études d'immunologie et leur mise à profit dans la recherche de plantes cultivées si possible peu sensibles aux attaques, voir même résistantes.

Le rappel ici des trois grandes orientations d'étude n'a pas qu'une seule valeur historique.

Il permet de préciser qu'il apparaît de plus en plus nettement que des rapports d'interdépendance doivent exister entre les trois catégories de travaux cités si nous cherchons à obtenir la limitation naturelle la plus marquée des dégâts du Doryphore.

La solution écologique de lutte avec le minimum d'interventions chimiques onéreuses se montre comme devant être le plus souvent le fait d'une s y n t h è s e bien équilibrée entre ces éléments divers et non résulter de l'action exclusive d'un seul d'entre eux.

En d'autres termes, le problème des plantes échappant d'elles même aux dégâts sérieux du Doryphore, sera plus aisé à résoudre pratiquement si on envisage parallèlement l'action au mieux des facteurs abiotiques et de la lutte biologique.

En conclusion: d'abord tout progrès en lutte biologique intéresse indirectement nos travaux et réciproquement; ensuite, dans les études d'immunologie, une part de liaison pour information régulière s'impose avec divers travaux zoologiques; les zoologistes peuvent seuls l'établir avec les interprétations appropriées, et ce fait apporte un élément de plus montrant que pour l'immunologie et ses applications, le travail de base revient obligatoirement à des équipes mixtes: généticiens-zoologistes.

Abordons maintenant, en le détaillant plus, à titre documentaire, un secteur restreint: les affinités existant entre le Doryphore et les Solanacées diverses, notamment les non tubérifères.

Les recherches dans cette voie ont été abordées en France peu après l'arrivée du Doryphore, mais elles furent amplifiées à partir de 1930, surtout entre 1934 et 1939 lorsque dans le cadre de notre Laboratoire de campagne d'Ahun (France), des équipes venant d'autres pays — Allemagne, Pologne — s'ajoutèrent à la nôtre.

Certaines plantes furent étudiées à nouveau après 1945, non exclusivement en France cette fois. Leur travail principal a porté alors sur des Solanacées non tuberifères; de ce fait il sera développé dans d'autres notes. Toutefois, sur les Solanacées non tuberifères pendant cette période, citons d'intéressantes observations effectuées les unes aux Pays-bas, sur les *Hyosciamus* en particulier, d'autres en Espagne, France et Italie, sur *S. melongena* et les *Lycopersicum*.

Les travaux d'immunologie conduits en France avant 1940, sont surtout l'oeuvre de FEYTAUD, puis de TROUVELOT, DUSSY, THENARD, GRISON et BUSNEL, du côté francais; de MULLER-BOHME, SELLKE et SCHAPER, du côté allemand; de Mlle BOCZKOWSKA pour la Pologne.

Lorsqu'on regarde les relevés d'attaques par le Doryphore effectués sur des plantations expérimentales de Solanacées diverses, les uns répétés pendant plusieurs années en un même lieu, les autres réalisées dans des ambiances variées la même année, deux remarques principales s'imposent rapidement.

En premier lieu, il n'existe pas d'une part des plantes nettement attaquées par le Doryphore et d'autres échappant à ces attaques, c'est à dire deux catégories bien tranchées de végétaux. En fait, on note très nettement un échelonnement continu dans les degrés d'attaques, donc d'affinités, depuis les Solanacées dont les peuple-

ments sont toujours très rapidement anéantis, conduisant en même temps à de fortes pullulations d'insectes, jusqu'à celles qui pratiquement ne sont pas attaquées et n'assurent aucune vie au Doryphore.

Ensuite, pour tous les végétaux, mais à des degrés assez variables selon les espèces ou formes, s'il y a possibilité d'attaque, celle-ci présente des oscillations dans son niveau, oscillations qui se révèlent en rapport avec l'ambiance écologique et avec l'état physiologique de la plante hôte, au moment de l'attaque: organe consommé et son âge; développement végétatif de la plante; sol et fumure; insolation reçue;...

En présence de faits aussi complexes on voit le manque de portée que peuvent avoir les observations ne portant pas sur des relevés continuels minutieux et se bornant, comme certaines personnes l'indiquent parfois, à laisser une attaque de Doryphore se produire sur des petits groupes de plantes situés les uns près des autres, puis noter en fin de travail ceux qui seuls subsistent. De tels relevés non seulement sont peu instructifs, mais encore se montrent souvent trompeurs; ils peuvent éliminer des nuances dans les modalités d'attaque, des effets marqués mais avec délais, dans les développements, comme on pourra s'en rendre compte par la suite.

Pour analyser le jeu, le mécanisme d'éléments d'immunité présentés par des plantes, l'observation conduit à examiner la serie suivante des rapports insectes-plantes hôtes.

Les rapports essentiels *imago*-plantes apparaissent de trois ordres. D'abord, les éléments „d'affinité" réglant l'arrivée, le stationnement, de l'*imago* sur des plantes hôtes. Une attraction, surtout à distance, à notre connaissance n'est pas démontrée. Par contre, des retentions existent, en rapport avec le micro-climat, causées par tel port et nature du feuillage, surtout avec des influences tactiles (notamment par l'intermédiaire des palpes) et gustatives. Sur ces dernières influences un parallèle serait à rechercher avec les observations récentes japonaises, vérifiées en France, sur le rôle limitateur très accentué du contact des palpes avec les feuilles, l'attaque de plantes, sans lui, serait beaucoup plus variée pour le Ver à soie, nombreuses plantes assurant la croissance aux chenilles privées de leurs palpes.

Pour les *imagos* du Doryphore, des plantes se sont montrées nettement retentives; telles sont (la classification suivante concerne des végétaux ayant vécu en plein soleil): *S. melongena* et *S. dulcamara:* des Solanées epineuses comme *S. rostratum* certaines tubérifères comme *S. edinense, S. andigenum, S. maglia, S. demissum*; enfin les *Petunia,* les *Salpiglossis.*

Après des échelons intermédiaires progressifs offerts par exemple par *S. heterodoxum, S. sisymbriifolium,* les *Nicotiana alba, sandere,* nombreux *Lycopersicum,* dans l'échelle décroissante des plantes retentives, nous rencontrions comme termes inférieurs, nombreuses formes du *S. nigrum* les *S. rantonetti, aculeatissimum.*

La seconde phase de comportement à examiner dans l'interaction Doryphore-plantes hôtes est la prise du premier aliment par l'imago, l'intensité dans l'attaque au départ puis la persistance de cette attaque. Le jeu de cet ensemble de facteurs de comportement, donne un classement des plantes à peu près analogue au précé-

dent mais certaines différences intéressantes apparaissent. Sur des plantes comme *S. demissum* en plein soleil, nous avons vu, bien des fois, l'*imago* rester très longtemps, bien retenu en quelque sorte par le végétal, mais après de premières morsures faites rapidement et étendues, assez vite l'attaque se ralentit puis demeure faible ou très faible.

Si l'on a vu en plein champ des pousses, souvent petites, de *S. demissum* disparaitre, dévorées, c'est que les attaques vives d'insectes nouveaux arrivants s'additionnaient, les anciens perdant peu à peu leur appetit.

Des plantes caractérisées par de mauvais stationnements étaient souvent mangées avec avidité, dès qu'on empêche l'insecte de les quitter: tels apparurent dans nos essais, la variété Wolthmann du *S. tuberosum*, divers *Lycopersicum*.

La troisième phase à considérer, est l'action, avec délais, du végétal sur la physiologie de *l'imago*: aptitude à l'hivernation et surtout, comme le détaillera GRISON, taux de fécondité. Rapidement nous avons reconnu qu'il y avait là un facteur des plus marqués, réglant les taux d'attaques possibles de plantes.

Pour cette phase, le classement présentera certaines différences encore plus accentuées avec le premier classement cité, même de véritables inversions pour des plantes comme *S. demissum*, les *Petunia*, alors que pour d'autres plantes il y a parallèlisme étroit des niveaux de classement. C'est ainsi le cas, parmi les plantes très attaquées, pour *S. melongena, S. dulcamara* (surtout sous sa forme maritime naine), *S. edineuse* qui est tuberifère; parmi les plantes peu attaquées cette catégorie comprend les *Nicotiana rustica* et *tabacum*, certains *Lycopersicum*.

Physiologiquement, le Doryphore au stade *imago* parait donc très sensible à la nourriture prise, celle-ci considérée à la fois sous l'angle de la qualité et de la quantité.

L'examen de trois liens *Imago*-Plantes hôtes, montre qu'il y a, en quelque sorte, des plantes supérieurement „favorables" à l'insecte parfait, tous les caractères d'affinité se présentant, pour elles, simultanément avec leur degré élevé ou le plus élevé. A un tel niveau, ce sont placés, dans nos essais, *S. edineuse, S. dulcamara* (en plein champ, non dans les haies ombragées), leurs affinités dépassant celles rencontrées chez la plupart des variétés de *S. tuberosum*.

Dans les plantes à affinités faibles ou très faibles, ce niveau bas peut résulter soit de caractères faibles partout, soit de la présence sur une seule phase d'un caractère ayant un très faible niveau, celui-ci suffisant à annuler les affinités par contre bien meilleures sur d'autres caractères.

Les affinités et immunités jouant pendant la vie larvaire, se précisent en étudiant les réactions de contact de la larve à l'égard des feuilles (tact, gustation, circulation ou immobilité, vitesse et intensité des attaques de tissus foliaires, etc....), puis les courbes de survie et les vitesses de croissance. Comme pour les *imagos*, l'aliment ingéré, par sa quantité et sa qualité, influe avec délais sur la physiologie des stades venant après la vie larvaire.

Nous avons eu d'excellentes courbes de développement sur les *Solanum marginatum, dulcamara, gilo, rostratum*; elles furent moins bonnes avec *heterodoxum, aviculare, sisymbriifolium, balbisii, N. sandere*; moins bonnes encore avec avec *demissum, commersonii, jamesii, polyadenum, nigrum, auriculatum;* le plus

souvent nulles ou toujours nulles, avec les *Petunia, Salpiglossis, Hyosciamus, Datura, Nicotiana tabacum, glauca* et *rustica, S. aculeatissimum.*

Dans les deux dernières catégories, il y a parfois attaques par les jeunes larves très rapides et intéressées, puis celles-ci cessent et la mort rapide intervient le qui plus souvent (*Petunia, Salpiglossis* notamment); d'autres fois on observe des morsures très limitées ou nulles (*Datura*); enfin, dans le cas extrême, on note même l'absence de toute morsure marquée (*S. aculeatissimum*).

Les quelques exemples montrés rapidement indiquent que c'est en considérant tout un ensemble de facteurs, opérant toutefois comme un tout par leurs effets finaux, que l'on peut fixer par végétal et pour un milieu donné, une *résultante cumulative* (se jugeant seulement sur une *suite de plusieurs générations*) qui définit une sorte de „coefficient de prolifération," caractéristique, avec ses limites de variations possibles, de ce végétal.

L'étude comparative de tels coefficients est particulièrement instructive sur le plan de l'immunologie lorsqu'on l'applique à l'ensemble de la famille des Solanacées; aussi ne peut-on que souhaiter une reprise un jour prochain en détail et en tenant compte des enseignements récents obtenus sur Solanacées tuberifères, de l'étude des caractères d'immunité chez des espèces et des genres nombreux, pris parmi les Solanacées non tuberifères.

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HOST PLANT SELECTION IN THE COLORADO BEETLE LARVA

(LEPTINOTARSA DECEMLINEATA SAY)

(An ethological approach to food finding in insects)

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In the food finding behaviour of the Colorado beetle larva, random searching and random biting are two apparently spontaneous behaviour elements belonging to different motor systems. Locomotion is directed by light and by host odour, biting is slightly inhibited by indifferent substrates like glass and filter paper. Initiation and maintenance of feeding are partly dependent upon different stimuli, as shown by the behaviour of the larva on *Solanum demissum* Lindl. Substances inhibiting the ingestion of food do not necessarily interfere with locomotory responses causing the insect to remain on the plant, nor do they prevent the feeding action from being released. This may lead to the cyclic process represented in fig. 6. The factors regulating intensity of motivation and threshold releasing stimulus are discussed.

In the Colorado beetle, host plant selection is primarily performed by the ovipositing female. Nevertheless, the larva is not entirely devoid of the ability to discriminate between food plants. Indeed, its abilities in food selection are such as to make the larva the preferred stage to be used in screening experiments with resistant potato hybrids.

The work of CHIN (1950) has provided a firm basis for the understanding of host finding in Leptinotarsa. It has seemed useful to discuss briefly the facts revealed by CHIN, supplemented by new ones, in the light of some ethological concepts.

A PROVISIONAL ANALYSIS OF FOOD-FINDING

The young larva, soon after hatching, consumes the remaining egg yolk. After this, it enters a period of random searching, during which it generally does not start feeding in the immediate vicinity of the former egg pod, but is apparently set in motion by some internal factor, for which we will use the ethological term motivation.

During this random searching, resulting in a more equal distribution of the larvae over the food plant, the pathway of the larva can be orientated by several environmental factors, one of which is light. The larva is equipped with two groups of six stemmata, the axes of which point in different directions (fig. 1). While the larva pursues its pathway, its head makes pendulating horizontal movements. DETHIER (1943), who observed the same movements in caterpillars, has pointed out that their function is to improve the perception of form and distance.

When the larva is placed in a gradient of light, a clear positive phototaxis is apparent.

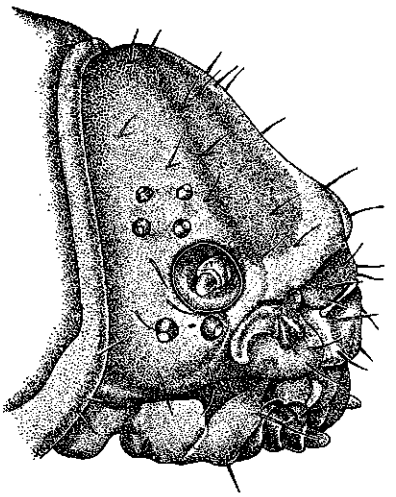

Fig. 1. Lateral view of the stemmata and position of the (retracted) antenna (DE WILDE & PET, 1957)

When a potato leaf is placed near the head of a larva removed from its food-plant, it only reacts at a close distance. Optical as well as olfactory stimuli are involved. The first is shown by the preference for green as a colour (DE WILDE & PET, 1957), which is especially clear when green is in contrast with other shades (Table I).

TABLE I

The Colorado beetle larva shows a preference for green when in competition with red, both colours being equal to the same shade of grey (DE WILDE & PET, *1957*)

COMBINATION	Gr 27—R1	Gr 18—R1	Gr 22—R1	Gr 18-Grass Grn
CHOICE	37—63	70—30	50—50	65—35
$P < 0.05$	+	+	—	+
COMBINATION	Gr 22-Grass Grn.	Grass Grn.—R1		
CHOICE	53—47	67—33		
$P < 0.05$	—	+		
COMBINATION	Yellowgrn 2-Gr 6	Gr 6-Ochre-yellow	Yellowgrn 2-Ochre-yellow	
CHOICE	52—48	57—43	62—38	
$P < 0.05$	—	—	+	

Gr 27, Gr 28, etc. = numbers of the Rietschel grey series.
R 1, Grass Grn., Yellowgrn. 2 = colours of the Hering series.
The result of 100 choices is given. The difference was tested for significance by means of binominal probability paper.

The attractiveness of the scent of solanaceous leaves is shown in the "screen test" (fig. 2) (CHIN, 1950). This simple method has the advantage that use is

made of still air. Anemotactic responses need not to be taken into account. Table II demonstrates the olfactive attractiveness of the leaves of different species of Solanaceous plants.

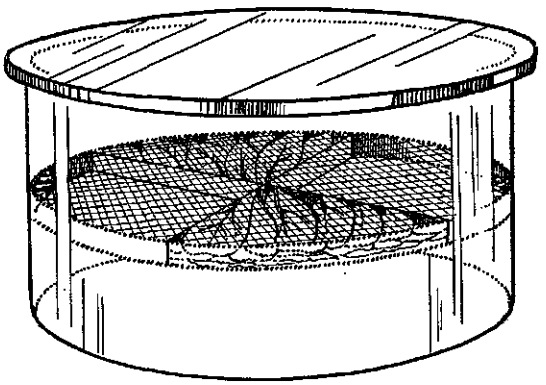

Fig. 2. Apparatus for the "screentest". The larva is placed upon a screen of wire gauze of fine mesh, to avoid optical or tactile contact with the leaves placed in the lower compartment (CHIN, 1950)

TABLE II

Preference for host plant odour by the Colorado beetle larva, as demonstrated by the screen test (CHIN, *1950) (% of positive choices with mean error, 50% being neutral)*

Solanum tuberosum	58,2 ± 2,1
S. demissum	59,2 ± 2,5
Petunia hybrida	57,2 ± 2,4
Doronicum sp.	49,2 ± 2,2
S. tuberosum (antennae amputated)	51,0 ± 2,5
S. tuberosum (antennae + palpae amputated)	48,8 ± 2,6

When the larva is removed from the plant, both factors may now be responsible for host finding. When it is on the food plant, they result in a tendency to stick to it, and move to places with highest illumination.

Next to "random searching", there is another type of activity which can occur without the interference of specific substrata, though it may happen more readily in the presence of such. This is „random biting"[1]). The searching larva bites a variety of substances. When a new substratum is offered to the larva, the time elapsing before biting occurs may be used as a measure of its attractiveness. We then see, that leaves of very different plants as *Solanum, Doronicum* and *Pelargonium* are bitten more readily than neutral substrates like glass or filter paper. Remarkable enough, removal of the palpae and antennae enhances the biting responses towards neutral substrates, suggesting an inhibitory influence of the last-named. We will call this inhibitory factor the "palp factor" (Table III).

[1]) "random" is used here in the sense of "whereever the larva walks". It occurs only in the underlying substrate and is not "random" in this sense.

TABLE III

Enhancement of biting in the underlying substrate brought about by the amputation of palpae and antennae (CHIN, *1950)*

SUBSTRATA	NORMAL LARVA					OPERATED LARVA				
	1	2	3	4	5	1	2	3	4	5
GLASS PETRI DISH COVER	—	—	—	+	—	+	+	—	+	—
DRY FILTER PAPER	—	—	—	—	—	+ +	+++	—	+ +	+
WETTED FILTER PAPER	+	—	—	+	—	+ +	+++	+++	+ +	+++
DORONICUM LEAF	+	+	+	—	—	+++	+++	+ +	+	+++
GERANIUM LEAF	+ +	+++	+++	+ +	+++	+++	+++	+++	+ +	+++
PETUNIA LEAF	+++	+ +	+++	+ +	+++	+++	+++	+++	+++	+++
DEMISSUM LEAF	+++	+	+ +	+	+++	+ +	+++	+++	+ +	+++
TUBEROSUM LEAF	+++	+++	+++	+	+++	+++	+++	+++	+++	+++

— 30 minutes elapsed without any biting action
\+ biting action more than two minutes after the larva was introduced on the substratum
++ biting action between one and two minutes after the larva was introduced on the substratum
+++ biting action less than one minute after the larva was introduced on the substratum

As soon as the larva bites a leaf belonging to its host plant series, a new type of action is released. We may now observe the larva "shaving" a certain part of the surface, if a dense pilosity is present, as in some lines of *Solanum demissum.* The larva subsequently perforates the leaf epidermis. The beginning of the feeding action is marked by the withdrawal of the antennae into the head capsula (fig. 3). During searching, these telescope-like appendages are held in a stretched position, thus enabling the sensilla basiconica on the terminal segment to fulfill their function.

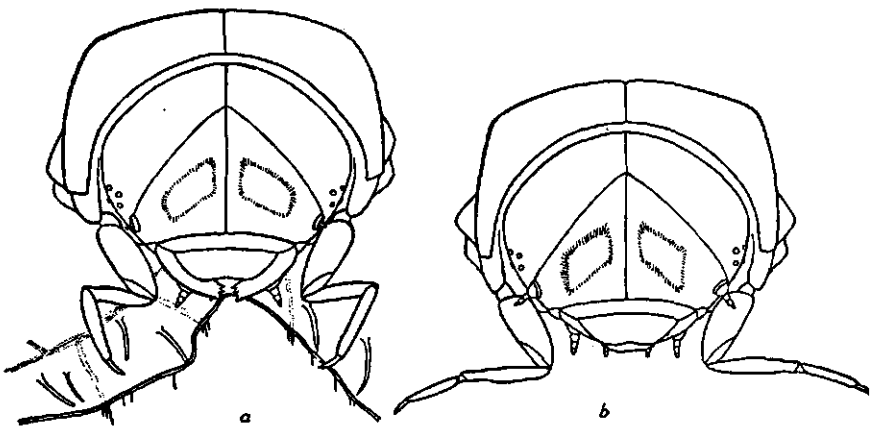

Fig. 3. Colorado beetle larva showing antenna
a. during feeding; b. when searching (CHIN, 1950)

After the feeding action has started, the behaviour of the larva is still greatly

influenced by the internal condition of the leaf, as is shown when different *Solanum* species are compared (fig. 4).

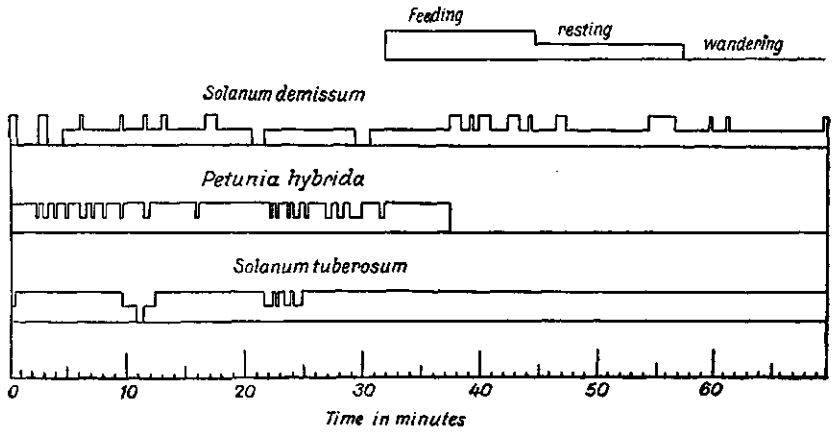

Fig. 4. The activities of the fourth instar larva on the leaves of Solanum tuberosum and Solanum demissum (CHIN, 1950)

On potato leaves we observe continuous feeding, interrupted by short periods of rest. On resistant lines of *Solanum demissum,* most of the time is spent resting and wandering, interrupted by short feeding periods. This results in quite different feeding patterns (fig. 5).

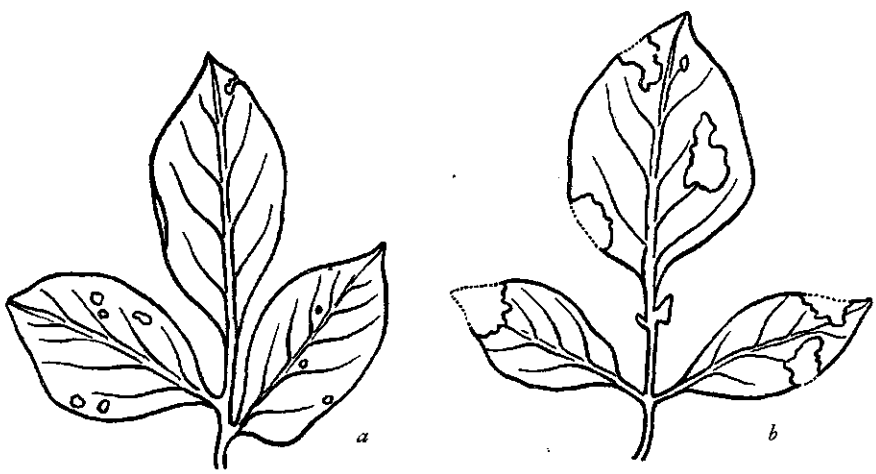

Fig. 5. Feeding pattern of the third instar larva a. on S. demissum; b. on S. tuberosum

Apparently the sense organs on the mouth parts are involved, for removal of the palpae results in enhanced feeding on plants rejected before. Furthermore, we may produce the same pattern as obtained on *Solanum demissum* by infiltration of potato leaves with one per cent acetic acid.

Putting the above-named observations into a scheme, we find that host selection is chiefly made on a chemosensory basis (fig. 6).

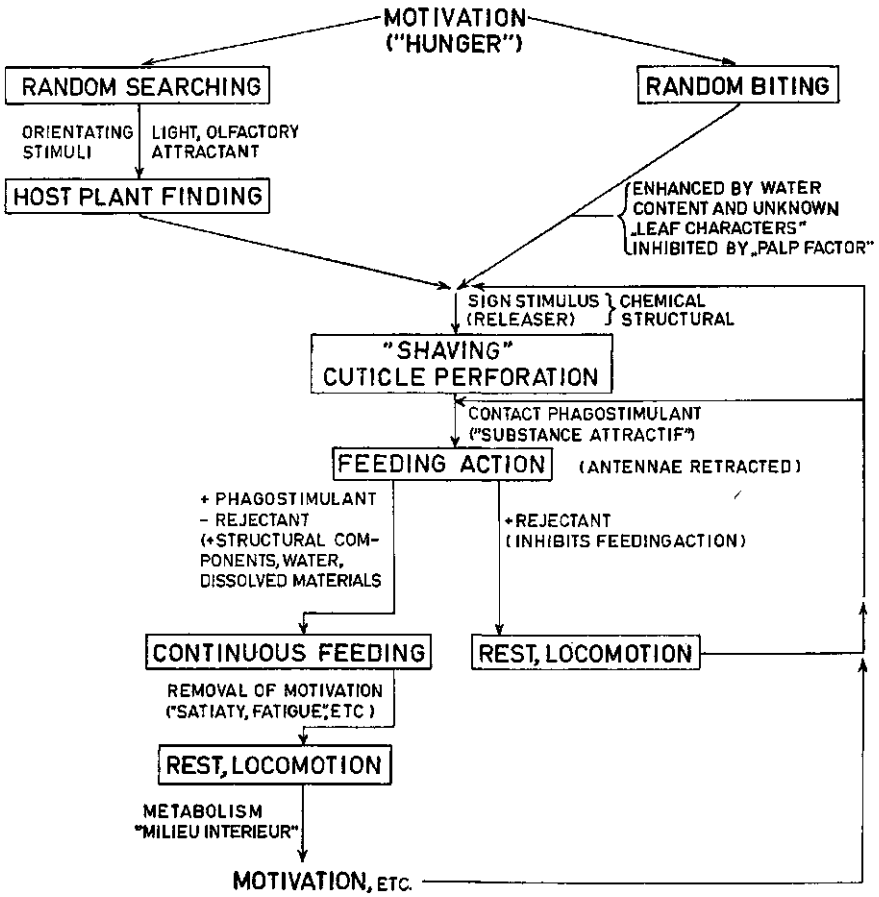

Fig. 6. Scheme of the sequence of activities leading to host finding and acceptance or rejection by the colorado beetle larva

PHAGOSTIMULANTS AND REJECTANTS

As regards the substances involved, much has still to be discovered. The olfactory attractive substance is unknown. It is certainly not identical with the "substance attractif" as mentioned by RAUCOURT & TROUVELOT (1936). This last-named substance is very probably a releaser of the feeding action [1]). It has been purified to some extent by CHAUVIN (1952), who defines it as a hygroscopic flavoglucoside. Much more is known of the chemical nature of the "resistance-factors" present in *Solanum demissum, Solanum chacoense* Bitter, the tomato and

[1]) The term "phagostimulant", as proposed by THORSTEINSON (1957) has been adopted by us, though to our opinion the term "releasing substance" would fit better. Similarly, BAERENDS (1950), discussing the behaviour of flower-visiting insects, states that ".....to some insects odour..... means a releasing stimulus."

some other Solanaceae. It appears that, contrary to the statements of HESSE & MEIER (1950) the attractive principles present in these species are much the same as in the potato. However, while the alkaloid solanine in the concentration present in *Solanum tuberosum* L. apparently does not provide a chemical stimulus to the beetle, the alkaloids demissine, tomatine, and leptine are strongly rejected in the concentration present in the plant (KUHN et al. 1947, 1950, 1957). Their presence adds a repellent (perhaps we might better use the term rejectant, as proposed by THORSTEINSON) to the otherwise acceptable food.

DISCUSSION

In our attempt at an analysis of host finding and recognition in this oligophagous beetle, we have been primarily interested in the type of information which the insect obtains from its food. This information can only be obtained through the sense organs. These in their turn select from the "Umwelt" the stimuli which, separately or in combination release or direct locomotory or feeding responses.

It seems of importance to stress once more the difference between the properties of plant substances as nutritional elements and as factors in host finding and initiation and maintenance of feeding. In dealing with the last category, DETHIER (1953) distinguishes between 1. orientation, 2. biting responses and 3. maintenance of feeding. In addition we would like to stress the role of plant substances as releasers and inhibitors of the feeding action. We also find that, in the colorado beetle larva, initial biting is probably an innate activity requiring internal motivation.

What may be the role of the nutrients in this complicated behaviour pattern ?

To KENNEDY & BOOTH (1951, p. 60), stating "..... one might expect aphids to have evolved some tastes for things that are good for them, in an immediate nutritional sense, and other tastes for things that are less or not at all good for them, but are valuable instruments of ecological specialization", one may object that even man cannot taste proteins or lipoids, nor the many vitamins that are indispensable parts of his nutrition.

Though the statement by FRAENKEL (1953, p. 99): ".....That it is the presence of odd chemical substances in which plants differ, such as glucosides..... which renders the plant a suitable or unsuitable food" is, according to the same author"..... an admittedly extreme and onesided view", it has the value that a sharp distinction is made here between the behaviour components of food selection and nutrition as such.

As found by THORPE c.s. (1947), the movements of polyphagous wireworms like *Agriotes lineatus* L. and *A. obscurus* L. are orientated, and their feeding action released, by substances generally occurring in plants like glucose and asparagine. Oligo- or monophagous insects require for their food intake much more specific chemical substances which, in the relation plant-insect have an orientating and releasing function.

The necessity of such "odd substances" does of course not exclude that components of a more general occurrence, used as nutrients by the insect, may also act along the sensory pathway and play a role in food selection. There are

numerous observations to prove the contrary (see THORSTEINSON, 1958). It may even represent the more primitive situation, the specialized feeding habits of oligophagous insects being due, as it were, to the formation of a conditioned reflex on the "odd substance", which in the course of their evolution has become part of their inherited behaviour pattern.

Once ingested, the nutrients most probably intervene by either deminishing the intensity of motivation ("hunger condition"), or increasing the threshold for the releasing stimuli of the feeding action. Recent experiments by DETHIER throw some light on the general aspects of this problem.

An accurate analysis of the factors controlling the ingestion of carbohydrates by the blowfly, *Phormia regina* Meig., has led DETHIER to the conclusions, that (a) there is no relation between the amount of sugar taken in and its nutritional value (DETHIER, 1956) and (b) raising the level of glucose in the haemolymph by means of injection does not influence the threshold concentration of glucose with regard to the sucking response (DETHIER, 1957). This would mean that in the blowfly, neither the feeding motivation nor the liminal releasing stimulus for the feeding action are regulated by the nutritional substance via the haemolymph.

We have no data to confirm or to reject either of these points in phytophagous insects.

RÉSUMÉ

1. Rapidement après l'éclosion, le premier stade larvaire de *Leptinotarsa* fait au hasard des mouvements d'exploration.

2. Ces mouvements peuvent être dirigés par la lumière (intensité, couleur) et par l'odeur de la feuille de *Solanum*.

3. Chez une larve en exploration, la morsure faite au hasard est un autre élément du comportement spontané, qui peut être inhibé par les impulsions reçues par les palpes.

4. Le début et le maintien de l'alimentation dépendent partiellement de différents stimuli, comme le montre le comportement de la larve sur *Solanum demissum*. Les substances inhibant l'ingestion d'aliment n'empêchent pas nécessairement l'acte alimentaire d'être déclanché.

5. Ceci peut aboutir à un cycle fermé représenté dans le diagramme.

6. Le rôle de constituants nutritifs dans la sélection de la plante-hôte est discuté.

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DISCUSSION

B. TROUVELOT asked whether the reaction to the stimuli mentioned occurred periodically, in relation to the rhythm of illumination. The answer was, that this is indeed the case; it has been proved that the beetle as a whole shows a diurnal rhythm of activity. It must be concluded that the feeding motivation shows a similar rhythm.

J. S. KENNEDY wondered whether the "palp factor" would consist of tactile stimuli provided by hard or brittle substances like glass or paper. He further asked whether prolonged stimulation of this kind would result in a lack of "hungry" behaviour shown by the starved larva. The author is indeed inclined to think that the inhibitory "palp factor" results from tactile stimuli unbalanced by host odour or taste. The fact that a palpectomised larva resembles in its behaviour a starved larva means that in the latter case the threshold for the palp factor is probably increased.

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THE CHEMOTACTIC INFLUENCE OF PLANT CONSTITUENTS ON FEEDING BY PHYTOPHAGOUS INSECTS

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The available evidence is in agreement with the postulate that all phytophagous insects require chemotactic (usually gustatory) stimuli to release feeding activity. Some of the soluble nutrients in plants serve this function. In the case of oligophagous insects food selection is further guided by perception of non-nutritive substances (token stimuli) of limited botanical distribution. The food range of all phytophagous insects is circumscribed by the distribution of repellants, rejectants and toxins. These concepts are developed and outlined in symbolic form.

Food plant selection or botanical specificity is brought about through two phases of insect behaviour. *viz* (1) food finding and (2) food acceptance. The odourous constituents of plants may assist insects in finding their food plants but little is known about the identity of chemicals that serve this function nor about the precise behaviour mechanisms displayed by insects in response to such stimuli.

Food acceptance is manifest either by the act of oviposition or by feeding. Only the second of these two types of response will be considered in this discussion. The term "acceptance" is preferable to "selection" since it is applicable whether or not a choice is available and does not imply any psychological behaviour on a higher level. As the ethological aspects of feeding by phytophagous insects have already been discussed in this symposium in a highly satisfactory manner by Professor DE WILDE, it will be appropriate to focus attention on the chemical plant constituents and the nature of their influence on feeding responses.

In the past, more notice has been taken of the token feeding stimuli provided by the non-nutritional constituents of plants such as the glycosides. The rather striking responses that can be evoked by token stimuli are illustrated in Table I. Similar data have been given previously (THORSTEINSON, 1953). It is evident that the diamond-back moth larva does not accept a mixture of nutrients unless it contains at least a small amount of a specific token phagostimulant (mustard oil glucoside) which occurs in its natural food plants.

However, it would be rather rash to generalize from the behaviour of one or even several insect species and, *a priori*, it should seem strange that nutrient plant constituents do not affect the palatability of food to many insects. Indeed, one of the universally present substances in plants, namely sucrose, has long been known to be avidly acceptable to numerous phytophagous and other insects species (BECK, 1956). Although several investigations have studied the responses of insects to sugars, quinine, salts and acids the question as to whether insects perceive

the taste of important nutrient constituents of their food such as amino acids, amides, vitamines etc. has been largely neglected. An important exception is the work of THORPE ET AL (1947) on the food finding of wireworms.

TABLE I

Feeding Responses to a token phagostimulant (sinigrin) by Plutella maculipennis Curt. as Measured by Frass Pellet Count

DIET	MEAN FRASS COUNT (5 Replicates)	STANDARD ERROR
1. Nutrient Agar*	117.8	15.8
2. Nutrient Agar + 0.1% Sinigrin	306.4	16.1
3. Black mustard leaf powder (2%) Agar	311.8	21.4

* Nutrients comprise glucose 6%, soluble casein 2%, triolein 0.5%, yeast 2%.

Of course, the physical obstacles confronting wireworms in their search for food would seem to demand some special compensation in the form of chemotactic guidance and THORPE ET AL have demonstrated elegantly that nature has provided them accordingly. It is not improbable however that leaf-eating forms also have a compelling need to appraise the quality of food such as would be provided by a perception of soluble constituents. As the event has proved, it has been worth looking for such effects. Indeed, the number and variety of substances that stimulate feeding by insects has exceeded our expectations.

By the use of "artificial leaves" prepared from thin discs of elder pith (a technique first introduced by the president of this Symposium, Professor TROUVELOT) it is possible to obtain satisfactory estimates of the phagostimulant activity for chewing insects of any soluble substance that may be of interest. By the use of this method including the refinements introduced by CHAUVIN (1952) we have studied the feeding response of several phytophagous insects including oligophagous and polyphagous forms (especially grasshoppers). An outline of the salient findings follow:

As we expected, polyphytophagous insects are stimulated to feed by sucrose which is a more active phagostimulant than glucose. For grasshoppers the optimum concentration is about 0.02 M under the conditions of our tests. An unexpected finding was that one oligophagous insect, *Leptinotarsa decemlineata* Say, is stimulated to feed by sucrose even when no token phagostimulant is present and, as for the grasshoppers, 0.02 M is the approximate optimal concentration but in this case little feeding response is obtained below this concentration.

As was reported previously (THORSTEINSON, 1956), a number of metabolic constituents of plants possess phagostimulant activity for various phytophagous insects. These substances include amino acids, amides and vitamines such as thiamine and ascorbic acid.

In view of these results and the recent finding by R. H. DADD (1957) that ascorbic acid may constitute a vitamine requirement for locusts one is tempted to speculate that the gustatory sense of insects may assist them in "selecting" food of

suitable nutritional quality. Of course it is too improbable that all the varied nutritional components could be appraised by insects in this way. However, it is not entirely implausible that some of the nutrient substances perceived by the gustatory sense of insects may serve as indicators of nutritional quality. If so, such substances, although of nutritional consequence, would nevertheless serve as „token" phagostimulants. This suggests that the distinction between token and nutritional feeding stimulants is not distinct. At any rate, it seems clear that there is a considerable correlation between the chemotactic stimuli that govern feeding and the nutritional requirements of insects. This conclusion is sufficiently probable *a priori* but there has been a reluctance to accept it (LIPKE and FRAENKEL, 1956) pending actual experimental evidence. This evidence appears now to be forthcoming.

In view of the fact that some chemical constituents of plants function both as nutrients and as phagostimulants for insects it is of interest to consider what other dual effects may be manifested by plant constituents. Table II suggests some possibilities:

TABLE II

Dual Effects of Chemical Plant Constituents on Feeding and Metabolism of Insects

	Feeding Stimulant or Acceptant *	Nutrient	Rejectant *
Feeding Stimulant or Acceptant *	T		
Nutrient	Ns		
Rejectant *	—	?	
Toxin	?	?	+

* The terms "acceptant" and "rejectant" have been adopted in place of "attractant" and "repellent" where the responses of the insects do not involve locomotion towards or away from the substrate, following discussions with R. H. DADD and D. GOODHUE, Imperial College, London.

A substance that stimulates feeding but has no other effect is a token phagostimulant (T). If however, it is also a nutrient (for example, sucrose) we may call it a sapid nutrient (Ns) to distinguish it from nutrients that are vapid or tasteless and do not affect feeding responses. A feeding stimulant cannot be also a repellent since this is a contradiction in terms. A substance that is both a feeding stimulant and a toxin is an interesting possibility since it should have valuable applications as an insecticide but we do not know of such a substance. A repellent nutrient seems somewhat unlikely, yet NUORTEVA (1952) has demonstrated that leafhoppers avoid sap of high sugar concentration. A toxic nutrient would seem to be a contradiction in terms unless we recall that any substance is likely to be toxic in sufficiently high dosage. Toxic effects of sugars have been reported by FRAENKEL (1955). Finally, a toxic repellent is not an unlikely combination and demissin is probably an example of such a substance for *Leptinotarsa.*

In order to summarize the conditions required for normal feeding and development by phytophagous insects, the following symbolic scheme is presented.

$F \underset{\leftarrow}{\longrightarrow} I$

Normal feeding (F) implies ingestibility (I) but I is not sufficient to ensure normal feeding. Similarly,

$F \underset{\leftarrow}{\longrightarrow} P_T$ (token stimulus factor in acceptability)

$F \underset{\leftarrow}{\longrightarrow} P_{N_s}$ (sapid nutrient factor in palatability)

$F \underset{\leftarrow}{\longrightarrow} pH$ (optimum pH)

$F \underset{\leftarrow}{\longrightarrow} -R\ -P$ (absence of rejectants and poisons)

$F \underset{\leftarrow}{\longrightarrow} I + P_T + P_{N_s} + pH - R - P$

Normal feeding implies all the factors on the right which *together* are sufficient to induce normal feeding.

Furthermore,

$D \underset{\leftarrow}{\longrightarrow} F$ (Normal development implies normal feeding but normal feeding is not sufficient for normal development).

Similary,

$D \underset{\leftarrow}{\longrightarrow} N_c$ (complete complement of nutrients)

$N_c \underset{\leftarrow}{\longrightarrow} N_s + N_v$ (complete complement of nutrients implies both sapid and vapid nutrients)

$D \underset{\leftarrow}{\longrightarrow} F + N_c$ (normal development implies normal feeding and a complete complement of nutrients)

But N_s is common to both F and N_c

Hence,

$D \underset{\leftarrow}{\longrightarrow} F + N_v$ (normal development implies normal feeding on a diet that includes both sapid and vapid nutrients)

In an earlier paper (THORSTEINSON, 1953), I pointed out that we should normally expect all insects to require some gustatory stimulus to initiate and sustain feeding activity. If such stimuli for a given insect species include token stimulants with a restricted botanical distribution, the insect will be oligophagous or monophagous. The phagostimulants required by polyphagous insects must necessarily have a wide botanical distribution. In the latter group, host plant acceptance will be governed by the botanical distribution of toxic or rejectant substances that mask or interfere with the effect of the phagostimulants but this should not obscure the significance of the phagostimulants to an understanding of the problem of host plant relationships of insects.

RÉSUMÉ

L'importance des stimulants alimentaires "token"[1]) a été clairement démontrée par exemple chez *Plutella maculipennis* Curt., *Pieris brassicae* L. et *P. rapae* L., lesquels, à l'état larvaire, reconnaissent leur plante-hôte, les Crucifères, au goût de glucosides de l'huile de moutarde tels que la sinigrine et la sinalbine. Bien qu'elles soient presque les seules démonstrations indiscutables de l'action de ces stimulants "token" parmi les insectes phytophages il a été admis que ce phénomène explique en général la sélection de la plante hôte par les insects

[1]) "Token" = Stimulus favorisant la reconnaissance de la nourriture, mais qui lui-même n'a pas de valeur nutritif.

oligophages et que la présence de substances désagréables au goût dans les végétaux est suffisant pour expliquer les préférences de plantes alimentaires des insectes polyphages. Cependant, ces vues ne rendent pas compte du fait que ces „phagostimulants "token" peuvent exiger la présence de constituants nutritifs afin d'obtenir une réponse alimentaire chez les insectes oligophages. En outre les insectes polyphages demandent des stimuli chimiotactiques positifs pour déclancher une activité alimentaire. Aucun effet inhibiteur de substances végétales désagréables n'est simplement superposé à ce phénomène de base.

Un autre fait significatif est que le saccharose, constituant universellement distribué chez les végétaux, est avidement goûté par de nombreux insectes, et on pourrait penser *à priori* que son rôle est important dans la nature comme stimulus effecteur d'alimentation chez les insectes phytophages. De récentes investigations ont montré que d'autres substances nutritives variées telles que le glucose, des acides aminés, des amides et des vitamines peuvent aussi déclancher d'une manière indépendante l'activité alimentaire.

Une interprétation totale de la connaissance utile à présent est que de nombreuses conditions sont exigées pour rendre compte des phénomènes d'alimentation chez les insectes et de la sélection de l'hôte. Ceci inclut la présence de stimulants alimentaires "token" (si nécessaires), la présence de certains constituants sapides et l'absence de substances répulsives ou désagréables au goût. Tandis que ces conditions, admettant un substrat ingestible, sont nécessaires et suffisantes pour une alimentation normale un développement satisfaisant exige en plus la présence de constituants nutritifs essentiels autres que les substances sapides déjà mentionnées et l'absence de toxines.

Certains constituants végétaux peuvent avoir plusieurs fonctions dans ce schéma et quelques exemples sont considérés.

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ÜBER EINIGE INHALTSSTOFFE DER SOLANACEEN UND IHRE BEDEUTUNG FÜR DIE KARTOFFELKÄFERRESISTENZ [1])

VON

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Unsere heutigen Kenntnisse über die natürlichen pflanzlichen Resistenzstoffe gegen den Kartoffelkäfer (*Leptinotarsa decemlineata* Say), insbesondere auf dem Gebiet der *Solanum*-Alkaloide, werden zusammengestellt. Gleichzeitig wird versucht, den Wirkungsmechanismus dieser Stoffe zu deuten.

Chemische Faktoren werden in zunehmendem Maße zur Deutung von Resistenzerscheinungen herangezogen. Eine auf die chemische Zusammensetzung der Pflanzen zurückführbare Widerstandsfähigkeit gegenüber Schädlingen kann folgende Ursachen haben:

1. Ausreichendes Vorkommen von entwicklungshemmenden sowie toxisch oder „futtervergällend" wirkenden Inhaltsstoffen,
2. unzureichender Nahrungswert der Wirtspflanze und
3. das Fehlen wirksamer „Lock-" und „Fraßstoffe".

Der Kartoffelkäfer und seine Larven sind im Rahmen der Pflanzenfamilie der Solanaceen oligophag. Durch das Zusammenspiel der genannten Faktoren tritt bei den verschiedenen Gattungen, Arten, Formen und Herkünften eine graduell sehr unterschiedliche Kartoffelkäferresistenz in Erscheinung.

Über das Nahrungsbedürfnis phytophager Insekten im allgemeinen und des Kartoffelkäfers im besonderen sind unsere derzeitigen Kenntnisse noch sehr unzureichend, vor allem da die Untersuchung der Frage, welche Bedeutung speziellen Nährstoffgruppen oder gar bestimmten, chemisch definierbaren Nahrungsbestandteilen zukommt, ausserordentlich schwierig ist. Ebenso offen ist das Problem der Fraß- und Lockstoffe, obgleich gerade hier in letzter Zeit, insbesondere in physiologischer Richtung, beachtliche Fortschritte erzielt wurden.

Einfacher dagegen ist die chemische Bearbeitung der zusätzlich in widerstandsfähigen Pflanzen vorkommenden, resistenzbedingenden Inhaltsstoffe, da geeignete Testmethoden auch bei phytophagen Insekten möglich und bekannt sind.

Als von KUHN und Mitarbeitern (KUHN und LÖW, 1947; KUHN und GAUHE, 1947; KUHN, LÖW und GAUHE, 1950) vor einigen Jahren mitgeteilt worden war, dass die Kartoffelkäfer-Larvenresistenz der mexikanischen Wildkartoffel *Solanum demissum* sowie einiger *Lycopersicon*-Arten auf die in diesen Pflanzen vorkommenden Alkaloidglykoside Demissin (VII) bezw. Tomatin (XII) zurück-

[1]) Vgl. K. SCHREIBER: Natürliche pflanzliche Resistenzstoffe gegen den Kartoffelkäfer und ihr möglicher Wirkungsmechanismus, *Der Züchter* 27, 289—299 (1957).

zuführen ist, wurde den chemischen Resistenzstoffen im allgemeinen und den Alkaloiden im besonderen ein erneutes starkes Interesse entgegengebracht.

Im Gegensatz zu Demissin (VII) und Tomatin (XII) üben das α-Solanin (II) und α-Chaconin (III) unserer Kulturkartoffel (*Solanum tuberosum*) diese Schutzwirkung nicht aus (KUHN und GAUHE, 1947; KUHN und LÖW, 1955; STEPANOWA und MIRONOWA, 1955; SCHREIBER, 1955; BUHR, TOBALL und SCHREIBER, 1957). Eine gleiche Unwirksamkeit muss den in einigen nicht knollentragenden *Solanum*-Arten (*Solanum sodomeum, Solanum aviculare, Solanum auriculatum, Solanum nigrum*[1]) u.a.) vorkommenden Alkaloiden Solasonin (IX) und Solamargin (X) zugesprochen werden (BUHR, TOBALL und SCHREIBER, 1957).

Demissin und Tomatin führen nicht zu einer akuten Vergiftung der Larven, sondern sie wirken als Abschreckstoffe, die das Futter für das Insekt „vergällen" und es so dem Hungertode ausliefern. Darüber hinaus wurden jedoch bei gelegentlich überlebenden Tieren, vor allem an nicht vollresistenten Pflanzen, die nur einen geringen Demissin- bzw. Tomatingehalt aufwiesen, Entwicklungsstörungen beobachtet (TROUVELOT und GRISON, 1935; CHIN, 1950).

Bei der Betrachtung der Strukturformeln (I—XIII) fällt auf, dass die resistenzbedingenden Alkaloide Demissin und Tomatin im Gegensatz zu den unwirksamen Verbindungen eine aus v i e r Monosacchariden (1 Mol. D-Galaktose, 2 Mol. D-Glukose und 1 Mol. D-Xylose) aufgebaute Kohlenhydratkomponente besitzen, also T e t r a o s i d e darstellen. In Demissin und Tomatin kommt somit die Pentose D-Xylose vor, in den T r i o s i d e n α-Solanin, α-Chaconin, Solasonin und Solamargin dagegen neben D-Galaktose und D-Glukose die Methylpentose L-Rhamnose. Außerdem ist die in den unwirksamen Alkaloiden auftretende $\triangle^5$-Doppelbindung in den beiden wirksamen Verbindungen nicht vorhanden.

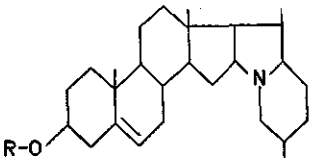
Ein uns zur Verfügung stehendes, sehr umfangreiches Sortiment an *Solanum*-Arten bot Gelegenheit, die oben aufgeworfene Frage nach den möglichen Beziehungen zwischen der chemischen Konstitution der *Solanum*-Alkaloide und ihrer Kartoffelkäferwirksamkeit durch die chemische Untersuchung weiterer, insbesondere resistenter Arten, zu überprüfen.

So konnten wir zum Beispiel aus dem sehr heterozygot resistenten *Solanum polyadenium* 2 Alkaloidglykoside isolieren, die in der Zusammensetzung ihrer Kohlenhydratkomponenten den bereits genannten Alkaloidpaaren α-Solanin-α-Chaconin (II und III) bzw. Solasonin-Solamargin (IX und X) entsprechen; allerdings enthalten diese *polyadenium*-Glykoalkaloide keine L-Rhamnose, sondern D-Xylose.

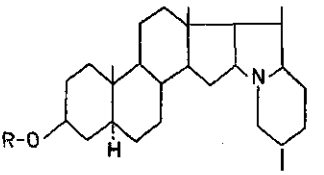
Während das resistenzbedingende Tetraosid, das nach unseren Befunden nur gelegentlich in den Pflanzen vorkommt, aus dem Aglykon Tomatidin (XI), 1 Mol. D-Galaktose, 2 Mol. D-Glukose sowie 1 Mol. D-Xylose aufgebaut und mit

[1]) In zahlreichen Varietäten und Herkünften von *Solanum nigrum* und verwandten Arten, die im allgemeinen eine gute Kartoffelkäferresistenz zeigen, konnten wir papierchromatographisch insgesamt 6 Alkaloidglykoside nachweisen, die wir vorläufig mit alpha-, bèta-, gamma-, delta-, epsilon- und zèta-S o l a n i g r i n bezeichnen möchten. Das Aglykon dieser Glykoalkaloide, die sich von den *tuberosum*-Alkaloiden eindeutig unterscheiden, ist mit Solasodin identisch. Delta- und gamma-Solanigrin, die vermutlich Hauptalkaloide darstellen, konnten nach präparativer Gewinnung als Solasonin und Solamargin identifiziert werden (SCHREIBER, 1957 b).

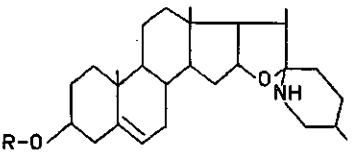
dem Tomatin (XII) der *Lycopersicon*-Arten identisch ist, besitzt das Triosid, über das wir bereits vor einiger Zeit kurz berichteten (SCHREIBER, 1954 a; BUHR, 1955), nur eine geringe Larvenwirksamkeit (BÜHR, TOBALL und SCHREIBER, 1957).



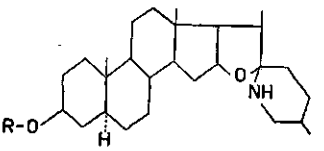
I R = H . . . . . : Solanidin
II R = L-Rhamnosyl-D-glukosyl-D-galaktosyl . . . : α-Solanin
III R = L-Rhamnosyl-L-rhamnosyl-D-glukosyl . . . : α-Chaconin
IV R = D-Xylosyl-D-xylosyl-D-glukosyl . . . . : Solacaulin
V R = D-Xylosyl-D-glukosyl-D-glukosyl-D-galaktosyl : Tetrosid aus der Ser. *Acaulia*.



VI R = H . . . . . : Demissidin
VII R = D-Xylosyl-D-glukosyl-D-glukosyl-D-galaktosyl : Demissin



VIII R = H . . . . . : Solasodin
IX R = L-Rhamnosyl-D-glukosyl-D-galaktosyl . . . : Solasonin
X R = L-Rhamnosyl-L-rhamnosyl-D-glukosyl . . . : Solamargin



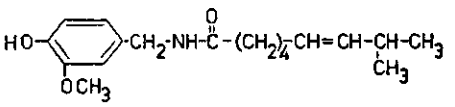
XI R = H . . . . . : Tomatidin
XII R = D-Xylosyl-D-glukosyl-D-glukosyl-D-galaktosyl : Tomatin
XIII R = D-Xylosyl-D-xylosyl-D-glukosyl . . . . : Polyadenin

Dieses *polyadenium*-Triosid, für das wir die Bezeichnung Polyadenin (XIII) vorschlagen, ist bisher in der Natur noch nicht aufgefunden worden. Es ist aus Tomatidin (XI) und den Kohlenhydraten D-Glukose (1 Mol.) und D-Xylose (2 Mol.) zusammengesetzt.

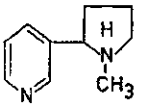
Ein ähnliches xylosehaltiges Alkaloidpaar isolierten wir aus der Wildkartoffelserie der *Acaulia*. Neben Solacaulin (IV) (SCHREIBER, 1954 b; SCHREIBER, 1956) kommt hier in geringen Mengen, vor allem in den Wildkartoffelarten *Solanum*

schreiteri und *Solanum punae*, ein zusätzlich D-Galaktose enthaltendes Tetraosid (V), dem ebenfalls eine Larvenwirksamkeit zugesprochen werden muss.

Während es nach unseren Mühlhäuser Untersuchungen gesichert erscheint, dass für die Resistenz der *Capsicum*-Arten das auch in den Blättern dieser Pflanze vorkommende Säureamid Capsaicin (XIV) und für die Widerstandsfähigkeit der Gattung *Nicotiana* das hier stark verbreitete und für die Kartoffelkäferlarven überaus toxische Alkaloid Nicotin (XV) verantwortlich zu machen sind, führten die an zahlreichen Instituten durchgeführten Arbeiten zur Auffindung und Identifizierung der resistenzbedingenden Stoffe der Wildkartoffelserie der *Commersoniana/Glabrescentia* bisher nur zu Teilergebnissen.



XIV Capsaicin



XV Nicotin

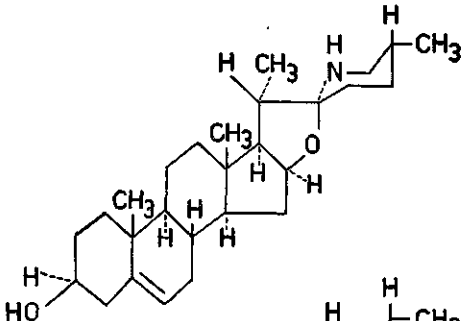
Von uns konnten aus einem homozygot larven- und käferresistenten *Solanum parodii* zwei vermutlich unterschiedliche resistenzbedingende Stoffe angereichert werden (durch Diäthyläther- bzw. Methanol-Extraktion der getrockneten Blätter). Ob einer dieser Resistenzfaktoren mit den von KUHN und LÖW (1957) kürzlich in *Solanum chacoense* aufgefundenen „Leptinen" identisch ist, konnte noch nicht geklärt werden. Die Leptine sind sehr gut wasserlösliche, durch Ammoniak nicht fällbare, zwitterionähnliche Alkaloidglykoside, die nach Abspaltung einer sauren Komponente in sogenannte „Leptinine" übergehen. An dem Aufbau dieser Glykoside sollen neben dem Aglykon „Leptinidin" (vermutlich ein Dihydroxyalkamin) D-Galaktose, D-Glukose und L-Rhamnose beteiligt sein.

Ein durch Ammoniak ebenfalls kaum fällbares Alkaloidglykosidgemisch konnten wir aus einer kleinblättrigen Herkunft von *Solanum dulcamara* isolieren. Papierchromatographisch wurden in dem Gemisch 3 Alkaloide nachgewiesen, die wir mit α-, β- und γ- Soladulcin bezeichnen (SCHREIBER, 1957 c). Das Aglykon dieser Glykoside ist identisch mit 5α-Solasodanal-(3β) (Dihydro-solasodin, XVII), das mit Tomatidin (5 α-Tomatidanol-(3β), XVIII) stereoisomer und aus Solasodin ($\triangle^5$-Solasodenol-(3β), XVI) durch katalytische Hydrierung darstellbar ist (SCHREIBER, 1957 a; SCHREIBER, 1957 c; BRIGGS, NEWBOLD und STACE, 1942; BRIGGS, HARVEY, LOCKER, MCGILLIVRAY und SEELYE, 1950; ROCHELMEYER, STÜTZEL und CHEN, 1944). An dem Aufbau der Kohlenhydratkomponenten der Soladulcine sind neben D-Galaktose und D-Glukose sowohl D-Xylose als auch L-Rhamnose beteiligt. Das Soladulcin-Gemisch besitzt wie die Leptine (KUHN und LÖW, 1957) eine hohe Larvenwirksamkeit (BUHR, TOBALL und SCHREIBER, 1957).

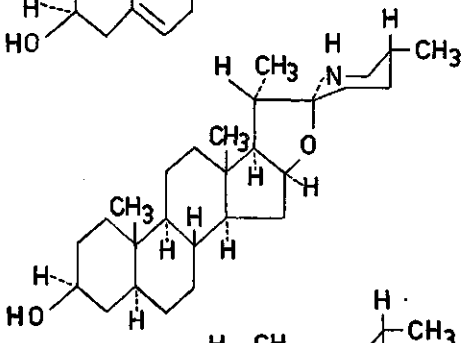
Diese Übersicht zeigt, dass zahlreiche natürliche Inhaltsstoffe der Solanaceen, vor allem verschiedene *Solanum*-Alkaloide, eine Widerstandsfähigkeit gegenüber dem Kartoffelkäfer bedingen können. Bemerkenswert ist allerdings, dass chemisch so verwandte Alkaloide wie Demissin, Tomatin, das *Acaulia*-Tetraosid, die Leptine und die Soladulcine auf der einen Seite und zum Beispiel α-Solanin, α-Chaconin,

Solasonin und Solamargin auf der anderen Seite eine so unterschiedliche Wirkung auf den Kartoffelkäfer ausüben.

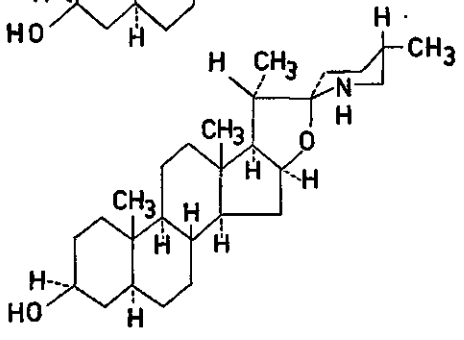
Den oben herausgestellten, chemisch-konstitutiven Merkmalen der wirksamen Alkaloide — Tetrasaccharidkomponente, Xyloseanteil und gesättigtes Aglykon — kann unseres Erachtens jedoch nur eine formelle Bedeutung beigemessen werden, denn die besondere chemische Konstitution der wirksamen Verbindungen steht sicherlich nicht unmittelbar mit der Resistenzausbildung in Verbindung. Wir sind vielmehr der Meinung, daß biophysikalische Vorgänge, wie sie bereits von DETHIER (1956) und anderen Forschern zur Deutung von Geruchs- und Geschmackserscheinungen bei Insekten herangezogen wurden, auch für die spezifische Wirksamkeit der *Solanum*-Alkaloide und somit für die Ausbildung der Kartoffelkäferresistenz verantwortlich zu machen sind.



XVI Solasodin
Δ^5-Solasodenol-(3β)



XVII Soladulcidin
5α-Solasodanal-(3β)



XVIII Tomatidin
5α-Tomatidanol-(3β)

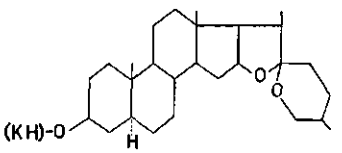
Durch die chemisch-konstitutiven Besonderheiten der wirksamen Alkaloide wird die „Polarität" der Moleküle erhöht, und zwar sowohl durch die Tetrasaccharidkomponente im Vergleich zum Trisaccharid und durch den Xyloseanteil im Vergleich zum Rhamnoseanteil, als auch durch das gesättigte Aglykon im Vergleich zum ungesättigten. Wir verstehen hier unter „Polarität" den intramolekularen

Gegensatz zwischen hydrophilen und hydrophoben Molekülteilen. Je größer dieser Gegensatz ist, desto stärker tritt die Kartoffelkäferwirksamkeit dieser Alkaloidglykoside in Erscheinung.

Diese Überlegungen stehen mit den Ergebnissen der Untersuchung der Oberflächenaktivitäten der Alkaloide im Einklang. Eine erhöhte Oberflächenaktivität macht sich besonders in der vergrößerten Schaumkraft, Benetzungs- und Emulgierfähigkeit der wässrigen Lösungen bemerkbar, und sie ist bei den Tetraosiden aufgrund der erhöhten Polarität ihrer Moleküle größer als bei den larvenunwirksamen Triosiden.

Derartige Oberflächen- bzw. Grenzflächenerscheinungen, die auch für die Höhe der Oberflächen- bzw. Grenzflächenpotentiale von Bedeutung sind (vgl. KIMOTO, 1953 und 1954), könnten darüber hinaus natürlich mannigfache Permeabilitäts- und weitere zellphysiologische Vorgänge beeinflussen. Ob hierdurch nun eine allgemeine Beeinflussung des „chemischen Sinnes" des Insektes möglich ist, und zwar insbesondere in Richtung einer geschmacksbedingten Abschreckung, bedarf einer weiteren experimentellen Nachprüfung.

Bekanntlich sind auch Steroidsaponine (XIX), die mit den *Solanum*-Alkaloidglykosiden chemisch und biogenetisch nahe verwandt sind, oberflächenaktiv. Durchgeführte Testungen bestätigten unsere Vermutung (SCHREIBER, 1954 a), dass auch Saponine — genauso wie zum Beispiel Demissin und Tomatin — eine zum Teil beachtliche futtervergällende Wirkung auf Kartoffelkäferlarven ausüben können (BUHR, TOBALL und SCHREIBER, 1957).



XIX Steroidsaponin

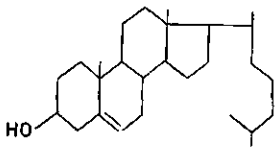
Von verschiedenen Seiten wurde gelegentlich beobachtet, dass bei Kartoffelkäfern, die mit larvenresistentem Pflanzenmaterial, also zum Beispiel mit *demissum*- oder *polyadenium*-Blättern ernährt wurden, Entwicklungs- bzw. Fertilitätsstörungen auftraten. Wir möchten diese Erscheinungen auf eine Beeinflussung des Steroidstoffwechsels der Tiere oder auf eine Beeinträchtigung der Resorption der für das Insekt lebenswichtigen Phytosterine durch die wirksamen Alkaloidtetraoside zurückführen.

Die *Solanum*-Alkaloidglykoside sind — wie die Saponine — hämolytisch aktiv, besitzen also eine Affinität zum Blutcholesterin (FISCHER, 1927; FISCHER und THIELE, 1929; KÖNIG und STAFFE, 1953; VELDSMAN und LOUW 1949; FONTAINE, SCHAFFER, DOUKAS, SCOTT, MA, TURKOT, DEEDS, WILSON und DOOLITTLE, 1955). Darüber hinaus wurde beobachtet (WINDAUS, 1909; SANDER, 1956; SCHULZ und SANDER, 1957), dass diese Alkaloide — und wiederum in besonderem Maße die käferwirksamen — wie zahlreiche Saponine auch in vitro aus alkoholischer Lösung mit Cholesterin schwer lösliche Fällungen geben. Es kommt zur Bildung von Molekülverbindungen (Molverhältnis 1 : 1), die man zum Beispiel als Cholesterin-Tomatid, -Demissid usw. bezeichnen könnte. Bemerkenswert ist,

dass nach eigenen Untersuchungen aus schwach saurem Milieu nur die käferwirksamen Tetraoside, nicht aber die Trioside mit Cholesterin eine Fällung geben.

Cholesterin (XX) gehört zur Gruppe der Zoösterine, jedoch werden auch die in Pflanzen vorkommenden Phytosterine, die wie das Cholesterin eine 3β-ständige Hydroxylgruppe besitzen, zum Beispiel die Sitosterine, Stigmasterin usw., durch Saponine und Alkaloidglykoside gefällt.

Aus der Literatur ist bekannt (vgl. SEDEE, 1956), dass Insekten für ihre Entwicklung Steroide benötigen. Während sie im allgemeinen in der Lage sind, neutrale Fette aus andersartigen Nährstoffen zu bilden, kommt ihnen diese Fähigkeit hinsichtlicht der Steroide, vor allem der Sterine, nicht oder in nur untergeordnetem Maße zu. Das Insekt ist darauf angewiesen, seinen Steroidbedarf aus der Nahrung zu decken.



XX Cholesterin

Von zahlreichen Arbeitskreisen (BERGMANN und LEVINSON, 1954; BLOCH, LANGDON, CLARK und FRAENKEL, 1956; BRUST und FRAENKEL, 1955; LEVINSON und SILVERMAN, 1954; NOLAND 1954 a und b; SILVERMAN und LEVINSON, 1954) wurden in den letzten Jahren Untersuchungen durchgeführt, um die Beziehungen zwischen der chemischen Konstitution dieser Steroide und ihrer wachstumsregulierenden Fähigkeit bei Insekten zu klären. Diese Arbeiten führten bei einigen Insektengruppen zu dem Ergebnis, dass insbesondere ungesättigte Steroide, zum Beispiel $\triangle^5$-Steroide, einen positiven Wachstumseffekt besitzen. Fehlen diese Steroide, so tritt eine Entwicklungsverzögerung auf. Gesättigte Steroide sind im allgemeinen nicht in der Lage, diese Entwicklungsbeeinträchtigung aufzuheben, im Gegenteil, sie bewirken zusätzlich eine wesentliche Wachstumshemmung. Das Insekt ist vermutlich nicht imstande, gesättigte Steroid-Ringsysteme zu dehydrieren.

Auch die *Solanum*-Steroidalkaloide Demissidin (VI) und Tomatidin (XI) besitzen ein derartiges gesättigtes Ringsystem. Es wäre somit denkbar, dass die ringgesättigten Steroidalkaloidtetraoside vom Typ des Demissin und Tomatin neben der oben erwähnten „Blockierung" der Phytosterine zusätzlich eine hemmende Wirkung auf den Kartoffelkäfer ausüben.

SUMMARY

A summary report is given of naturally occurring substances in plants which confer resistance to the Colorado potato beetle (*Leptinotarsa decemlineata* Say). Alkaloid-glycosides occurring in many *Solanum* and *Lycopersicon* varieties are discussed in some detail, and results are reported of some recent investigations of the alkaloids in *Solanum dulcamara, S. nigrum, S. polyadenium* and *Acaulia*. An attempt is made to explain the mechanism by which these substances affect insects. The harmful effects of alkaloid-tetraosides of *Solanum* on the taste of fodder are attributed to biophysical processes, particularly to surface and interface phenomena. In addition it is assumed that the alkaloids in question induce a blocking of the steroide metabolism and affect the resorption of the phytosterines that are indispensable for the insects, thus explaining frequently observed disturbances in development and fertility.

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DISKUSSION

M. TORKA, J. DE WILDE, H. J. TOXOPEUS, H. J. DEN HERTOG, K. SCHREIBER.

Von WALL und Mitarbeitern[1]) wurden kürzlich aus *Solanum tuberosum* β-Sitosterin und Stigmasterin isoliert, und es ist anzunehmen, daß auch in weiteren knollentragenden *Solanum*-Arten diese Phytosterine bevorzugt vorkommen. Auch β-Sitosterin und Stigmasterin werden — wie Cholesterin — in vitro durch die Alkaloidglykoside Demissin und Tomatin gefällt. Eine „Blockierung" dieser Sterine für den Kartoffelkäfer durch das im Überschuss vorhandene Alkaloid tritt jedoch voraussichtlich erst bei der Zerstörung der pflanzlichen Zelle, zum Beispiel während des Fraßaktes, ein. Die Hauptwirkung der resistenzbedingenden Glykoalkaloide auf das Insekt wird aber in der biophysikalisch zu deutenden Beeinflussung der „Geschmacksvorgänge" zu sehen sein.

Es wird darauf hingewiesen, dass in speziellen Fällen die biologische Wirksamkeit einer Substanz durch Glykosidierung erhöht werden kann. So konnte auch von THORSTEINSON festgestellt werden, dass Sinigrin auf *Plutella maculipennis* stärker wirksam ist als Allylisothiocyanat.

[1]) SCHWARTZ, J. J. u. M. E. WALL (1955), Steroids and steroidal sapogenins. XXIX. Isolation of the sterols of the white potato. *J. Amer. chem. Soc.* 77 : 5442—5443.

Untersuchungen über die Frage, ob im Verlauf der Vegetationsperiode der Pflanzen neben quantitativen Unterschieden auch qualitative Veränderungen im *Solanum*-Alkaloidgehalt auftreten, wurden bisher kaum durchgeführt. Die gelegentlich gemeinsam mit den Tetraosiden Demissin und Tomatin in geringen Mengen auffindbaren Trioside β-Demissin bzw. β-Tomatin scheinen nicht zu einem bestimmten Vegetationszeitpunkt bevorzugt aufzutreten.

Das Vorkommen resistenzbedingender Tetraoside kann papierchromatographisch leicht erkannt werden. Diese Methode, die unter standardisierten Bedingungen auch eine gewisse quantitative Auswertung erlaubt, kann in der züchterischen Praxis ohne wesentliche Schwierigkeiten auch als Serienanalyse angewendet werden.

Ent. exp. & appl. 1 (1958): 38—40. *North-Holland Publishing Co., Amsterdam*

DIETETIC REQUIREMENTS AND INTERMEDIARY PROTEIN METABOLISM OF AN INSECT (*CALLIPHORA ERYTHROCEPHALA* MEIG.)

BY

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SUMMARY

Calliphora erythrocephala commonly known as the Bluebottle belongs to the Diptera. The larvae of this insect feed on food which is heavily infected with bacteria. Unlike most other terrestrial insects which excrete uric acid, the larvae of *C. erythrocephala* excrete ammonia, the most toxic end product of nitrogen metabolism. In this direct excretion of ammonia the larvae therefore behave like many aquatic animals. Under natural conditions the larvae grow very rapidly. After a growth period of six or seven days they become mature, stop feeding, migrate to a dry place and then pupate.

Although the larvae of *C. erythrocephala* usually live in an environment heavily contaminated with bacteria, it is possible to rear the larvae from the egg under aseptic conditions. When reared on adequate diets the aseptic larvae grow as well as those under natural conditions and metamorphose into normal adult flies.

As in its mode of feeding and living the larvae of the Bluebottle are extreme specialists, it was to be expected that these specialisations may influence its dietetic requirements and intermediary metabolism. In how far these expectations came true was studied in a series of experiments in which the larvae were reared under aseptic conditions so that the intestinal bacteria could not interfere with the results of the feeding experiments and those of the study of the intermediary metabolism.

DIETETIC REQUIREMENTS. After repeated trials it was found that the larvae could be reared from the egg aseptically on a medium consisting of vitamin-free casein, cholesterol, cystine, B-vitamins and water. Larvae reared on this medium pupated and metamorphosed into normal adults. Any addition of carbohydrates or fats to this medium did not improve their growth. By omitting in turn one of the added B-vitamins, it was possible to determine which of these vitamins are necessary for good growth of the larvae. Among the B-vitamins studied the following are required for good growth and development of the larva of *C. erythrocephala*: thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid, choline and biotin. On the other hand the significance of inositol or vitamin B_{12} in the nutrition of the larvae could not be demonstrated. Hereupon a series of experiments was carried out to determine the quantitative B-vitamin

requirements. It was shown that the larvae require for their optimal growth approximately the following quantities of B-vitamins per gram of medium: 3 μ g. of thiamin, 3 μg. of riboflavin, 35 μg. of nicotinic acid, 4 μg. of pyridoxine, 12 μg. of pantothenic acid, 100 μg. of choline, 0.4 μg. of folic acid and 0.04 μg. of biotin.

With the aid of media containing either an acid casein hydrolysate or a mixture of pure amino acids as the nitrogen source, the qualitative amino acid requirements were studied. The following ten amino acids were found to be essential for growth of the larva of *C. erythrocephala*: arginine, histidine, lysine, phenylalanine, tryptophane, methionine, leucine, isoleucine, valine and threonine. Dispensable amino acids are: alanine, glutamic acid, aspartic acid, proline, glycine, serine, tyrosine, and hydroxyproline. Furthermore it was found that D-serine is extremely toxic to the larvae of *C. erythrocephala*. The natural form of this amino acid did not exert any toxic effect.

In addition to the compounds mentioned above the larva of *C. erythrocephala* requires cholesterol and minerals for its growth; nucleic acid has a growth promoting action.

The results of these feeding experiments show that the nutritional requirements of the larvae of the Bluebottle are not very different from those of other insects. Like other insects the larvae require only the B-vitamins, whereas vitamin A, C, D, and E are of no importance. The amino acid requirements of the larvae have also been found to be much the same as for other insects.

INTERMEDIARY PROTEIN METABOLISM. The intermediary protein metabolism of the larvae was studied with the aid of the stable isotope of nitrogen (N^{15}). The larvae were reared from the egg on food to which a N^{15} labelled compound was added. A medium consisting of casein, cholesterol, cystine, B-vitamins, and water served as food. After the larvae were grown up, they were killed, hydrolysed and a number of their amino acids isolated. The isotope concentrations in these amino acids were analyzed with a mass spectrometer[1]).

Two experiments were carried out. In the first experiment the rôle of glutamic acid in the metabolism of the larvae was studied (N^{15} labelled glutamate was added to the food). The second experiment was carried out to investigate the utilization of dietary ammonia for amino acid synthesis in the larvae. In this experiment the larvae were reared on a medium to which N^{15} labelled ammonia was added as the nitrate.

The results of the experiment in which N^{15} labelled ammonia was fed, offer definite proof that the larvae of *C. erythrocephala* like the rat are able to utilize dietary ammonia for amino acid synthesis.

From the results of the isotope studies it can be concluded that glutamic acid, alanine, and aspartic acid play a central part in the protein metabolism of the larvae. An intensive breakdown and formation of these amino acids takes place during larval life. The breakdown of these amino acids takes place via an intensive deamination. By means of the resulting α-keto acids the larva is able to

[1]) The N^{15} analyses were carried out by Dr. D. H. W. den Boer, at the laboratory of Organic Chemistry, University of Utrecht.

build up a considerable reserve of fat for use during the pupal stage. Among the isolated essential amino acids (phenylalanine, lysine, histidine, arginine, leucine and valine), only leucine and valine are involved in the reversible intermediary N transfer reactions. This finding shows that the essential nature of these amino acids is due to the fact that this insect is unable to synthesize the carbon chains of these amino acids. Furthermore it was found that the larva of *C. erythrocephala* has more inert amino acids than the rat. In the rat only two amino acids, lysine and threonine, are not involved in the continuous reversible processes of nitrogen shift, but in the larva of *C. erythrocephala* at least five amino acids do not exchange their amino groups with other amino acids. These amino acids are proline, phenylalanine, histidine, arginine and lysine (threonine was not isolated). The finding that proline, which is a non-essential amino acid, is not involved in these processes is especially interesting. In the metabolism of the rat it has been demonstrated that proline, arginine, glutamic acid and ornithine are mutually interconvertible. It is very doubtfull whether these amino acids are also interconvertible in the metabolism of the larvae. However, more experiments will have to be carried out to give a definite answer to this problem. Finally we may conclude from the results of the isotope studies that phenylalanine, known to participate in transamination reactions in mammals, does not participate in these processes in the larva of *C. erythrocephala.*

A detailed evaluation of these experiments is given in: PH. D. J. W. SEDEE: Dietetic requirements and intermediary protein metabolism of the larva of *Calliphora erythrocephala. Thesis Univ. Utrecht* 1956. Ed. Van Gorcum Co., Assen.

DISCUSSION

J. H. STEGWEE : Since it was mentioned, that the larvae of *Calliphora* cannot synthesize the carbon-chains of the amino acids valine and leucine, what is then the mechanism by which the rather considerable amount of N15 is incorporated ?

ANSWER : The N15 found in the isolated leucine and valine has been incorporated by means of transamination processes between the α-keto analogues of the amino acids and glutamic acid or (and) glutamine.

H. J. DE FLUITER : Can we conclude from your experiments as well from the experiments made by HOUSE and FRIEND that all insect larvae need a certain number of the same essential nutrients, but that the amounts of these nutrients acquired are different in the different species ?

ANSWER : Yes, I believe that all insects require for their growth and development B-vitamins (thiamin, riboflavine, nicotinic acid, pantothenic acid, folic acid, biotin, pyridoxine and choline, and the following amino acids: lysine, histidine, arginine, methionine, isoleucine, leucine, valine, threonine, tryptophane and phenylalanine). In some cases it was found that certain insects do not require certain B-vitamins but in those experiments the synthesizing action of the micro-organisms in the gut or food will be responsible for these results.

On the basis of our present knowledge it seems probable that the quantitative requirements for the B-vitamins do not differ much. About the quantitative amino acids requirements in insects not much can be said since only for the honeybee (DE GROOT) this subject has been studied thoroughly.

J. DE WILDE : Did you try to replace cholesterol by other sterols ?

ANSWER : No, only cholesterol was studied. Since HOBSON studied the nutritive value of different kinds of sterols for the sheep blow fly, no further experiments were made in this field.

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DIE ROLLE DER SPEICHELSEKRETE IM WECHSELVERHÄLTNIS ZWISCHEN TIER UND NAHRUNGSPFLANZE BEI HOMOPTEREN UND HETEROPTEREN

VON

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Im Speichel der Rhynchoten gibt es verschiedene Verdauungsenzyme, die eine weitgehende Anpassung hinsichtlich der Art der Nahrung aufweisen und bei der Verdauung wichtig sind. Zugleich gibt es im Rhynchotenspeichel auch Pflanzenwuchs hemmende Stoffe, Auxine und Viren, die alle Krankheitszustände bei den Nahrungspflanzen verursachen welche ihrerseits die Lebensbedingungen der Schädlinge fördern.

Die zu Stechwerkzeugen umgebildeten Mundteile bilden die ausgeprägteste Baueigenschaft, welche die zu den Homopteren und Heteropteren gehörenden Insekten miteinander verbindet. Man kann ohne zu übertreiben sagen, dass auch in der Biologie dieser Tiere der bezeichnendste gemeinsame Zug in der Art und Weise liegt, in welcher der Saugrüssel und die anschliessenden grossen Speicheldrüsen im Dienst der Nahrungsaufnahme gebraucht werden. Diese vollzieht sich bei sämtlichen Homopteren und Heteropteren prinzipiell auf die Weise, dass die Tiere ihr Stechborstenbündel in das Nahrungsobjekt versenken, enzymhaltigen Speichel in dasselbe absondern und dann die dem Speichel beigemengte oder von ihm aufgelöste Nahrung durch die feinen Gänge der Maxillen in den Verdauungskanal aufsaugen. Kennzeichnend ist hierbei der reichliche Aufwand von Speichel, eine Eigenschaft, die sowohl die räuberischen Formen wie auch die Phytophagen auszeichnet. DAY und IRZYKIEVICZ (1953) haben in Australien mit Hilfe von radioaktivem Phosphor festgestellt, dass die Kohlblattlaus, *Brevicoryne brassicae* (L.), bei der Nahrungsaufnahme in die Nährpflanze eine Speichelmenge absondert, die sich auf etwa 1% der aufgenommenen Nahrung beläuft. Diesem so reichlich in das Nahrungsobjekt abgesonderten Speichel kommt eine Schlüsselstellung in den Beziehungen zwischen den Rhynchoten und ihren Nahrungspflanzen zu. Er vermittelt nämlich erstens die Beförderung der aus der Pflanze aufgenommenen Nahrung in den Verdauungskanal. Anderseits wiederum werden die durch die Nahrungsaufnahme dieser Insekten bei den Pflanzen hervorgerufenen Krankheiten hauptsächlich durch die im Speichel enthaltenen phytotoxischen Stoffe oder die mit ihm in die Pflanze geratenden Viren verursacht.

Obwohl diesen Einwirkungen des Speichels der Homopteren und Heteropteren überall in der Welt jährlich recht beträchtliche Mengen von Kulturpflanzen zum Opfer fallen, sind unsere Kenntnisse über dessen Zusammensetzung noch sehr mangelhaft. Am eingehendsten sind in dieser Hinsicht die hydrolytischen Enzyme

studiert worden. Von diesen hat man bei den phytophagen Arten Proteasen, Amylasen, Saccharase, Maltase, Pektinase und Lipasen festgestellt (siehe BAPTIST 1942; GOODCHILD 1952; NUORTEVA 1954, 1956 a und b; SAXENA 1955; ADAMS und MCALLAN 1956; u.a.). Diese Enzyme sind jedoch nicht alle in den Speichelsekreten ein und derselben Art enthalten, sondern kommen gewöhnlich nur zu zweit oder dritt vor. Die enzymatische Zusammensetzung des Speichels variiert von Art zu Art; und wenigstens im Auftreten der Proteasen haben sich auch jahreszeitliche oder entwicklungsbedingte Schwankungen ergeben. So wurde gefunden, dass in den Speicheldrüsen der auf Weizen lebenden Wanze *Eurygaster integriceps* Put. in derjenigen Jahreszeit keine Proteasen vorkommen, in der die Tiere ihre Nahrung aus grünen Teilen der Pflanze aufnehmen. Sobald aber die Wanzen zur Nahrungsaufnahme aus dem heranreifenden Korn übergehen, erscheinen in den Speicheldrüsen solche Enzyme (KRETOVICH, BUNDEL und PSHENOVA 1943; KRETOVICH 1944). Bei der Wanze *Lygus rugulipennis* Popp. hinwieder gibt es Proteasen in den Speicheldrüsen der Larven, aber nicht in denen der erwachsenen Tiere (NUORTEVA 1954 b). In diesem Falle dürfte man annehmen können, dass das Vorkommen der Proteasen mit dem grossen Proteinbedarf der heranwachsenden Larve verknüpft ist, dessen Deckung durch ihre Gegenwart erleichtert wird. Bei der Wanze *Miris dolabratus* L. herrscht indessen das entgegengesetzte Verhältnis, indem bei dieser Art die Proteasen den jüngsten, 1.3—2.5 mm langen Larven fehlen und erst auftreten, wenn die Larven eine Länge von 2.6—3.3 mm erreicht haben und allmählich im Begriff sind, sich zu Imagines zu entwickeln. Interessanterweise finden sich jedoch Proteasen nur in den Speicheldrüsen der Weibchen, nicht aber in denen der Männchen (NUORTEVA 1956 c). Letzteres ist vielleicht ein Hinweis darauf, dass die Rolle der Speicheldrüsenproteasen bei *Miris dolabratus* mit der Eiproduktion verknüpft ist. Hinreichend grosse Proteinmengen stellen ja eine notwendige Voraussetzung zur Bildung entwicklungsfähiger Eier in den Ovarien des Weibchens dar. Die in die Nahrungspflanze abgesonderten Speichelproteasen erleichtern dabei zweifelsohne die Gewinnung von Proteinen.

Erwartungsgemäss zeigt die Enzymzusammensetzung des Speichels bei phytophagen Homopteren und Heteropteren eine gewisse Abhängigkeit von der Beschaffenheit der aus der Pflanze aufgenommenen Nahrung. Hinsichtlich der Tätigkeit der hydrolytischen Enzyme sind die ausschlaggebendsten Unterschiede in der Nahrungsbeschaffenheit davon abhängig, ob das Insekt seine Nahrung aus den Siebröhren der Pflanze oder aus dem Mesophyll der Blätter oder des Stengels aufsaugt. Im Mesophyll liegen die Nährstoffe ja meistens in Form von verhältnismässig grossmolekularen Verbindungen vor, während sie in den Siebröhren ziemlich weitgehend aufgespalten sind. Bei manchen mesophyllsaugenden Zikaden sind in den Speicheldrüsen sowohl Proteasen als Amylasen gefunden worden (SAXENA 1954; NUORTEVA 1954 a, 1956 a). Bei solchen Arten dagegen, die ihre Nahrung aus den Siebröhren saugen, fehlen diese Enzyme. Sie sind natürlich auch gar nicht notwendig, da ja die Pflanze durch die Mobilisierung der Nährstoffe vom Standpunkt der Insekten die vorläufige Verdauungsarbeit bereits geleistet hat.

Da nun im Auftreten der Speicheldrüsenenzyme der Rhynchoten eine deutliche Anpassung an die Art der benutzten Nahrung zu bestehen scheint und da

P. NUORTEVA : Speichelsekrete bei Homopteren und Heteropteren.

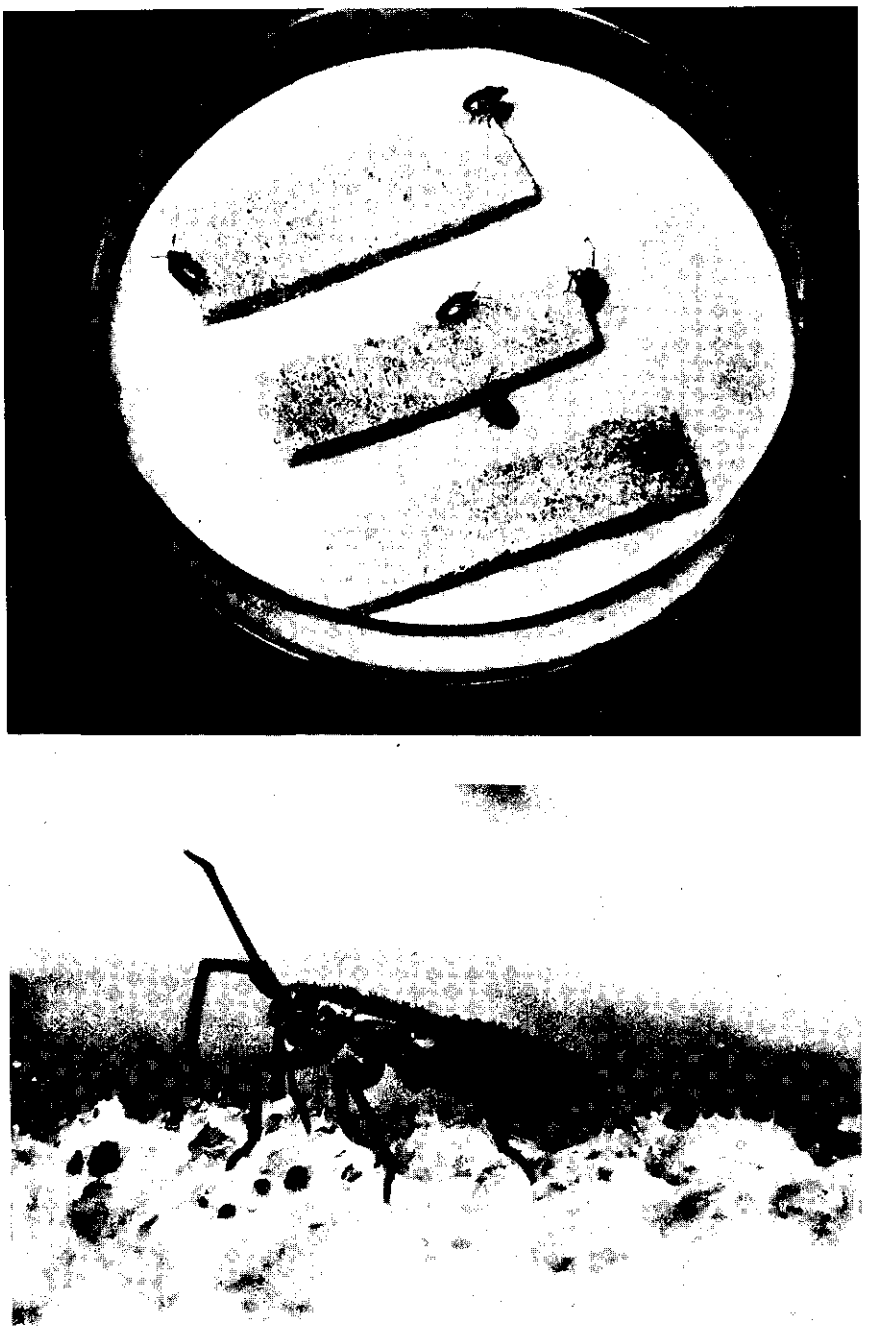

Abb. 1. Die benutzte Methode zur Fütterung von Heteropteren mit synthetischen Nährlösungen. — Man lässt die Nährlösung sich in eine poröse Zellulosemasse aufsaugen und bietet diese dann den Versuchstieren in eine Petri-Schale. — Oben *Aelia acuminata*-Individuen in einer Versuchsschale; unten eine Larve von *Miris dolabratus* beim Saugen an der Zellulosemassa. Aufn.: Teuvo Suominen.

die Enzymsekretion jahreszeitlichen Schwankungen unterworfen ist, war zu vermuten, dass Veränderungen in der Nahrungsbeschaffenheit entsprechende Veränderungen auch in der Absonderung der Enzyme hervorrufen würden. Einige vorläufig unveröffentlichte Ergebnisse meiner Untersuchungen scheinen jedoch zu erweisen, dass das nicht der Fall ist. So konnten bei im Freien gefangenen Wanzen, die allein mit Zuckerlösung gefüttert wurden, keine Veränderungen im Vorkommen der Speicheldrüsenproteasen beobachtet werden. — Diese fanden sich nicht in den Speichelsekreten von Arten, in denen sie von Natur aus fehlen. Andrerseits hörte die Ausscheidung von Proteasen auch bei solchen Arten nicht auf, in deren Speichel sie normalerweise enthalten sind. — Offenbar stellen die deutlich nahrungsbedingte Anpassung der Speichelsekrete und die jahreszeitliche Schwankung ihrer Sekretion phylogenetisch bedingte erbliche Eigenschaften dar, denen die Fähigkeit fehlt, sich bei ausnahmsweise veränderter Nahrung der neuen Lage anzupassen.

Wenn also die Speichelsekrete nicht imstande sind, sich an qualitative Abweichungen in der Nahrungsaufnahme anzupassen, so bedeutet dies jedoch nicht, dass den Bestandteilen der aufgenommenen Nahrung nicht ein Anteil an der Bildung und Beschaffenheit des Speichels zukommen könnte. In manchen Fällen können die Bestandteile der Nahrung als solche in die Speichelsekrete übergehen. So ist bei der Wanze *Plesiocoris rugicollis* Fall. festgestellt worden (NUORTEVA 1955), dass bei Verfütterung mit papain-haltiger Nahrung in den Speicheldrüsen alsbald eine Protease nachgewiesen werden kann, deren pH-Optimum auf der sauren Seite liegt, während bei dieser Art normalerweise nur eine Protease mit leicht alkalischem pH-Optimum zu finden ist. In diesem Falle ist das Papain ganz offenbar ohne Einbusse seiner Wirkung aus der Nahrung in die Speicheldrüsen übergetreten. Es mag manchem sonderbar erscheinen, dass ein Enzymmolekül ungespalten den gesamten Weg aus der Nahrung durch die Darmwandung in die Hämolymphe und dann noch durch die Wand der Speicheldrüse hindurch in den Speichel wandert. Man hat sich jedoch hierbei daran zu erinnern, dass auch so grosse „Proteine", wie das Tabakmosaikvirus, mit seinem Molekulargewicht von 10—20 Millionen, denselben Weg durchläuft. Gleicherweise hat WIGGLESWORTH (1943) gefunden, dass die Hämoglobinmoleküle unversehrt in die Speicheldrüsen einer blutsaugenden Wanzenart gelangen. Weiter verdient erwähnt zu werden, dass eine mit radioaktiver Kohle, Phosphor oder Polonium markierte Nahrung nach Versuchen an Wanzen und Blattläusen aus der Nährpflanze sehr rasch in die Speichelsekrete hinüberwandert (HAMILTON 1935; FLEMION u. Mitarb. 1951, 1952; DAY und IRCZYKIEVICZ 1953; NUORTEVA und REINIUS 1953). — In diesem Zusammenhang hat man auch zu bedenken, dass sich die Insekten von den meisten anderen Tieren und selbst von den Crustaceen dadurch unterscheiden, dass ihnen ein der Leber entsprechendes Organ fehlt, in welchem sich der intermediäre Stoffwechsel abspielen könnte. Angesichts dessen erscheint das Bestreben der Insekten, die aus der Nahrung erhältlichen Wirkstoffe als solche zu verwerten, in gewissem Sinne sogar natürlich.

Die durch den Stich der Rhynchoten bei der Wirtspflanze hervorgerufenen Krankheitssymptome äussern sich meistens in Form von verschiedenen Wachstumsstörungen, wie Gallenbildungen, Verkrümmungen, vorzeitigem Abfallen der Früchte und gesteigerter Wurzelbildung. Diese Symptome erinnern sehr an die-

jenigen, denen man in Verbindung mit verschiedenen wachstumshormonalen Störungen begegnet; und daraus ist gefolgert worden, dass der Speichel der Rhynchoten auch Auxine enthielte. Man hat zur Stütze dieser Auffassung auf mehrere Tatsachen hinweisen können. So hat der Amerikaner MARTIN (1930) durch Einspritzung eines aus homogenisierten Zikaden bereiteten Extrakts an den Versuchspflanzen Stengelgallen hervorgerufen. LINK u. Mitarb. (1940) wiederum haben beobachtet, dass aus gewissen Blattläusen bereitete Ätherauszüge Haferkoleoptilen zu zusätzlichem Wachstum stimulierten. Dem Franzosen NYSTERAKIS (1946, 1947, 1948 a—c) ist es schliesslich gelungen, aus Blattläusen der Gattungen *Phylloxera, Pemphigus* und *Anuraphis* β-Indolylessigsäure (Heteroauxin) zu isolieren. Ebenso hat er zeigen können, dass die durch diese Blattläuse bei den Pflanzen hervorgerufenen Krankheitssymptome jenen entsprechen, die sich mit Hilfe von Heteroauxin hervorbringen lassen. Besonders interessant ist die Feststellung von NYSTERAKIS vom Jahr 1946, wonach blattlausresistente Weinrebensorten amerikanischen Ursprungs gegen die Wirkung der Auxine nicht so empfindlich sind wie blattlausanfällige europäische Sorten. Nach seiner Auffassung gründet sich die Blattlausresistenz der amerikanischen Weinreben gerade darauf, dass ihre Gewebe auf das Heteroauxin, das ihnen durch die Blattlausstiche zugeführt wird, nicht mit Gallenbildung reagieren.

Alle diese Untersuchungen gründen sich auf Auxinbestimmungen aus homogenisierten Zikaden oder Blattläusen und ergeben darum keinen bindenden Beweis dafür, dass die Auxine auch in den Speicheldrüsen und ihren Sekreten enthalten wären. Im vergangenen Sommer untersuchte ich darum (NUORTEVA 1956 b) die Wirkung von Speicheldrüsen-Extrakten von sechs Wanzen-Arten und einer Blattlaus-Art auf das Wachstum von Erbsen. Auxine wurden in keinem einzigen Falle festgestellt. Dies ist natürlich immer noch kein absoluter Beweis für das allgemeine Fehlen von Auxinen in den Speichelsekreten der Rhynchoten, wohl aber zeigt das Ergebnis, dass sie jedenfalls nicht häufig vorkommen. Dagegen stellte es sich bei diesen Untersuchungen heraus, dass der Speichel dreier Arten, nämlich von *Miris dolabratus* L., *Dolycoris baccarum* L. und *Cinara piceae* (Panz.) Auxin-Inhibitoren oder jedenfalls Stoffe enthielt, die das Wachstum der Pflanzen hemmten. Ein Vorhandensein solcher Inhibitoren wurde auch in Amerika von FISCHER u. Mitarb. (1946) in der Wanze *Lygus oblineatus* Say entdeckt. Es gelang diesen Forschern auch, den durch die Wanze an der Nährpflanze hervorgerufenen Krankheitszustand durch Hormonbehandlung zu heilen.

Es könnte bisher noch nie bewiesen werden, dass Insekten oder andere Tiere imstande wären, in ihrem Körper Auxine zu synthetisieren. Darum erscheint es ganz natürlich anzunehmen, dass die im Rhynchotenspeichel möglicherweise vorhandenen Auxine aus den Nahrungspflanzen stammen. Bei meinen ersten Versuchen zur Klärung dieser Frage (NUORTEVA 1956 c) hat es sich gezeigt, dass bei Fütterung der Wanze *Stenodema calcaratum* Fall. mit heteroauxinhaltiger Zuckerlösung ihr Speichel bei Erbsen gesteigertes Wachstum hervorruft. Bei *Dolycoris baccarum* dagegen ist eine solche Wirkung nicht zu bemerken. Nun gehört aber diese Art zu denjenigen, in deren Speichelsekreten Auxin-Inhibitoren gefunden wurden (siehe oben), und darum ist ein solches Ergebnis bei ihr durchaus verständlich.

Die obige Beobachtung an *Stenodema calcaratum*, dass aus der synthetischen

Nährlösung Heteroauxin in die Speicheldrüsen hinüberwandert, dürfte die Annahme rechtfertigen, dass dies auch in der Natur geschieht, wenn in der Pflanze Auxine in genügender Menge vorhanden sind. Die Tatsache, dass in den Speicheldrüsen der im Freien zur Untersuchung gefangenen Exemplare keine Auxine gefunden wurden, mag als Hinweis darauf gelten, dass es solche Umstände nur selten gibt; — wahrscheinlich wohl nur in einer ganz bestimmten Entwicklungsphase der Pflanzen.

Oben ist in verschiedenen Zusammenhängen festgestellt worden, dass sowohl Papain wie Heteroauxin bei den Rhynchoten unbeeinträchtigt aus der Nahrung in die Speicheldrüsen wandern können. Dies bedeutet, dass wenigstens ein Teil der vom Tier in die Pflanze abgeschiedenen toxischen Substanzen als solche ursprünglich aus der Nahrungspflanze stammt. Die Insekten haben sie wahrscheinlich in ihren Speicheldrüsen nur in eine so hohe Konzentration gebracht, dass sie beim erneuten Eintritt in die Pflanze durch den Stich des Tieres bei dieser einen Krankheitszustand hervorrufen. Zumal was die Auxine betrifft, ist ihre Wirkungsweise bekanntlich völlig von ihrer Konzentration in den pflanzlichen Geweben abhängig. Für die Auffassung, dass die mit dem Speichel in der Pflanze abgeschiedene Toxine als solche von den Nahrungspflanzen herstammten, spricht auch die Feststellung von CARTER (1951) auf Hawaii, dass die Stärke der von der Schildlaus *Pseudococcus brevipes* Ckl. auf der Ananaspflanze hervorgerufenen Krankheitssymptome weitgehend davon abhängig ist, welcher Nährpflanze sich das Tier vor der Übersiedlung auf die Ananas bedient hat.

Die Rolle der in den Speichelsekreten der Rhynchoten enthaltenen phytotoxischen Stoffe und Viren ist als pflanzenpathologischer Faktor in recht vielen Untersuchungen behandelt worden. Weit weniger hingegen hat man sich ihrer Bedeutung vom Standpunkt dieser Insekten selbst zugewendet. Den Speicheldrüsenenzymen kommt unzweifelhaft ein nicht geringer Anteil an den Verdauungsprozessen zu. Dazu sind sie aber, gleichwie die übrigen pathogenen Bestandteile und Mikroorganismen des Speichels indirekt von Bedeutung. Vom Standpunkt der Rhynchoten bedeutet der in der Pflanze entstehende Krankheitszustand nämlich eine Veränderung ihrer Eignung als Nährpflanze. — Man hat nachweisen können, dass die Eignung der Pflanze als Nahrungsquelle für die Homopteren vor allem von der Menge der in den Siebröhren wandernden Nährstoffe — vor allem der stickstoffhaltigen — abhängt (vgl. z.B. LINDEMANN 1948; KENNEDY und BOOTH 1951; NUORTEVA 1952; FENNAH 1953; KENNEDY 1953). Die Nährstoffströmung wiederum ist in Verbindung mit der Mobilisation und Demobilisation der Reservenährstoffe am intensivsten, m.a. W. dann, wenn im physiologischen Zustand der Pflanze Veränderungen vor sich gehen. Dies ist zweifelsohne auch bei Krankheitszuständen der Pflanze der Fall. Dadurch, dass die Insekten durch ihre Speichelsekrete die Pflanze in einem krankhaften Zustand versetzen und so eine lebhaftere Saftströmung in den Siebröhren verursachen, fördern sie zugleich offenbar nicht wenig auch ihre eigenen Möglichkeiten zum Nahrungsbezug. Dies kann nicht ohne Bedeutung sein für das Gedeihen solcher Arten, die sich lange auf ein und demselben Pflanzenindividuum aufhalten oder die auf begrenzter Fläche in sehr dichten Populationen leben. Auch KENNEDY (1951) hat den günstigen Einfluss der Blattgallenbildung sowie der pflanzlichen Virosen auf das Gedeihen der Blattläuse aufgezeigt und ähnliche Beobach-

tungen sind auch an anderen Rhynchoten gemacht worden.

Auf Grund des oben Ausgeführten findet man, dass die Beziehungen der Rhynchoten zu ihren Nahrungspflanzen auf recht mannigfache Weise durch die Speichelsekrete dieser Tiere vermittelt werden. Erstens ist festzustellen, dass die in den Speichelsekreten enthaltenen hydrolytischen Enzyme bei der Nahrungsaufnahme und Verdauung der Rhynchoten eine wichtige Rolle spielen und eine weitgehende Anpassung hinsichtlich der Art der zu Gebote stehenden Nahrung aufweisen. Anderseits wieder verursachen die Enzyme, Auxine und Viren des Speichels Krankheitszustände der Nahrungspflanzen, welche ihrerseits wieder die Lebensvoraussetzungen der Schädlinge fördern. Weiter scheint es, als ob wenigstens ein Teil der im Rhynchotenspeichel enthaltenen phytotoxischen Substanzen als solche von den Pflanzen herrühre. — Man darf wohl sagen, dass die Zusammensetzung des Speichels bei den Rhynchoten einem Spiegel gleicht, aus dem man die wesentlichen Besonderheiten des nutritiven Verhältnisses der jeweils in Frage stehenden Art zu ihrer Nahrungspflanze ablesen kann. Vom Standpunkt der Erzeugung resistenter Kulturpflanzen hat man hierin einen Hinweis darauf zu erblicken, in welche Richtung die Veredlungsarbeit zu leiten wäre, um die Pflanzen als Nahrungsquelle für den Schädling weniger zusagend zu machen.

Da die mechanische Schädigung der Pflanze durch die Rhynchoten nahezu bedeutungslos ist, lässt sich eine hinreichende Restistenz gegen diese Insekten auch auf die Weise erzielen, dass man der Pflanze eine Widerstandskraft gegen die Toxine des Speichels anzüchtet, ohne dass die Pflanze an sich als Nahrungsobjekt untauglich würde. Diese Möglichkeit, bei Kulturpflanzen eine Toxinresistenz hervorzurufen, besteht namentlich im Hinblick auf die Rhynchoten. Im allgemeinen ergeben sich für die Resistenzzüchtung nur zwei Wege — entweder macht man die Pflanze als Nahrungsobjekt für den Schädling ungeeignet (durch Herabsetzen des Nährwertes, Erhöhen der Giftigkeit oder Herausbildung mechanischer Hindernisse für die Nahrungsaufnahme der Insekten) oder man unterbricht die Kette der Instinkthandlungen, die das Insekt zu seiner Nahrungspflanze hinführen.

SUMMARY

The above article is a short survey of the relation between the salivary secretions of the Hemiptera and their host plants.

In analyses of the composition secretions in the salivary glands of phytophagous Homoptera and Heteroptera, the most extensive work has been done on the digestive enzymes. Proteases, amylases, saccharase, maltase, pectinase and lipase have been detected, but most commonly only 2—3 of these occurs in any one species. An adaptation of the enzyme complement to the diet is evident and in this respect the feeding site used by the insect is especially decisive to the enzyme composition of the saliva. Proteases and amylases occur in the saliva of mesophyll feeders, but are absent in phloem feeders, for which they are useless because, from the insect's point of view, the food is already digested. The adaptation of the salivary enzymes to the nature of the food seems to be largely inherited, for a diet containing nothing but sucrose does not induce adaptive changes in the enzyme content of the salivary glands. By contrast, papain seems to be transferred from a synthetic food to the salivary glands.

The disease symptoms caused in plants by the salivary toxins of Homoptera and Heteroptera have many similarities to those caused by abnormal amounts of growth hormones. Indole-tri-acetic acid has been detected in extracts of crushed aphids and leafhoppers, although no growth stimulating hormones have been detected in salivary glands dissected from

several heteropterous bugs and one aphid. On the contrary, substances inhibiting plant growth exist in the salivary glands of many Heteroptera and in at least one aphid. In some experiments with a heteropterous bug, indole-tri-acetic was observed to have been transferred from a synthetic diet to the salivary glands. It seems possible that auxins, enzymes and other phytotoxic substances occurring in the salivary glands of insects may at last in some cases originate from the host plant and are not produced by the insect.

The salivary enzymes without doubt play an important role in the digestion of the insects secreting them. They are important also in the differentation of Homoptera and Heteroptera to different modes of life. The auxins or auxin inhibitors, as wel as all phytotoxic substances in the salivary glands of Homoptera and Heteroptera are significant for these animals by changing the physiological state of the host plant in such a direction that its suitability as a source of food increases.

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DISKUSSION

W. ROEPKE : Betreffs der im Vortrage zitierten Auffassungen von NYSTERAKIS sei bemerkt, dass gerade bei der amerikanischen Rebe durch den *Phylloxera*-Stich Blattgallen entstehen, während die Wurzeln nicht nennenswert geschädigt werden. Bei der europäischen Rebe entstehen keine Blattgallen, so dass man das Vorhandensein von Abwehrstoffen annehmen muss, die entweder die Läuse am Stechen und Saugen verhindern oder die Gallbildung unterdrücken.

ANTWORT : Leider sind mir die ziemlich komplizierten Verhältnisse zwischen den Phylloxeren und ihren Nährpflanzen nicht genügend bekannt, um hier eine Antwort geben zu können. Darum möchte ich nur auf die Argumente hinweisen, die NYSTERAKIS in seiner Publikation von 1946 gibt. Doch bin ich der Meinung, dass die Auffassung dieses Forschers theoretisch betrachtet sehr interessant ist — selbst wenn sie in diesem Falle unhaltbar sein sollte.

H. J. MÜLLER : Ich möchte in diesem Zusammenhang darauf hinweisen, dass nach BÖRNER auf blattresistenten Europäer-Reben keine Gallen, sondern Nekrosen entstehen die zum Absterben der Reblaus führen.

H. J. DE FLUITER : Der Vortragende hat als Beispiel für ein grosses Proteinmolekül, das in Insekten durch die Leibeshöhle nach den Speicheldrüsen transportiert wird, das Tabakmosaik genannt. Das ist m. E. nicht richtig, weil das Tabaksmosaik ein nonpersistentes Virus ist.

ANTWORT : Die Bemerkung ist ganz richtig. Alle von den Aphiden übertragbaren persistenten Viren wie auch die von Zikaden übertragbaren Viren dürften in dieser Hinsicht ein zutreffenderes Beispiel sein. Ich habe das Tabaksmosaikvirus darum genannt, weil es das grösste pflanzliche Virus ist, das nach unserem Wissen durch die Darmwand in die Hämolymphe eines Insekts wandern kann (siehe M. F. DAY & M. J. BENNETTS : A review of problems of specifity in arthropod vectors of plant and animal viruses. 172 pp., Canberra 1954, p. 14).

J. H. STEGWEE : Hat der Vortragende eine Vorstellung über die Herkunft der Hemmstoffe, die in den Speicheldrüsen gewisser Insekten gefunden wurden ?

ANTWORT: Es wäre verfrüht darüber etwas zu äussern. So viel kann man jedoch sagen, dass es sich nicht um Auxine in übergrossen Konzentrationen handelt.

J. H. STEGWEE: Haben Sie einmal Symptome beobachtet, die auf die Wirkung dieser Hemmstoffe zurückzuführen wären?

ANTWORT: Bisher habe ich die Hemmstoffe nur bei Untersuchungen im Laboratorium gefunden durch Beobachtungen über ihre Wirkungen auf das Wachstum von abgeschnittenen Stückchen etiolierter Erbsenhypocotyle. Darum ist er verfrüht, etwas darüber zu sagen, durch welche Symptome sich diese noch nicht näher bekannten Stoffe an lebenden Pflanzen äußern.

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PHYSIOLOGICAL CONDITION OF THE HOST-PLANT AND SUSCEPTIBILITY TO APHID ATTACK

BY

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Familiar types of physiological variation in aphid host-plants, such as in the pattern of growth or the water economy, have a great influence upon the growth, development and behaviour of aphids, for reasons that we are beginning to understand. They would merit fuller exploitation for the purposes of aphid control.

The profound effect of physiological as distinct from botanical variation in plants, on their susceptibility to aphids, is exhibited in model form by the distribution of *Cryptomyzus ribis* L. over the surface of one Red Currant leaf (*Ribes rubrum* L.). The aphids do not feed on the general leaf surface but are confined to the inside of the raised 'blister' or 'pocket' galls they produce on these leaves. For a long time it seemed almost superfluous to ask why they should be there, since the galling occurs where they feed. The only other suggestions made, referred to incidental, extrinsic factors such as the shade and shelter provided by the gall cavities. In this case as in many others, intrinsic plant-physiological factors were the last to be considered. But in fact they constitute the main reason for the aphids confining their feeding and reproductive activities to the interior of the galls (Figs. 1 and 2; see also KENNEDY, 1951a, 1951b).

In themselves such correlations between plant-physiological condition and aphid susceptibility do not take us far: the analysis is at too superficical a level. But as a point of departure for the agricultural entomologist, they seem to me to offer certain advantages. They provide a series of pointers for work at all the other levels which must concern him, with many possibilities of cross-checking. First, at the level of insect physiology; secondly, back from that to insect ecology, by reference to the known or discoverable patterns of physiological variation in plants in the field; and thirdly, from that in turn eventually to better integration of aphid control (whatever the means) into the rest of agricultural practice, since the physiological performance of the crop plants is the one thing which all agricultural scientists in common are concerned to understand and control.

HOST-SELECTION THEORY AND ITS PRACTICAL CONSEQUENCES

It must be admitted that such a programme would be dismissed as completely unrealistic in the light of current theories of insect host-preference in general. This subject being "the very heart of agricultural entomology" (LIPKE and FRAENKEL, 1956) and the theme of the present symposium, some reference to general theory is called for. The correlation between aphid susceptibility and such

J. S. KENNEDY : Host plant condition and aphid attack.

Fig. 1. Distribution of *Cryptomyzus ribis* L. over the abaxial surface of a leaf of *Ribes rubrum* L., with the blister galls everted. See legend to Fig. 2.

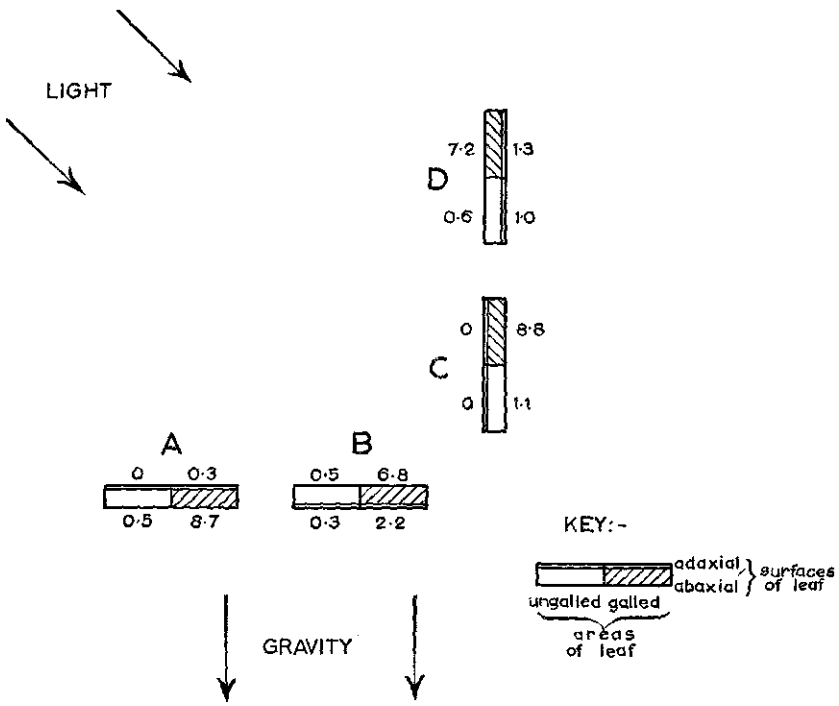

Fig. 2. Feeding preferences shown by *Cryptomyzus ribis* L. between different parts of the leaf surface of *Ribes rubrum* L., after eversion of the blister galls (Fig. 1). Before eversion the aphids were confined to the abaxial, interior surface of the galls, but were removed and released again at random on the leaves and allowed to re-settle before these counts were taken. Based on the distribution of 2079 individuals on 17 leaves with a total area of *c.* 1240 sq. cm. of which the galled regions covered *c.* 376 sq. cm. Eversion of the galls distorted the leaves, so that some parts of each were now abnormally disposed with reference to the light source and gravity (B, C and D), while others parts remained in the normal position (A). All areas of leaf possessing the same combination of intrinsic and extrinsic features were summed, together with the numbers of aphids feeding there, to give the mean densities which are shown in figures against each part of the diagram (aphids per sq. cm). Note the overwhelming preferences for intrinsic leaf features — galled and abaxial surfaces — almost regardless of exposure to light and gravity.

elementary universal physiological processes as leaf growth and senescence, led us some years ago to propose a *dual* discrimination theory of aphid host selection (KENNEDY & BOOTH, 1951; KENNEDY, 1953). This postulated that, in addition to specific stimulatory substances of no nutritive value governing botanical preferences, universal substances such as amino acids which are of fundamental metabolic (nutritional) importance to plants and insects alike, also played a major part in aphid host relations. This of course assumed that the supply of these substances varied greatly according to the physiological activity of the plant. Apart from their "direct" effects on aphid nutrition, we suggested that such common substances also acted as immediate sensory stimuli for feeding and other responses, either in themselves or through token stimuli physiologically associated with them. However, this has been strongly disputed by FRAENKEL (1953a,

1953b; LIPKE & FRAENKEL, 1956) and DETHIER (1947, 1953, 1954), who believe that metabolically unimportant "odd substances" or irregular occurrence among plants, are predominantly responsible for feeding preferences in phytophagous insects: "the curious glycosides, tannins, saponins, alkaloids and essential oils whose occurrence is unexplained by plant physiologists" (LIPKE & FRAENKEL 1956). In support of this, FRAENKEL (1953) collected a large body of evidence from bulk analyses of green leaves, from which he concluded that "sufficient levels of all the major nutrients are present in most, if not all leaves to satisfy phytophagous insects", and doubted whether physiological changes such as leaf ageing altered this situation appreciably. He reminded us that the non-nutrient theory of host selection offers an explanation for the occurrence of all those odd substances in plants, which may have been evolved by the plants specifically as a defence mechanism against insects.

This last is an attractive idea, and one should not overlook other phytophagous organisms here, including eelworms. And as DETHIER (1954) says, "while the recognition of food by nutritionally unimportant token stimuli is certainly well established, the relationship between nutritionally good and sensorially attractive materials remains an open question". But it is plain that DETHIER and FRAENKEL discuss the nutritional-sensory question only because it has been raised; they themselves consider it effectively closed already. The result could be a general disinclination to look for further evidence on the nutritional side: theories are very important at this early stage in the development of the subject.

There are other strictly practical consequences to be faced if these authorities are right that physiological variation in plant constituents (or tokens of them) which are of metabolic importance to both plant and insect, plays little part in determining plant susceptibility to insect attack. This means a definite limitation on our prospects of creating resistant plants. The causes of resistance must then be a highly specialized private problem of entomologists and chemists unaided by the other agricultural sciences. It will remain a special problem for the plant breeder in his turn, always called upon to incorporate a resistant factor which has no known relation to the familiar plant-physiological properties which it is his special skill to preserve and improve. It has been worth while tackling the two very serious potato pests, *Leptinotarsa* and *Heterodera*, in that way, but how many other pests would merit it ? And this kind of resistance based on substances which merely inhibit attack on an otherwise perfectly suitable plant, is just the kind to which the pest itself can be expected most easily to develop "resistance": with the appearance of a new strain which "requires the former repellent (now attractant) stimulus to induce feeding" — exactly as LIPKE and FRAENKEL (1956) suppose host specificity to have evolved in the past.

Fortunately, the nutritional question still remains open. We are not yet obliged to work on the assumption that resistance must be sought almost entirely in odd substances which are a law unto themselves. There is evidence that ordinary physiological differences can mean important differences in susceptibility to insects (PAINTER, 1951, 1958). There is also ample evidence that some nutrient substances stimulate feeding, and THORSTEINSON (1958) has given us fresh instances including glutamate and vitamins. No one would expect all nutrients to stimulate feeding themselves when other nutrient or non-nutrient token stimuli make this

unnecessary. Undoubtedly we require more evidence, especially on plant-physiological variation in nutritiousness and its effects on insect behaviour and nutrition. But for aphids, uniform nutritiousness certainly cannot be assumed on the basis of evidence concerning whole leaves. Aphids do not eat leaves; the only nutrients directly available to them are those dissolved in the sap and in transit from one part of the plant to another. This translocation of solutes is neither constant nor haphazard but patterned by the nature and nurture of each plant. To start from correlations between plant physiology and aphid susceptibility, hoping to carry through the programme outlined above, did not therefore seem to us unrealistic. What follows is an attempt to illustrate this approach by reference to three recent and largely unpublished pieces of work done in our laboratory.

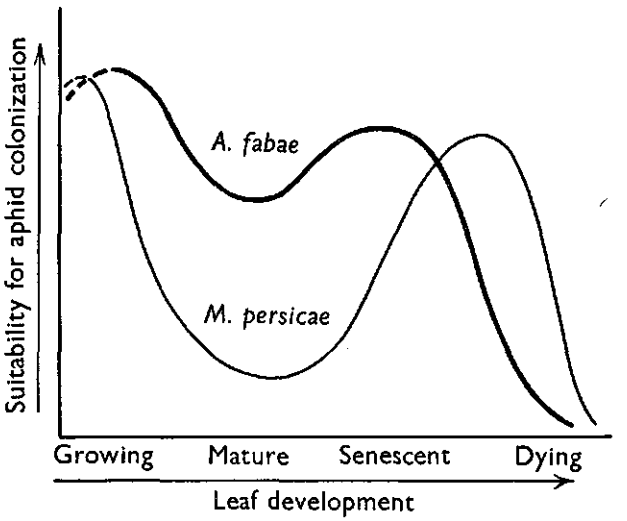

Fig. 3. Schematic representation of the relation between the age of leaves and their susceptibility to colonisation by *Aphis fabae* Scop. and *Myzus persicae* (Sulz.) (from KENNEDY & BOOTH, 1950). In practice the relationship varies greatly, depending among other things upon the rates of leaf growth and senescence, and upon the duration of the intervening mature stage when no growth or senescence is evident in the leaf.

PLANT GROWTH AND SENESCENCE

The starting point here was the observation that growing and senescing leaves of certain plants were more susceptible to *Myzus persicae* (Sulzer) and *Aphis fabae* Scopoli than mature green leaves of the same plants (Fig. 3). Three measures of "susceptibility" were used in the earlier work: simple counts of free infestations, cage measurements of feeding preferences and cage measurements of birth rates. Our hypothesis to account for the leaf-age effects was that the aphids' food (phloem sieve-tube sap) would be especially rich in soluble organic nitrogen compounds of high nutritive value — amino acids and amides — at or near places where growth and hence protein synthesis was going on in the plant, and again where senescence with protein breakdown was going on; but poor in these compounds where neither growth nor senescence was in progress, as in mature green leaves. LINDEMANN (1948) had put forward the same hypothesis in connection

with her work on leaf-feeding aphids. We still have no direct evidence that the postulated differences of phloem sap composition exist between leaves (and other organs) at different developmental stages, on the same plant at the same time. And we recognize that part of this hypothesis is at variance with the usual picture of translocation in the plant occurring d o w n a concentration gradient of organic nitrogen and other solutes, from their source in mature green photosynthetic leaves or in storage tissues, to growing organs functioning as "sinks". But that particular plant-physiological question can be left aside for the time being, since MITTLER (1953, 1954, 1957, and in press), following LINDEMANN (1948), has provided some direct quantitative evidence on the main insect-physiological question before us, by comparing plants at different stages of development rather than individual organs.

He used a different aphid, *Tuberolachnus salignus* (Gmelin), which feeds in the phloem sieve tubes in the woody stems of the willow, *Salix* spp. Samples of the aphids' actual food were obtained by collecting the sap exuding from their stylet stumps left embedded in the stem after the rest of the aphid had been cut away (KENNEDY & MITTLER, 1953). This sap was analysed qualitatively and quantitatively, and so was the honey-dew being excreted by intact aphids on the same stems at the same time. The composition of the two fluids was found to vary together. On one stem at one time, the total sugar content of the honey-dew was not appreciably lower than that of the sap, suggesting that little of the sugar was being assimilated (although some of it was chemically altered). But the nitrogen concentration was always lower in the honey-dew than in the sap. This difference, when measured and multiplied by the total volume of sap taken in by aphids feeding and growing on the same stem for a given time, approximated closely to the measured gain of nitrogen in the bodies of the aphids during that time in several detailed experiments. Thus the sap was apparently the only source of nitrogen available to the aphids (cf. TOTH, 1952).

TABLE I

Effect of growth in the host-plant on the feeding activity, growth and development of Tuberolachnus salignus (Gmelin) on stems of Salix for 7 days from birth (from MITTLER, *1954).*

	State of foliage	
	actively growing	mature
Total honey-dew output per nymph, cu.mm.	110	140
Total nitrogen assimilated per nymph, μg.	105	6
Instar reached	III—IV	III
Percentage of alate forms	,24	67

Moreover, the nitrogen content of the sap in the stem varied greatly through the cycle of growth, maturity and senescence of the foliage on a tree, as would be expected from the work of plant physiologists (e.g. ZIEGLER, 1956; TAMMES, 1958). And the amount of nitrogen assimilated by aphids feeding on the stems varied correspondingly. For example, Table I summarizes the results of one experiment using two potted willow plants, one in active growth and the other having completed its growth and not yet begun to senesce. New-born aphids were

left to feed on the stem of each plant for a week. Ninhydrin tests showed that the concentration of amino-nitrogen was many times greater in sap exuding from cut stylets in the tree with growing leaves than in sap from the tree with mature leaves. Correspondingly, the gain of total nitrogen (also fresh weight and dry weight) by the nymphs during the week was about 17 times greater on the tree with the growing leaves. The output of honey-dew was recorded continuously during the week as a measure of the relative intake of sap. It proved to be consistently rather higher on the mature plant with little nitrogen in the sap. In this case therefore one can say with more than usual confidence that the faster growth of the aphids on the growing plant was due, not to more feeding there, but to better nutrition in the strict sense of the term.

From a number of such experiments, comparing the same plant at different times as well as different plants at the same time, it was possible to build up this general picture. The nitrogen content of the sap was very high as soon as the buds began to swell and the aphids began to feed on the stems. Before that when the buds were still dormant the aphids probed the stems often but would not settle down to feed, so no sap or honey-dew could be obtained for analysis. As shoot growth proceeded and slowed the sap nitrogen content fell; when leaf senescence set in it rose again. The growth rates of aphids feeding on the stems began high, then fell and then rose again in parallel.

So much for nutrition; what evidence was there of sensory stimuli associated with the differences of nutritiousness ? MITTLER was most concerned with nutrition but he did observe both excretory and morphogenetic responses, presumably organized by the central nervous system upon receipt of some sensory stimuli. Thus in the first experiment mentioned above (Table I), the following additional differences were observed on the two plants. Associated with the faster feeding as indicated by the greater daily volume of honey-dew excreted where the sap was poor, the act of excretion was executed more frequently: smaller droplets being expelled, but 2-5 times more often. The aphids also developed more slowly on the poor sap, being about half an instar behind by the end of the week; and more of them turned out to be alates (Table I). LINDEMANN (1948) had earlier shown a correlation between sap nitrogen content and the parturition and wing-development responses, as well as the growth, of certain leaf-feeding aphids : *Cryptomyzus ribis* L., *Neomyzus circumflexus* (= *Aulacorthum* (*Neomyzus*) *circumflexum* (Buckt.)) and *Macrosiphon koehleri* (= *Macrosiphum euphorbiae* Thomas)). Although the sap analysed in this work was not obtained through aphid stylets but from cut stems or as contaminated exudate from petioles, etc., its nitrogen content, the nitrogen content of the aphids' honeydew, the aphids' growth, and aphids' responses, all showed the same relation as MITTLER found to the growth, maturation and senescence of the leaves. These workers' results do therefore appear to support the hypothesis that the host responses of aphids are governed partly by stimuli associated with sap rich in organic nitrogen and possibly even given by that sap itself. It would be surprising if the feeding and parturition preferences observed in *Aphis fabae* between young, mature and senescent leaves were not also governed by such "nutrient stimuli".

At the same time MITTLER's demonstration of 'true' nutritional differences associated with developmental changes in the plant overcomes what was certainly

a serious weakness (DETHIER, 1954; LIPKE & FRAENKEL, 1956) in the original case for the dual discrimination theory. We were quite unjustified in using the parturition rate of young adults over a few days as a measure of fecundity and hence of nutrition[1]). Parturition is itself a nervous response to external and internal stimuli no less than is feeding, as subsequent work has shown (JOHNSON, 1953, 1954; MITTLER, 1954; CARTIER & PAINTER, 1956; KENNEDY, 1958). The first offspring produced by an aphid (as by many insects) are not the product of its feeding as an adult but of the food taken in during its immature stages. Indeed, as UICHANCO (1924) first showed, and LAMB (1955) recently confirmed in *Aphis fabae*, embryonic development begins within an aphid before its birth, in the grandmother's body. Hence when we speak of an adult aphid responding to stimuli betokening nutritiousness, it is not only her own nutrition that is meant, but even more the nutrition of the young she deposits in response to those stimuli. The main significance of the host-selecting behaviour of mother aphids is for the next generation, as in other insects.

DETHIER (1954) brings forward this well-known fact that the female often "selects a plant or part thereof which is to feed the larvae and is not at all the proper food for herself", as an argument for the predominantly odd-substance theory of insect host-selection and against the idea that nutrients or tokens of them are important co-stimuli. But nutritional effects always come after the behavioural response, whether that be oviposition or feeding. In this context it is immaterial whether the nutritional effect is on the individual that makes the behavioural response, or on its progeny. Our concern is with the stimuli for the selective response: are some of them linked with nutritiousness or not ?

To take an extreme case, consider the selection of oviposition site by the sexual female of an heteroecious aphid such as *Aphis fabae* Scop. in the autumn. This certainly has nothing to do with food for the ovipositing female herself. Yet it appears to be a "nutritional" selection on her part (although the stimuli are most unlikely to be actual nutrients), for it is related to a change that occurs in the location of food supplies on the plant between her generation and the next. It is not primarily a "botanical" selection. These females do not have to find the right kind of plant, for they are born on it, apterous. They may well have "botanical" preferences (this has not been tested), but the major act of botanical selection is carried out by their mothers, the winged parthenogenetic viviparous *gynoparae* and by the winged males. The special act of selection carried out by the ovipositing sexual female is between parts of one plant, and is fitted to the developmental physiology of the plant. It consists of (i) ceasing to feed on and

[1]) The distinction made by DETHIER (1953, 1954) between active and passive host selection, and the similar one made by PAINTER (1951, 1953, 1958) between preference and antibiosis, presented not as loosely descriptive but as analytical and quasi-physiological, are also rather misleading. For these distinctions imply that while host finding and feeding are active responses to the host, the subsequent effects of the host plant — on parturition or oviposition, on growth, morphogenesis and survival — are not. The working notion of a 'truly' or 'strictly' nutritional effect is also one that begs some fundamental physiological questions. Insects can only grow or ripen their eggs after ingesting certain substances in sufficient amount; but it is to say the least premature to assume that this effect is a passive one, different in kind from other effects of the external and internal environment which are known to be organized responses.

walking off the leaves which were senescing and therefore suitable for feeding while she was immature, but which will soon become unsuitably old and then fall from the plant, and (ii) moving on to the bark of the woody stems where no suitable feeding sites are now present, and depositing the eggs in crevices especially around the dormant buds which will be growing and suitable for feeding by the time the eggs hatch in the spring.

The larger phenomenon of seasonal host alternation (heteroecy) itself seems also to be at bottom a matter of seasonal selection by "nutrient" stimuli in this sense, even though the selections made are between different kinds of plant (LINDEMANN, 1948 ; KENNEDY, 1953). *It is the concomitant selection of a certain* number of winter and summer hosts as a set, in preference to all the other kinds of plant not colonized at any season by the given species of aphid, that constitutes the true "botanical" selection which we may reasonably ascribe to odd substances. The different forms of one aphid species differ in the relative strength of their 'botanical and 'nutrient' preferences, in accordance with their differing ecological roles (KENNEDY & BOOTH, 1954). The primary (winter) host is preferred to other plants even by the summer forms of the aphid which rarely colonize it in nature; it is their "nutrient" preference which takes them elsewhere.

PLANT WATER RELATIONS

The study of another kind of plant-physiological "nutrient" factor for aphids, physical rather than chemical, started from the correlations observed between aphid behaviour and plant water status. A good deal of seemingly contradictory evidence has been published on the effects of plant water shortage on aphids: unfavourable effects such as the aphids becoming restless or developing wings (e.g. RIVNAY, 1937), and the reverse — mass multiplication in droughts (e.g. MARKKULA, 1953). In neither case has the mechanism of the effect on the aphids been clear. The effects on the plant are known to be complex: loss of turgor, arrest of growth, and hydrolysis in the leaves resulting in premature senescence. During the original experiments on leaf ageing as a factor in the host alternation of *Aphis fabae*, the outdoor results were much less consistent than the greenhouse results using well-watered plants (KENNEDY & BOOTH, 1951). In warm dry weather when the senescent leaves of the outdoor Sugar Beet plants wilted most, they ceased to be preferred by the aphids to the mature leaves. The more rigid leaves of *Euonymus europaeus* L. never wilted visibly outdoors. But discs punched out of them showed that they also lost water and they also became then less acceptable to the aphids. Indeed they became relatively more unacceptable than the beet leaves under similar conditions.

Experimental comparisons were therefore made of leaves on watered and unwatered plants under otherwise identical conditions both outdoors and in the greenhouse using *Euonymus* and *Vicia faba* L. (LAMB, 1955; KENNEDY & BOOTH, unpublished). When the plants were not watered, consistently negative effects were recorded on feeding activity, parturition rate, survival and form (production of alates). Tables II and III exemplify the results obtained. The question then was, what made the water-deficient leaves unfavourable ? Several experiments showed that the effects were readily reversible in the same leaves by restoring the water

TABLE II

Effect of water supply to the host-plant, Euonymus europaeus L., on colonisation by Aphis fabae Scop. in the field. Two similar lots of four potted plants were exposed for 10 days during which lot A was kept watered and lot B left unwatered. After counting and removal of the aphids, the same plants were exposed for a second period of 21 days with the watering treatment reversed, and fresh counts then made. Figures refer to mature leaves only, present through both periods (KENNEDY & BOOTH, *unpublished*).

		Alate mothers per leaf	Progeny per leaf
First period	Lot A watered	1.7	18.5
	Lot B unwatered	0.5	8.0
Second period	Lot A unwatered	0.2	0.7
	Lot B watered	1.9	5.5

TABLE III

Effect of wilting of the host-plant, Vicia faba L., on parturition by apterous Aphis fabae Scop. caged singly on the leaves of potted plants. Mean number of young produced per adult by three adults in three days (from LAMB, *1955).*

	Age of leaf		
State of plant	young	mature	old mature
Turgid	14.7	5.0	2.3
Wilted	5.7	2.3	1.0

supply to the roots. Moreover, aphids caged on all ages of leaf, young, mature and senescent, were affected in the same reversible way at the same time. Thus the negative effects of water shortage cut right across the effects of leaf age, and they could not be related to chemical changes in the leaves (LAMB, 1955). It appeared that the immediate physical effect of loss of turgor might be responsible. In other words the aphids might depend upon the sap pressure to maintain their sap intake, and, in addition, respond actively to changes in it.

It was originally to test this hypothesis that the stylets of feeding aphids were cut, to see if sap would continue to come up without any assistance from the aphid. It did so in a few cases when scissors were used on *A. fabae* on beans, but the stylets here were easily dislodged. MITTLER found that much more repeatable results were obtained when the stylets could be cut guillotine-fashion against a rigid woody stem, and developed this as a routine technique for the work already mentioned. He measured the rate of sap exudation from *Tuberolachnus* stylet stumps in a willow stem, and compared it with the rate of honey-dew excretion by intact specimens feeding on the same stem at the same time, on a number of occasions. The two rates were not significantly different, which confirmed the dependence of the aphids on the sap pressure within the plant, and showed how mistaken we had been in thinking of aphids as "sucking" insects. Furthermore, interrupting the water supply to the stem, or to a small area on it by local incisions down to the wood, caused the exudation from cut stylets to slow down and stop while at the same time intact feeding aphids withdrew their stylets and wandered away — like *A. fabae* from wilting leaves.

Turning from this back to ecology again, it was inferred that the invisible loss

of turgor by *Euonymus* leaves in fine summer weather was an additional factor preventing *A. fabae* from colonising them then, reinforcing the effect of the maturity of all the leaves. We obtained some indications that the mature leaves of *Euonymus* developed a day-time water deficit earlier in the season than sugar beet leaves of the same age; and later on in the season when the beet leaves wilted daily but recovered their turgor and water content at night, the *Euonymus* leaves did not regain water at night, being apparently in a chronic state of water deficiency (Cp. WEATHERLEY, 1951, on cotton plants). Secondly, there are indications that the growth and also the senescence of *Euonymus* may be restricted by water shortage in the summer even in our supposedly wet climate. The general absence of growing and senescing leaves on many shrubs and trees in the summer, which we have suggested lies at the root of the phenomenon of host alternation, may not be so fixed a property of such plants as we thought. It may be imposed upon them to some extent by water shortage.

The herbaceous secondary hosts of *A. fabae* on the other hand, bean and sugar beet, manage to continue growing to some extent when the drought is not too severe, apparently at the expense of the old leaves. The leaves start senescing as soon as they cease growing or even before, so that the middle-aged leaves become acceptable to the aphids instead of being truly mature and unacceptable (KENNEDY, IBBOTSON & BOOTH, 1950). The growing leaves are the last to lose water and droop in a drought, while the older leaves which wilt more readily also recover readily, as at night. Continuous wilting of course quickly kills the leaves; but intermittent wilting favours the progressive type of senescence which in turn favours aphids, perhaps because of an increase in translocatory amino-nitrogen (Cp. GATES, 1957, on tomatoes). Leaf senescence brought on in this way may well be involved in the reported cases of drought favouring aphid multiplication. Usually too many other factors are varying at the same time to assess the part played by the condition of the plants. But in a randomized plot experiment with potatoes planted at a succession of dates, TAYLOR (1955) observed that when premature senescence was induced in one of the plantings by a temporary drought, there was a rapid multiplication of *Myzus persicae* on these particular plants only, so that in this case extrinsic factors were probably not responsible.

It is therefore not very surprising that the literature on this subject appears self-contradictory. The plant-physiological consequences of water shortage may, in fact, have contradictory effects on aphids, reducing the quantity of sap they can get but at the same time improving its quality. Clearly we shall not be able to predict or control which way the balance of effect will go in any given case in the field until much more quantitative work has been done. But we should eventually be able to obtain aphid resistance through altering the water economy of the plants, by cultural or plant breeding methods.

VARIETAL RESISTANCE

It is patently absurd to think of trying to create resistance in field crops such as beans by altering their growth pattern to resemble that of *Euonymus*, with no growth or senescence going on in the summer. The present physiological properties of crop plants are, with present techniques, what we require of them as crop

plants. This truism might suggest that physiological differences as of growth pattern play little or no part in the observed varietal differences of resistance to aphids. That is often assumed and it fits the odd-substance theory of insect host-relations. But there is some evidence that it is not always true. Thus BALD, NORRIS and HELSON (1946) in Australia and then TAYLOR (1955) in England have compared a number of potato varieties for aphid susceptibility. Both came to the conclusion that the main reason for the observed differences was the different growth patterns of the varieties. For example TAYLOR found that *Myzus persicae* with its marked preference for senescent potato leaves, attained the biggest populations on the variety which developed such leaves first, that is, the earliest variety (Table IV). He found the same correlation between the incidence of senescent leaves and of *persicae* populations, whether he was comparing the same variety planted at different dates, or at the same date in different years, or different varieties planted together.

TABLE IV

Comparative susceptibility of potato varieties to infestation by Myzus persicae (Sulz.) and Aphis nasturtii (Kltb.). Maximum populations per plant in a trial by TAYLOR *(1955). M. persicae shows a marked preference for senescing leaves on potatoes, and in this trial the early-maturing variety Arran Pilot bore a much higher proportion of such leaves than the other varieties, when the aphids arrived. A. nasturtii apterae colonize leaves of all ages, so that the number of leaves available for them is greatest on late-maturing varieties such as King Edward and Majestic.*

Maturation class of varieties:	'First early'	'Second early'	'Maincrop'	
Variety:	Arran Pilot	Ulster Chieftain	King Edward	Majestic
M. persicae	1410	340	920	490
A. nasturtii	270	490	1310	1410

Such information suggests there may be possibilities of increasing aphid resistance physiologically, at any rate by cultural methods (e.g. water control). But since early and late varieties of potatoes are both needed in practice, neither can be eliminated merely to avoid aphid attack; and there might appear to be no prospect of breeding for resistance along these lines. Perhaps that is what BALD *et al.* (1946) meant when they drew the strange conclusion that there was no evidence of aphids preferring any of the varieties they tested. Nevertheless, where aphids seriously limit the growing of a crop, genetical differences in physiological characters have been used for aphid resistance, or so it is believed. It is a general if unproved assumption that Winter (autumn-sown) varieties of Field bean which are less heavily infested and less damaged by *A. fabae* than Spring varieties, derive their resistance from the simple fact that they have out-grown the most susceptible juvenile stage before the aphids arrive (e.g. RADEMACHER, 1939). In this case, it is believed that the grower is employing a genetical difference in one kind of physiological property, frost resistance, to permit earlier planting to obtain another kind of physiological difference, in age, which in turn confers aphid resistance.

But undoubtedly the most interesting problem is presented by varieties which are more alike in their agricultural performance, such as varieties within the general class of Spring beans. Here, if anywhere, one might expect that differences quite unconnected with growth patterns and aphid nutrition would be entirely responsible for any differences of aphid susceptibility that exist. H. TAMBS-LYCHE (unpublished) studied such a case in a small mixed planting of two varieties of Field (Spring) beans: *Rastatt* which is relatively resistant and *Schlanstedt* which is very susceptible to *Aphis fabae* (MÜLLER, 1951, 1953, 1956, 1957). As expected from MÜLLER's extensive studies, the natural incidence of *A. fabae* alatae was consistently lower on Rastatt than on Schlanstedt in repeated counts made throughout the season. This original difference was reflected in the relative size of the colonies which built up on the plants in the intervals between sprayings to kill off all the aphids.

As usual on beans, the majority of feeding alatae were counted in the young furled leaves at the shoot tips and in the crevices between the developing flower buds near the tip. The colonies started by the alatae were still more concentrated into these flower bud crevices. The records kept of the growth, flowering and fruiting of the plants showed that this preferred site among the loosening flower buds became available for colonisation *later* on Rastatt than on Schlanstedt (Table V). For several weeks in the early and most critical part of the season, Rastatt continued to offer fewer such sites for colonisation, because the first flower buds developed on Rastatt about two nodes higher than on Schlanstedt. In this and other respects Rastatt behaved in this trial as a later variety than Schlanstedt, differing from it in the same way as Field beans as a class differ from Broad or Garden beans (CHAKRAVARTY, DRAYNER and FYFE, 1956). Here then was a difference in developmental physiology which did appear to be helping in some measure to make Rastatt more resistant, if only in a rather mechanical fashion as far as the eye could judge.

TABLE V

Comparative growth and development of two varieties of Field bean (Vicia faba L.): Schlanstedt which is very susceptible to Aphis fabae and Rastatt which is relatively resistant. Mean number of organs developed, and final weight yield of beans (seeds), per plant (TAMBS-LYCHE & KENNEDY, *unpublished*).

		Schlanstedt	Rastatt
May	Leaf-bearing nodes	7.1	7.2
	Inflorescence-bearing nodes	3.5	1.6
July	Leaf-bearing nodes	75	99
	Pod-bearing nodes	33	43
August,	Filled pods	32	47
when	Beans	94	130
harvested	Weight of beans, g.	66	61

However, this obvious difference did not last beyond the first weeks. The flower buds ceased to be preferred as they opened in June, and Rastatt soon bore as many unopened ones as Schlanstedt. By July Rastatt offered more favoured sites than Schlanstedt. Yet the aphids continued to prefer Schlanstedt; and we

confirmed MÜLLER's (1953) finding that the individual organs of Schlanstedt were preferred, even the expanded leaves, and not just the plants as a whole. From this one might be tempted to conclude that the sole cause of the preference was some subtle difference between all parts of the plants which was quite unconnected with growth pattern and nutrient supply. Such a conclusion, however, still seems premature when the other aspects of the lateness of Rastatt are considered.

During the period of flowering and fruiting in June and July, Rastatt was engaged in the production of more shoots, more leaves, more flowers (of which many fell without opening) and more pods, per plant, than Schlanstedt (Table 5). The final result at harvesting was more numerous but smaller beans from Rastatt than from Schlanstedt, with about the same total weight yield of beans per plant. There is no reason to suppose that the total assimilatory product of Rastatt was any greater than Schlanstedt's. But it does appear to have been distributed differently. On Rastatt, growth was going on at more points at the same time; hence less at each. It would seem to follow that an aphid feeding in the phloem sieve tubes supplying any one growing organ of Rastatt, say a flower bud, would be getting sap less rich in nutrients than it could get on a similar organ of Schlanstedt.

This is still only a suggestion; but it invites further work in several directions. AUCLAIR and MALTAIS (1950) and MALTAIS (1951) found a lower amino-nitrogen content in pea varieties resistant to the pea aphid, than in susceptible varieties. Since it was not the whole plants but only the tops of growing shoots that they assayed for amino-nitrogen, the question arises whether this resistance, too, may have been due to a small difference of growth pattern. The postulated difference in sieve-tube sap composition between varieties with slightly different growth patterns (Cp. the difference between vegetative and flowering shoots of alfalfa: DAHMS and PAINTER, 1940) could be checked directly, by comparing the composition of sap exuding from cut aphid stylets on the two plants. Again, it would be interesting to see how far Rastatt might lose its resistance if earlier flowering were induced by vernalising the seed (CHAKRAVARITY et al., 1956), and how far Schlanstedt might become more resistant if more "bushing" were induced by destroying the first shoot. The results might be negative and so fail to give the hoped-for hints to the plant breeder. But it was encouraging to find anything worth further investigation from the plant-physiological point of view in this case. The physiological difference between Rastatt and Schlanstedt is not apparently of any other agricultural significance. If it is responsible for the difference in aphid resistance, this is the kind of physiological, nutritional resistance, which could be selected or bred for without losing any other desirable character in the plants.

ZUSAMMENFASSUNG

1. Es ist bekannt, daß unregelmäßig auftretende Sekundärstoffe unbekannter Funktion in den Pflanzen oft die Reize darstellen, mit deren Hilfe die Insekten ihre Wirtspflanzen unterscheiden. Es wird behauptet: I. daß vorwiegend solche Reize für die Wirtswahl verantwortlich seien, II. daß alle für die Insekten notwendigen Grundnährstoffe von fast allen Blättern in ausreichendem Maße geliefert würden und daß deshalb III. die für

den Stoffwechsel der Pflanzen wie der Insekten als wichtig bekannten häufigen Verbindungen eine geringe Rolle als Ursache der Präferenzerscheinungen spielten (sowohl als unmittelbare Reize wie als physiologisch mit ihnen verbundene Signalfaktoren). Die Theorie der „doppelten Unterscheidung" bei der Wirtswahl der Blattläuse, andererseits, fordert, daß solche „Ernährungs"reize eine bedeutende Rolle spielten, zusammen mit spezifisch pflanzlichen Reizen; und daß der Nährstoffgehalt der Pflanzensäfte, von dem sich die Aphiden ernähren, dem physiologischen Zustand der Pflanzen entsprechend, in weiten Grenzen schwankt.

2. Es wird hier darauf hingewiesen, daß das Studium des für die Ernährung wichtigen pflanzenphysiologischen Aspekts der Beziehungen zwischen Aphiden und ihren Wirtspflanzen heutzutage wissenschaftlich und praktisch weit fruchtbarer sein dürfte als das Studium des auf die speziell pflanzlichen Sekundärstoffe bezogenen Aspekts, da bereits viel über die physiologische Variabilität bei den Pflanzen bekannt ist und da dies den Sammelpunkt aller landwirtschaftlichen Wissenschaft darstellt.

3. Zur Unterstützung dieser Auffassung wird über drei (weitgehend unveröffentlichte) Arbeiten über Aphiden (*Tuberolachnus salignus* Gmelin and *Aphis fabae* Scopoli) zusammenfassend berichtet. Sie zeigen, daß gewöhnliche Formen der physiologischen Verschiedenheit unter den Pflanzen, wie etwa Eigentümlichkeiten in Wachstum und Entwicklung oder im Wasserhaushalt, sowohl die Ernährung wie das Verhalten der Blattläusse beeinflussen. Mit der Zunahme weiterer Kenntnis solcher Unterschiede könnten wahrscheinlich im Hinblick auf die Aphidenresistenz von Pflanzen viel größere Fortschritte erzielt werden, als bisher durch Anbau- und Pflanzenzuchtmethoden für möglich gehalten wurde.

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DISCUSSION

A. J. THORSTEINSON suggested that the departure of aphids from leaves that have lost turgor might be due, in part, to a repellent high concentration of (for example) sugar.

J. S. KENNEDY agreed, mentioning some supporting experimental evidence, and pointing out that this would equivalent to the leaves becoming more mature.

Ent. exp. & appl. 1 (1958): 66—72. *North-Holland Publishing Co., Amsterdam*

THE BEHAVIOUR OF *APHIS FABAE* IN SELECTING ITS HOST PLANTS, ESPECIALLY DIFFERENT VARIETIES OF *VICIA FABA*

BY

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The different initial infestation of the two field bean varieties Rastatter and Schlanstedter (in the relation 1 : 3 till 1 : 5) by *Aphis* (*Doralis*) *fabae* Scop. is caused by a preference behaviour of the alates, which land on both varieties of bean in equal numbers, but whereas 99% leave Rastatter in a few minutes, only 90% leave Schlanstedter.

According to PAINTER (1951) the hereditary resistance of plants against infestation by insects may depend on three different ecological principles: Firstly on the t o l e r a n c e of the host plant, that is not weakened or damaged by the infestation. Secondly on a n t i b i o s i s, that is on the check of the growth or reproduction of the pest by an insufficient nutritive quality or by toxic effects caused by special properties of the plant or thirdly on p r e f e r e n c e, that is on individual choice of the insects, which prefer distinct species or varieties of plants more or less refusing others.

The fact, that the different varieties of beans are infested by the black aphids in a very different measure, has been known for a long time, but nearly nothing is known about the causes of these differences. No tolerance could be observed as yet. As the bean aphid must be looked upon as an extremely polyphageous insect, it seems improbable that the more or less distinct resistance of field bean varieties might be based on preference. DAVIDSON (1922, 1925) who, in the twenties, was the first to investigate the causes of the resistance of beans against aphids, therefore based his research entirely on an examination of the antibiotic effects of the plant. He placed a certain number of young adults on plants of the tested varieties and after a fixed time he counted the numbers of their offspring. As these numbers were strikingly different in the tested varieties, he considered that they gave a measure of resistance.

With a bean assortment at Quedlinburg, too, it was not possible to get a reproduceable series from resistant varieties to highly susceptible ones based on the reproduction rate. It appeared possible that there existed some mechanism of preference. In this we confined our investigations on the much susceptible variety Schlanstedter (S) and on the variety Rastatter (R) considered to be relatively resistant, thirty plants of either being cultivated repeatedly in an alternating double row of fifty to fifty centimetres. By a daily sampling of all alate aphids and the colonies of young larvae left by them respectively, we determined on every morning the mean infestation resulting during a period of 24 hours on each plant.

All repetitions yielded the difference of 1 : 3 to 1 : 5 between Rastatter and Schlanstedter. In the course of 147 test-days during the growing period in 1950, 695 initial infestations have been established on 60 Rastatter plants but 3427 on 60 Schlanstedter, that means 83% of the entire infestation being found on Schlanstedter. How constantly this primary settlement kept appearing is also made clear by the graph (fig. 1). Considering the frequency distribution on the days on which observation were made during the test years 1949 and 1950 of the height of the percentage infestation of both varieties, there is a maximum near 85% of S (sample mean 69,5 $\pm$ 29,1%). Obviously the winged aphids are able to distinguish the two varieties from one another and at the initial infestation they prefer the S 3 to 5 times more than the R. Considering the progressive reproduction of aphids it is evident that this different preference is the primary cause of the resistance observed by growers and farmers, even if an antibiotic effect could be proved as well.

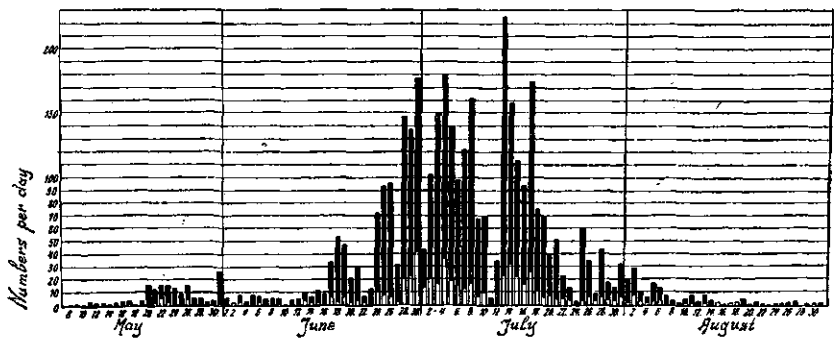

Fig. 1. The daily numbers of the initial infestations by *Aphis fabae* on 60 Rastatter (white) and 60 Schlanstedter (black) beans during the summer 1950

These results raise the question, how these differences of preference come about. Till now we have not succeeded in analysing the behaviour of the bean aphid in choosing its host plant in the laboratory or in the greenhouse by experiment, as it is hardly possible to keep winged aphids in the infestation stage. The young winged aphids are easy to breed, but they show a strong flying impulse connected with a distinct positive phototaxis and do not react either on host plants or on any stimulation to land. But while outdoors after a shorter or longer flight this phase turns over again to negative phototaxis and to a tendency to settle on host plants, the alates in the greenhouse will stay in the brightest place of the roof, starving rather than showing normal reactions to their host plants.

Therefore the further analysis of the preference resistance of *Vicia faba* to *Aphis fabae* could be tried only in the field, where the experimental conditions are not constant and the opportunities for observation are seldom favourable, but where instead of that the normal natural conditions prevail.

First of all we proved whether the preference applied to certain parts of the bean plant, or if all organs equally caused the difference in the initial infestation of the two varieties. It became evident in a series of tests with 30 decapitated R-

and S-plants each, that plants deprived of all vegetation points indeed showed a smaller total infestation, but the S — as in the control — kept showing 90% of the initial infestation. The same result came about when, reversely, all leaves were removed and only the sprouts were left intact. The suspicion that the extra-floral nectaries might cause the difference, was removed by experiments on plants without stipulae, for here also 80% of the initial infestation developed in S-plants. Even the reduction to one single primary leaf did not change the difference. There was the same result with cropped leaves freshened in water and arranged in the field in the usual mixed double row. Not matter whether primary or secondary leaves were used as a material, the S got about 80 to 83% of the daily found colonies.

Neither does the age of the leaves influence the difference in the preference of the two varieties. It is true: on tests on plants with 3 young, middle aged (ripe) and older (yellowish) leaves each, the total infestation decreased to 49, 33 and 18% respectively. But the S unvariably attracted 75 to 80% of initial infestations. Neither particular organs nor the physiological stage of the plants or leaves change the preference relation.

Before we know anything about the behaviour of the aphids infesting a new host plant it appeared possible that the initial infestation observed in 24-hour intervals was coming about by a primary choice, that is: at first arrival, at the first landing on a plant. One could believe it might be caused by the different smell of the varieties, so that the corresponding organs of the aphids allowed them to make a choice at a distance.

In the course of the last decade however some observations of KENNEDY (1950 a, b), and MOERICKE (1955) and the author (H. J. MÜLLER 1951 u. 1953) showed us that there is a great restlessness among the aphids in the infesting stage, and that of the numerous landing aphids only very few settle there definitely. The first hint in the direction of a secondary choice was brought about by the comparison of the initial infestation of the single plants with catches by yellow dishes that were observed simultaneously in a R- as well as in a S-plot at 24 hours' intervals. While the initial infestation was as usual with S, the number of *Aphis fabae* in the yellow dishes of both plots were practically always the same (fig. 2). Both plots had obviously been flown over by the same number of aphids in the infestation stage; however the S had been settled 5 times more frequently than the R. It remained still undecided whether the aphids had landed in equal numbers on both varieties and then most of them had left the R or if they had primarily avoided the latter.

Shortening the control intervals to 1 hour and finally to 20 minutes did not lead to any final proof. The percentage of the initial infestation on the S went down accordingly from 87 to 70 and 65% respectively, but this difference is not statistically significant.

All we could do was to catch uninterruptedly all the bean aphids alighting on two comparable plants of the two varieties over a period of many hours. During a period of more than 40 hours a total of 874 alate aphids were captured, 53,3% on R and 46,5% on S. The same plants, when exposed to colonisation by aphids both before and after these observations on alighting had been made showed the typical differences, i.e. 85% of the total infestations were on the S plant. This

was definite proof that both varieties were primarily infested by equal numbers of infesting stage aphids and that the difference of preference exclusively arose from secondary choice, namely from "trying" or testing. This conception was confirmed by 27 hours' continued observation of, altogether, 600 alates alighted on two similar plants of both varieties during 9 different control periods in July

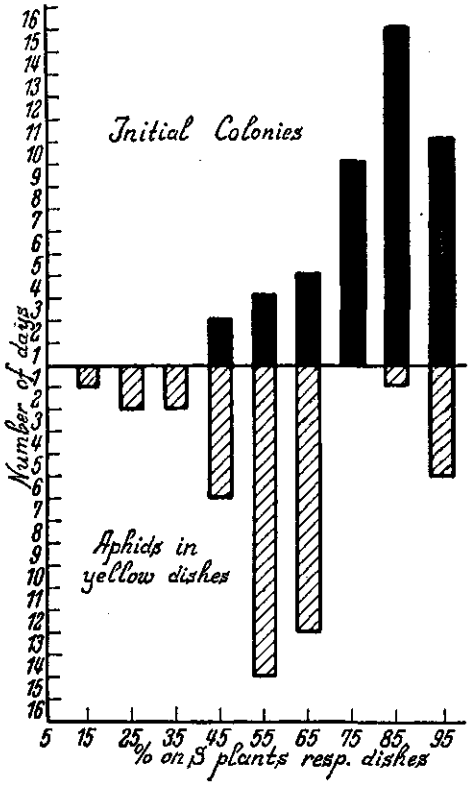

Fig. 2. Comparison between the daily numbers of the initial colonies on beans and the alate aphids (*Aphis fabae*) captured in yellow dishes in separated Rastatter (R) and Schlanstedter (S) plots (of 32 plants each) by the distribution of the control days according to their percentage of colonies on S-plants (black) respectively of specimens in the dishes of the S-plot (hatched).

1952. Out of 312 alates alighted on R, only 3 definitely settled, that is: they bore some larvae (2 other cases remaining doubtful because of the nightfall), all but 1% left sooner or later. But on the S-plant 29 out of 289 alighting alates (+ 5 doubtful cases) remained for birth of larvae, that means 10%. It is therefore secondary choice that decides the difference of preference, but it is not simply that on the preferred variety all alighted aphids remain sitting and only from the less preferred plants the aphids fly away, but arrival and departure on both varieties are different in percentage. This significant difference of 1 : 10 of plants under constant observation is greater than at the daily counting of the initial colonies, where it is 1 : 5. This fact is clear if we consider that with the

latter method a great number of aphids are caught that have not yet definitely settled and that would soon have left. This amount however, being the same on both varieties, decreases the difference relation.

The fact that on the highly infestable and most preferred S bean only 10% of the alighted aphids remain sitting demonstrates that the choice of the host plant must be masked in a highly effective and variable manner by other factors or other ways of behaviour. The final settlement will always be smaller than the arrival. We know however that the intensity of the infestation flight is strongly dependent on the temperature, on the humidity and on the wind-speed. Accordingly the host preference is strongly dominated by two antagonistic reactions: the flying impulse and the infesting impulse, both of them being influenced contrarily by factors of environment. High temperature and humidity increase the flying impulse, lower ones increase settling. Low wind-speeds make possible unrestricted flying off and high wind-speeds may force an aphid to settle when it would have left the plant under calm conditions. A tired aphid on the contrary may settle under favourable flying conditions on a plant that it would have refused in a former phase.

It is very difficult therefore to tell anything definite about the real causes of the acceptance or refusal of one of the two varieties by the alighted aphids. The searching time of the future settlers, that is the time between the landing and the final settling, was with the 3 R-settlers 13, 14 and 46 minutes; with the 29 S-colonists it varies between 2 and 32 minutes with an accumulation between 2 and 17, especially between 2 and 10 minutes, the mean being 11 minutes. The mean staying time of visitors, that is the time between landing and taking off again, was on the contrary longer for those on S beans ($6\frac{1}{2}$ minutes) than for those on R beans ($3\frac{1}{2}$ minutes); these differences, notwithstanding the variability of the periods in different cases, are statistically significantly different. Therefore most of the visitors are leaving R-plants sooner than S-plants (fig. 3).

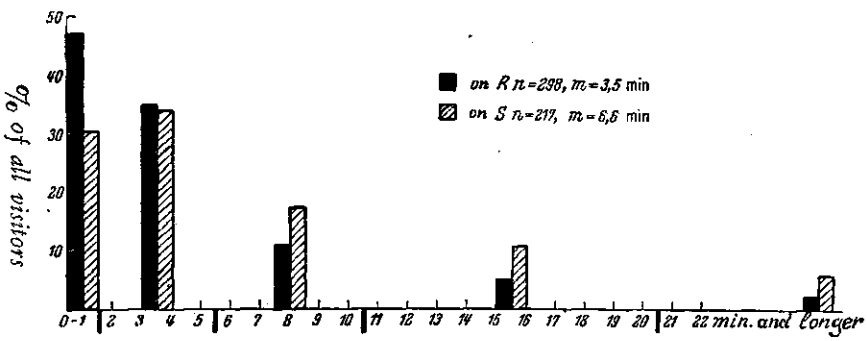

Fig. 3. Frequency distribution of the staying times of alatae (from alighting till take off) on a Rastatter and a Schlanstedter bean plant in percent of the total numbers of visitors during altogether 27 hours of flight.

During the searching and the staying time the alates wander about a great deal on the surface of the plant, however interrupting their wandering very often, setting up their proboscis and laying back their antennae. We could see by the

remaining salivary sheaths that the so-called test feeding punctures (which mostly do not take more than 20 tot 40 seconds) very seldom penetrate beyond the epidermis and obviously penetrate into the intercellular tissue and constantly end blindly in the intercellular spaces. It seems improbable that on doing so they might suck up or taste anything.

The significance of the test feeding punctures is still unknown. As they end blindly it is doubtful whether the aphids imbibe by them any material for tasting. According to unfinished histological investigations the real feeding punctures pass always intercellularly to the phloem through tissues with no or little intercellular spaces. Therefore I should suppose the test feeding punctures to be a search for tissues having no intercellulars. These are normally found in young organs and in the surroundings of the vascular bundles, which are enveloped by sclerenchymatic or collenchymatic sheaths reaching to the epidermis. These are the very places, where aphids like to settle finally. Here the punctures automatically lead to the phloem. Probably the stylets are able to indicate by lever effects minute differences of pressure in the tissues. Therefore the aphid continues to insert its stylets only where it feels a close contact with the neighbouring cells. If we could prove this, we could suppose that the causes of the difference of preference were to be seen in the size and structure of the intercellular systems of the varieties. We could assume that the aphids succeed more often on susceptible varieties, less often on resistant ones to get to tissues with no or very few intercellular spaces, in order to find the phloem and so to be induced to settle down. In this way the statistical distribution of the infestation would be understandable.

Some of the aphids (alates and apterae) will change places once more or twice after the birth of the first or of a few young larvae. There is a distinctly perceivable tendency with the aphids to leave R-plants more frequently than S-plants, and at the next choice even the R-infestors prefer S-plants. Here perhaps an antibiotic effect is to be observed.

As we can now see the differences in resistance of the tested varieties of field beans arises first by a different host preference of the aphids. Moreover antibiotic effects may play a minor role too. The causes of the different preference are not clear as yet. It is sure only that the preference behaviour with varieties develops by a secondary choice not before the landing and is influenced by manifold exogenous factors (as plant condition, weather and so on) and by endogenous ones too (as age, physiological stage and motivation of the aphids).

ZUSAMMENFASSUNG

Als primäre Ursache des unterschiedlichen Befalls der beiden Ackerbohnensorten Rastatter und Schlanstedter durch die Schwarze Bohnenlaus, *Aphis (Doralis) fabae* Scop., wird Präferenzresistenz nachgewiesen. In mehrjährigen Beobachtungsreihen ergab sich bei 24-stündigen Kontrollen ein Unterschied in der Anzahl der Initialkolonien von 1 : 3 bis 1 : 5 auf Rastatter und Schlanstedter. Direktes Abfangen bzw. eingehende Beobachtung der auf den Pflanzen gelandeten Geflügelten zeigte, daß diese Initialbefallsdifferenz erst sekundär entsteht. Die Pflanzen beider Sorten werden statistisch in gleicher Häufigkeit beflogen, jedoch siedeln sich auf Rastatter nur 1%, auf Schlanstedter 10% der Gelandeten für längere Zeit (bis zum Absetzen wenigstens einer Junglarve) an. Die Bedeutung der sogenannten Probesaugstiche sowie der Größe und Verteilung der Interzellularräume in den Pflanzengeweben für das Auffinden des Phloems und somit für die Wirtswahl wird diskutiert.

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DISCUSSION

H. J. DE FLUITER : Wie tief versenken die Blattläuse beim „Probieren" ihre Stechborsten ins Pflanzengewebe, bevor sie sie wieder zurückziehen ?

H. J. MÜLLER : Die Probesaugstiche enden fast ausschließlich zwischen den Epidermiszellen bzw. in den Interzellularräumen der unmittelbar darunter liegenden Zellschicht, jedoch niemals in den Zellen selbst. Sie erreichen in keinem Falle ein Leitbündel.

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L'INFLUENCE DE LA PLANTE-HÔTE SUR LA FÉCONDITÉ DE L'INSECTE PHYTOPHAGE

PAR

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Pour étudier l'influence des constituants organiques de *Solanum tuberosum* sur la fécondité de *Leptinotarsa decemlineata* il faut d'abord tenir compte de la ration alimentaire d'entretien, et établir la relation entre fécondité et quantité d'aliment consommé.

Les variations du rapport C/N sont moins importantes que celles de la teneur en lipides choliniques sur la fécondité du Doryphore; cependant un supplément de caséine augmente le taux de ponte si la feuille est pauvre en azote. Le glucose ralentit l'activité ovogénétique mais son effet est plus difficile à interpréter car il joue un rôle énergétique important dans l'activité musculaire.

L'effet des lipides phosphatidiques est plus nettement mis en évidence dans les tests biologiques et dans les tests biochimiques.

Les facteurs alimentaires de la fécondité ont été moins étudiés que ceux de la croissance et ces derniers sont moins bien connus chez les Insectes phyllophages que chez les espèces dont le régime alimentaire peut être artificiellement constitué (Diptères saprophages et hématophages, Coléoptères et Lépidoptères détritiphages ou granivores); ce que l'on constate en consultant les revues générales récentes de W. TRAGER (1953) et de Z. H. LEVINSON (1955).

L'influence du régime alimentaire, caractérisé par l'espèce botanique de la plante-hôte, sur la ponte est toujours marquée chez les Insectes oligophages mais n'est pas négligeable chez les polyphages. TAUBER et al. (1944) établissent une variation de fécondité de 1 à 7 chez les femelles de *Melanoplus bivittatus* Say nourries sur 29 végétaux différents; l'indice de fécondité (ou "quantité moyenne d'oeufs pondus par femelle et par jour") est le suivant pour quelques plantes:

Lycopersicon esculentum	1.0
Alopecurus pratensis	1.8
Cucurbita pepo	2.6
Cannabis sativa	3.3
Melilotus sp.	3.9
Trifolium sativa	4.9
Medicago sativa	6.0
Lactuca sp.	6.9

Sur un autre Acridien, *Melanoplus mexicanus mexicanus* Sauss., O. L. BARNES (1955) étudie l'influence de quelques plantes-hôtes fondamentales; il conclut que la Luzerne est moins favorable qu'on ne le pensait pour le développement et la reproduction de cet Insecte, et il donne comme quantité d'oeufs par femelle nour-

rie zur Luzerne (*Medicago sativa*): 13, sur *Chenopodium murale*: 43, sur „Johnson grass": 89 et sur *Sisymbrium irio*: 196.

Chez *Leptinotarsa decemlineata* Say, TROUVELOT et GRISON (1935) ont montré l'influence de diverses espèces d'un même genre botanique; le nombre total de pontes déposées par 6 couples répartis en trois cages est :

— sur *Solanum edinense* Berth.	=	35	pontes	comptant	en	moy.	35	oeufs	par	ponte
— sur *S. tuberosum* L.	=	29	—	—	—	—	25	—	—	—
— sur *S. utile-demissum* Lindl.	=	13	—	—	—	—	12	—	—	—
— sur *S. Jamesii* Torr.	=	8	—	—	—	—	8	—	—	—
— sur *S. Commersonii* Dun.	=	0	—							

Enfin DAHMS et al (1936) constatèrent des phénomènes analogues chez *Blissus leucopterus* Say vivant en présence de variétés différentes de Sorgho:

— sur la var.	„Dwarf Yellow milo"	la	ponte	moy.par	jour	est	de	3.5	oeufs		
— — —	„Blackhull kafir"	—	—	—	—	—	—	1.3	—		
— — —	„Atlas sorgo"	—	—	—	—	—	—	0.4	—		
— — —	„Common feterita"	—	—	—	—	—	—	0.2	—		

Bien entendu, selon le type d'évolution des gonades, il y a lieu de distinguer entre l'influence du régime alimentaire de l'imago et l'influence de la nutrition larvaire sur l'ovogénèse et la fécondité.

Ce dernier cas est celui du Coléoptère *Bruchus obtectus* Say dont les femelles n'exigent pas, dans les conditions normales, une alimentation spéciale pour pondre (G. M. HERFORD, 1935), bien que, chez *Bruchus quadrimaculatus* Fab., A. O. LARSON (1924) ait constaté un accroissement de fécondité en offrant expérimentalement aux femelles de l'eau sucrée (33 oeufs par femelle nourrie contre 23 oeufs par femelle jeûnant). Il en est de même chez *Pectinophora gossypiella* Saund. (LUKEFAHR, 1956).

Le fait est assez fréquent chez les Lépidoptères; chez *Lymantria monacha* L. la fécondité croît en fonction du poids des nymphes résultant d'une alimentation larvaire variable : avec le Sapin elle est de 0 à 137 oeufs et avec le Chêne de 178 à 374 oeufs (H. SATTLER 1939).

Nous avons étudié plus particulièrement l'action de l'état physiologique de la plante-hôte *Solanum tuberosum* L. et celle de certains constituants biochimiques du feuillage, c'est-à-dire l'influence des facteurs alimentaires intrinsèques, sur la fécondité du Doryphore, *Leptinotarsa decemlineata* Say.

Ces études ont été précédées par l'analyse de l'influence des facteurs externes sur la fécondité de l'Insecte, qui a été publiée ailleurs (GRISON, 1948, 1957), mais dont il faut tenir compte dans l'interprétation des résultats obtenus en expérimentant sur les constituants alimentaires.

METHODE EXPERIMENTALE ET RATIONS ALIMENTAIRES

Les méthodes et techniques d'essais que nous avons utilisées font l'objet d'une description détaillée ailleurs (1957). Il est fondamental de rappeler que la vie imaginale du Doryphore comporte ordinairement trois phases caractérisées par des besoins physiologiques, des états éthologiques et des exigences écologiques différents; ce sont: la phase de maturation de courte durée au cours de laquelle l'insecte parfait achève, après la mue imaginale, le développement de

ses gonades; la période de diapause qui peut ne pas se manifester dans certaines circonstances (GRISON, 1944a; DE WILDE, 1954); et la phase de reproduction qui se prolonge d'avril en août sous le climat parisien.

Tous les animaux d'expériences rapportées ici ont été prélevés au début de la phase reproductrice; ils étaient placés par couples séparés, ou le plus souvent même les femelles étaient isolées après accouplement, sous des petites bonnettes de toile métallique parfaitement aérées.

La ponte et la consommation quotidiennes de chaque femelle étaient évaluées au cours des essais dont la durée était le plus souvent limitée à des périodes de „test biologique" de 10 à 20 jours après une période de contrôle préalable de la fécondité. La fécondité est généralement exprimée par un „indice de fécondité" représentant la ponte moyenne par femelle et par jour. La signification de cet indice est discutée ailleurs; il y a une corrélation hautement significative entre la quantité d'oeufs et le nombre de pontes; en outre la tendance présentée par une femelle d'être plus ou moins féconde se maintient pendant toute sa vie, en sorte qu'il y a intérêt à établir, dans les tests biologiques, la comparaison entre deux traitements dont un sert d'étalon avec les individus d'un même lot.

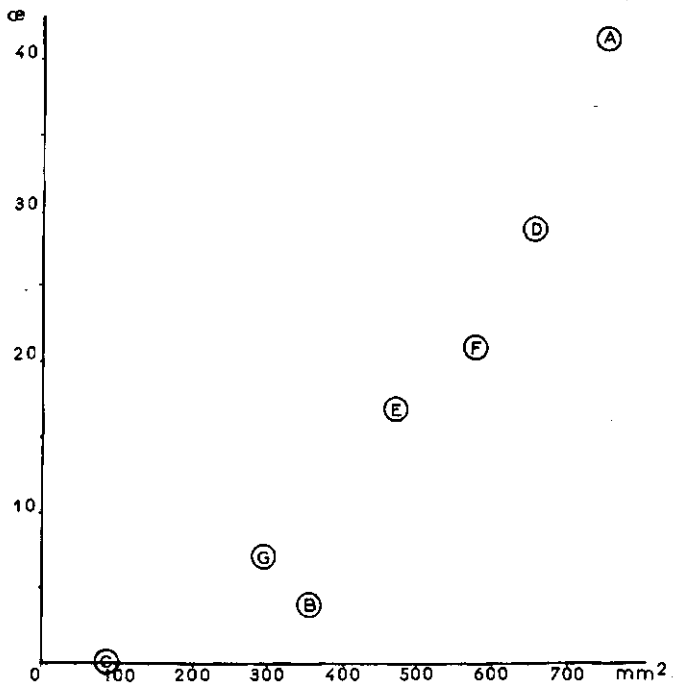

Fig. 1. Relation entre la fécondité moyenne (en oeufs) et la quantité d'aliment consommé (en mm² de surface foliaire) chez *L. decemlineata,* par individu et par jour.

Nous établissons d'une manière analogue un „indice de consommation" pour chaque femelle représentant approximativement la quantité moyenne d'aliment consommé par individu et par jour et exprimée en surface foliaire. Dans les expériences de base cette évaluation a été faite d'une manière rigoureuse.

Nous reproduisons à nouveau le graphique montrant la corrélation élevée entre fécondité et consommation (fig. 1). Cependant, si l'on fait porter l'analyse sur six périodes hebdomadaires successives, l'analyse de covariance indique que la fécondité par période reste significative après avoir tenu compte de la consommation tandis que la fécondité par individu dans les mêmes conditions devient non significative. Ceci traduit le fait que, pendant les dernières semaines, la consommation est tombée au-dessous d'un „seuil quantitatif" assurant l'ovogénèse et la ponte ou encore que les besoins alimentaires sont réduits simultanément avec la diminution du potentiel reproducteur.

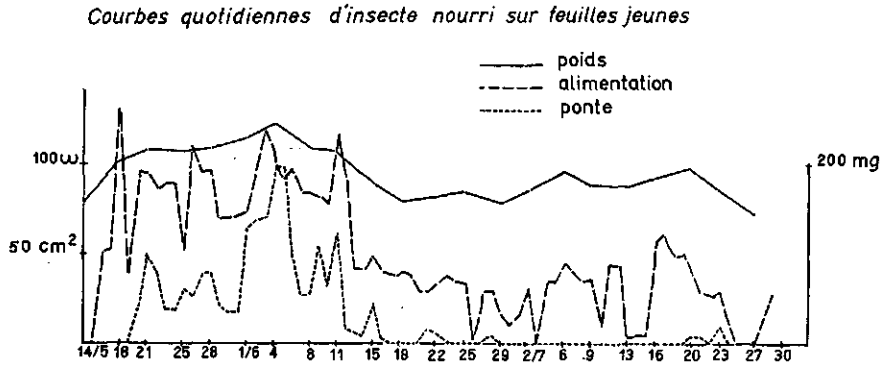

Fig. 2. Courbes quotidiennes de consommation, de ponte et courbe bi-hebdomadaire de poids d'une femelle de *L. decemlineata* nourrie sur feuilles jeunes de Pomme de terre.

Ce sont deux aspects d'un même problème que nous étudierons plus tard mais que schématisent les graphiques des fig. 2 et 3; ils sont empruntés à une expérience dans laquelle l'évolution pondérale des individus a été suivie à raison de deux pesées par semaine et parallèllement à l'observation quotidienne rigoureuse de la ponte et de la consommation: la fig. 2 en donne un exemple pour l'une des dix femelles de l'essai. Elle met très bien en évidence les faits suivants :

1. la phase reproductrice comprend deux périodes distinctes : l'une d'activité ovogénétique pendant laquelle les besoins énergétiques correspondant à cette fonction physiologique se traduisent à la fois par une consommation élevée et un accroissement appréciable du poids de l'insecte; l'autre de sénescence ou d'épuisement ovogénétique au cours de laquelle le poids de l'animal se maintient un peu au-dessus du poids initial jusqu'à une brusque diminution a n t e - m o r t e m correspondant à la sénescence organique totale (GRISON et LE BERRE, 1953);
2. la consommation durant cette deuxième période représente approximativement la r a t i o n a l i m e n t a i r e d ' e n t r e t i e n y compris les besoins énergétiques requis par une activité musculaire modérée;
3. le seuil quantitatif de consommation, établi dans les expériences interprétées par le graphique de la fig. 1, devrait correspondre à cette ration d'entretien.

Nous avons alors établi, pour 4 femelles ayant vécu et pondu assez régulièrement au moins pendant deux mois, les courbes de rendement ovogénétique de la fig. 3. E. TITSCHACK (1924) est, je pense, le premier auteur à avoir suggéré cette

notion: chez *Carausius morosus* Br. & Redt. il établit que pour produire 1 mg. d'oeuf, l'Insecte doit consommer 7,8 à 10,4 mg. de nourriture. Pour nous, cet indice correspond au rapport de la quantité d'oeufs pondus entre deux observations à la quantité d'aliment consommé, en cm² de feuillage, pendant le même temps. Il devrait être corrigé, ainsi que l'a fait LAFON (1950) chez le *Carausius*, en tenant compte de la ration d'entretien et en fonction du poids de l'animal par une analyse de covariance qui sera faite ailleurs, c'est-à-dire en établissant la „ration alimentaire de croissance ovulaire".

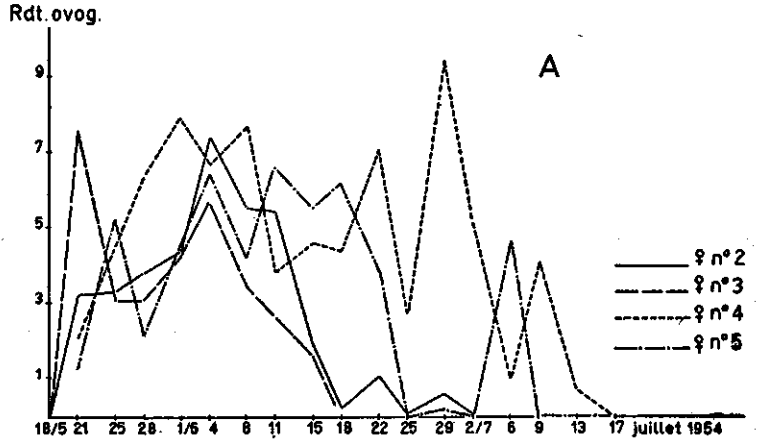

Fig. 3. „Rendements ovogénétiques" de 4 femelles de *L. decemlineata* au cours de leur phase reproductrice.

Néanmoins cet élément brut nous indique que le potentiel d'activité reproductrice, correspondant à des échanges métaboliques intenses, est maximum pendant une période de durée variable selon les individus; le rendement ovogénétique brut est en moyenne, pour les 4 femelles considérées dans le graphique de la fig. 3, au cours des huit semaines successives de : 3,8; — 4,0; — 5,8; — 4,5; — 4,3; — 3,8; — 1,8; — 0,9 oeufs par cm² d'aliment.

Il y a donc intérêt, du point de vue méthodologique, à réduire la durée des tests biologiques de fécondité et à prélever les femelles pendant la même période d'activité imaginale. Dans ces conditions le rendement ovogénétique brut peut être utilisé comme élément d'appréciation et de comparaison de la valeur de régimes alimentaires qualitativement différents, car il tient compte de la variation quantitative de la ration. Malheureusement les difficultés d'évaluation pondérale rigoureuse de cette dernière qui nous ont amené à utiliser de préférence l'estimation de la surface foliaire consommée, limitent la portée et l'utilisation du rendement ovogénétique.

Enfin il ne faut pas négliger de mentionner les différents processus sensoriels et humoraux grâce auxquels, sous l'influence de la croissance des gonades et de la multiplication des cellules germinales, l'organisme répond à des besoins énergétiques accrus par une augmentation de consommation: „la ponte stimule fortement l'appétit" (LAFON, 1951).

Ce stimulus peut être d'origine endocrine: JOHANSSON (1954) a obtenu le

déclanchement de la ponte chez *Oncopeltus fasciatus* Dallas par la transplantation dans les femelles immatures et à jeun, de corpus allatum normalement développés provenant de femelles alimentées. Il dépend aussi de mécanismes sensoriels dont DETHIER (1953, 1954) a étudié en détail le fonctionnement. Mais nous ne prendrons pas en considération dans ce travail les facteurs du comportement qui participent à la fois au rationnement quantitatif et à la sélection alimentaire, ce qu'a parfaitement analysé DE WILDE (1958) chez la larve de *L. decemlineata.*

VARIATION DU CHIMISME FOLIAIRE CHEZ SOLANUM TUBEROSUM

La plante subit des modifications biochimiques au cours de son développement et aussi selon les conditions de nutrition du végétal.

Les données analytiques fournies par la physiologie végétale ne sont pas négligeables lorsqu'on étudie les effets de la qualité de l'aliment offert aux Insectes sur la fécondité des femelles (TRAGER, 1941; WIGGLESWORTH, 1950); mais elles ne peuvent nous renseigner utilement sur l'efficacité d'un élément biochimique qu'à la condition de pouvoir établir des corrélations statistiquement significatives entre ces données analytiques et les résultats des tests biologiques de fécondité.

L'examen du rapport C/N a d'abord été pris en considération pour deux raisons: d'abord parce que les entomologistes ont attribué un rôle tantôt aux glucides, tantôt aux matières azotées dans la fécondité des insectes; ensuite parce que ce rapport, depuis KLEBS (1918), a pris une grande importance en physiologie végétale et qu'il est caractéristique de l'évolution du chimisme de la plante au cours de son développement.

Les conditions de culture sont susceptibles de modifier la teneur en azote total et en glucides du feuillage d'une plante et par conséquent de modifier le rapport C/N; c'est ainsi que le chimisme de végétaux, tels que des Crucifères (EVANS, 1938) ou des Légumineuses (BARKER, 1952; BARKER et TAUBER, 1954) a été modifié en relation avec l'étude de la reproduction chez les Aphides. Elles ne présentent probablement pas le même intérêt pour les Insectes broyeurs et les Insectes piqueurs.

TABLE I

Conditions de culture en 1953 (3ème expér.)	Sucres[1]) réducteurs	Sucres totaux	Azote total	C/N	Indice fécondité
terreau ombré	6,1	16,5	67	0,25	9,1 oeufs
terreau ensoleillé	16,8	47,0	58	0,81	10,8 —
limon ensoleillé	13,3	28,5	94	0,31	7,1 —
limon ombré	9,0	14,2	74	0,19	6,1 —

[1]) en p.p. 1000 du poids sec de feuilles de la var. „Bintje".

Les différences de fécondité que nous avons obtenues avec le Doryphore nourri sur des feuilles de Pommes de terre développées soit en terreau, soit en limon, soit sous ombrage, soit en plein soleil, n'ont pas été statistiquement significatives (Table I).

Il ne faut pas perdre de vue que les conditions de nutrition et de croissance des végétaux, sont affectées par les variations annuelles des facteurs climatiques, en sorte que celles-ci peuvent influencer indirectement le développement et la fécondité des Insectes phytophages par leurs effets sur les plantes-hôtes (UVAROV, 1931).

D'autre part, la composition chimique des feuilles est constamment modifiée au cours de chaque jour sous l'effet de la photosynthèse: à la fin de la journée, le feuillage est plus riche en sucres réducteurs qu'au lever du jour, tandis que la teneur en azote varie peu, en sorte que le rapport C/N s'en trouve assez considérablement modifié.

Des rameaux prélevés à des heures différentes n'ont donc pas la même composition chimique et peuvent être qualitativement différents pour les Insectes qui s'en nourrissent. DE JONG (1938) a montré ainsi que certains *Epilachna* pondent davantage lorsqu'ils sont nourris de feuilles de Thé cueillies le soir et riches en glucides, tandis que *Helopeltis theivora* Waterh. ne réagit pas de la même manière.

Nous avons opéré de même avec *L. decemlineata* après avoir constaté que les modifications chimiques étaient négligeables au cours de la journée dans les feuilles de Pomme de terre séparées des plantes et mises à tremper dans le petit tube d'eau de la cage d'essai. Il n'est pas nécessaire de reproduire les résultats peu significatifs publiés ailleurs (GRISON, 1952; 1957) et dont certains figureront plus loin à d'autres occasions.

Enfin, parallèlement aux études entreprises par COIC (1945) sur les variations de la constitution organique et minérale du feuillage de Pomme de terre au cours de la croissance du végétal, nous avons recherché l'influence de celle-ci sur la fécondité du Doryphore et constaté que la ponte est inhibée chez les femelles nourries avec du feuillage sénescent ou des feuilles de plantes âgées (GRISON, 1944b; 1947b).

F. PICARD (1926) avait déjà supposé que la sénescence des tissus foliaires constituait pour *Haltica ampelophaga* Guer une cause de diminution de fécondité.

LEGAY et Melle BAUD (1953) et LEGAY (1954) ont montré un phénomène comparable chez *Bombyx mori* mais, dans le cas de la plupart des Lépidoptères, c'est la nature de l'alimentation larvaire qui retentit sur la vitalité et la fécondité de l'imago: il en est ainsi chez le Bombyx Cul-brun, *Euproctis chrysorrhoea* Don. (GRISON, 1956) dont les femelles issues de larves nourries de feuilles sénescentes de Pommier depuis le troisième stade déposent des pontes de 100 à 200 oeufs tandis que celles provenant de chenilles avec régime de feuilles jeunes pondent chacune 350 à 400 oeufs.

BLAIS (1953) a constaté une relation analogue chez une Tordeuse, *Choristoneura fumiferana* Clem., dont la fécondité est réduite au moins de moitié sinon du tiers si les femelles sont nourries de feuillage d'Epicea de l'année précédente au lieu de s'alimenter aux dépens de celui de l'année.

En étudiant plus particulièrement l'apparition des différentes formes d'Aphides, KENNEDY et BOOTH (1951, 1954) ont étudié la signification physiologique et écologique de l'âge de la feuille sur le processus de la succession des formes.

En ce qui concerne le Doryphore, nous avons déjà précisé que la valeur qualitative de l'aliment a un effet immédiat sur la fécondité de l'Insecte (GRISON, 1952)

et que la feuille jeune a des effets présque semblables quel que soit l'âge du végétal sur lequel elle a été prélevée (GRISON, 1957).

Nous nous sommes alors attaché à caractériser les différences biochimiques pouvant exister entre feuilles jeunes et feuilles vieilles de *Solanum tuberosum*. Des résultats analytiques ont été publiés en ce qui concerne l'azote total, les glucides et la choline totale; mais nous en reproduisons les caractéristiques essentiels en un tableau dans lequel sont portés également les indices de fécondité des Insectes nourris avec du feuillage analogue à celui qui a été analysé (Table II).

TABLE II

Aliment : Ackersegen	Feuilles âgées		Feuilles jeunes	
Prélèvement à	9 h.	17 h.	9 h.	17 h.
en 1949				
sucres totaux	44,0	55,7	55,6	63,6
azote total	43,0	55,0	41,0	47,0
C/N	0,63	0,83	1,10	1,16
indice fécondité	*13,3 oeufs*	*3,6 oeufs*	*2,2 oeufs*	*2,3 oeufs*
en 1953				
sucres totaux	32,0	50,0	25,0	42,6
azote total	61,0	69,0	28,0	33,5
C/N	0,52	0,72	0,89	1,27
indice fécondité	*11 oeufs*	*20 oeufs*	*2 oeufs*	*1 oeuf*
en 1954				
indice fécondité	*26,7 oeufs*	*22,6 oeufs*	*7,8 oeufs*	*8,6 oeufs*
choline totale	0.274		0,079	

Il ressort de ces données que la choline totale symbolise le mieux la différence d'indice de fécondité: cette substance a retenu plus particulièrement notre attention. Les données analytiques nombreuses qui ont été recueillies (DUCET, KAHANE et GRISON, 1957) mettent en évidence:

a) — que la teneur en substances choliniques des tissus végétaux et notamment du parenchyme foliaire de *Solanum tuberosum* diminue progressivement au cours de la maturation et du vieillissement de ceux-ci ;

b) — que la choline, sous toutes ses formes, est constamment en plus grande quantité chez les femelles que chez les mâles de *Leptinotarsa decemlineata* ; ensuite, qu'elle subit des variations très importantes au cours de l'évolution de l'Insecte (de 0,535 à 1,540 mg de choline totale par gr. de poids frais de l'animal) et que c'est en période reproductrice que l'animal est le plus riche en choline totale.

RELATIONS ENTRE LA TENEUR DE L'ALIMENT EN CONSTITUANTS ORGANIQUES ET LA FECONDITE DU DORYPHORE

L'utilisation, par l'organisme du Doryphore, des groupes de substances organiques normalement présentes dans la feuille de Pomme de terre a été contrôlée sans étude systématique; ainsi le „coefficient de rétention azotée" a été évalué à 50%; pour les lipides choliniques la rétention est un peu supérieure à la moyenne (53%) chez les femelles après l'émergence printanière, tandis qu'elle est presque totale

après la mue imaginale ce qui caractérise bien le rôle d'"éléments constants" donné par TERROINE (1919) à ces substances.

Pour apprécier, dans les tests biologiques, l'effet d'un constituant sur la fécondité nous avons eu recours à deux procédés essentiels : soit d'offrir des feuilles physiologiquement différentes et caractérisées par l'analyse chimique faite simultanément sur des échantillons de plante prélevés à cet usage; soit d'offrir des feuillages aussi identiques que possible par leur origine et leur état physiologique mais recouvert d'une substance chimique définie : c'est la „supplémentation" de l'aliment naturel.

Nous commenterons quelques expériences inédites de supplémentation pour justifier les conclusions que nous avons émises (GRISON, 1957) dans un travail où sont rassemblés la plupart des essais et tests éxécutés pendant douze ans[1]).

Constituants azotés et glucidiques

La plupart des expériences reproduites ailleurs montrent des résultats non significatifs, notamment si l'on considère les cas où les suppléments biochimiques sont apportés à des feuilles jeunes. Le graphique de la fig. 4 schématise cette interprétation.

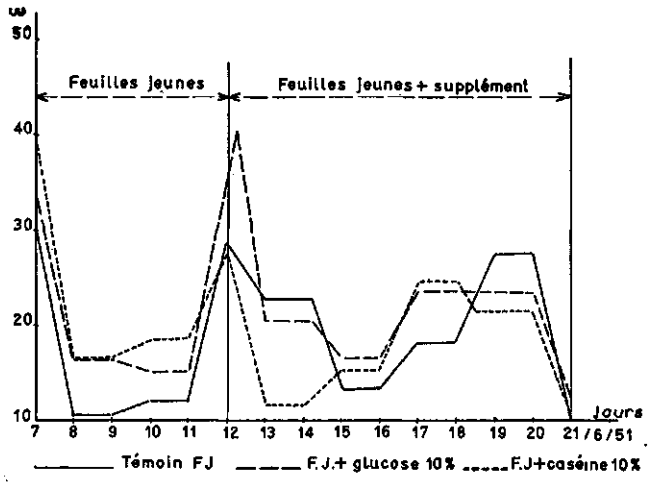

Fig. 4. Fécondité de trois lots de femelles de *L. decemlineata* pendant deux périodes successives avec et sans „suppléments".

Cependant, lorsqu'on supplémente des feuilles vieilles prélevées le matin ou le soir avec de la caséine on obtient une augmentation significative de la fécondité des Doryphores soumis à l'essai (Table III).

[1]) En douze ans, à la Station Centrale de Zoologie agricole de Versailles et pour la seule étude des facteurs alimentaires qualitatifs, notre Laboratoire a observé individuellement et quotidiennement la ponte et la consommation de plus de 4.600 femelles de Doryphores groupées en 510 lots ou séries d'aliments distincts et définis dont 85 étaient suivis pendant plusieurs périodes et parfois même pendant toute la vie imaginale avec pésée plus ou moins fréquente des Insectes.

Dans l'expérience 1953, l'unité de surface d'un cm² de feuille âgée pesant approximativement 30 mg., représente une teneur moyenne de 0,9 mg. d'azote total. Le supplément caséine étant d'environ 0,7 mg. par cm², l'unité de surface de feuille supplémentée représente alors une teneur de 53 pp. 1000 d'azote, voisine de celle de la feuille jeune.

TABLE III

Exp.	A. Régimes avec feuilles seules: Aliment support (Ackers.)	Teneur azote pp. 1000	Ponte totale oeufs	Indice fécond. oeufs	B. avec caséine: Ponte totale oeufs	Indice fécond. oeufs	Rapport indices B/A
1953	F. jeune 9h	61,0	715	11,1	478	7,5	0,7
	F. — 17h	69,0	1617	20,0	1688	26,3	1,3
	F. âgée 9h	28,0	128	2,0	527	8,2	4,1
	F. — 17h	33,5	61	1,0	288	4,2	4,2
1954	F. jeune 9h	—	2673	26,7	3091	33,1	1,2
	F. âgée 9h	—	781	7,8	1022	11,7	1,5
	F. — 17h	—	859	8,6	2082	20,8	2,4

Aussi peut-on admettre que l'apport d'un supplément azoté à la ration accroît la fécondité du Doryphore lorsque la teneur de l'aliment naturel (ici la feuille âgée de variété "Ackersegen") en azote est faible.

Dans le cas de supplémentation de feuilles jeunes naturellement riches en azote total, tout se passe comme si l'Insecte manifestait un besoin quantitatif minimum d'azote, et qu'il était susceptible de réduire son taux de consommation jusqu'à la satisfaction nécessaire et suffisante de ce besoin.

Ainsi dix individus consomment en 48 h. 52,6 cm² de feuilles normales pendant que la consommation de dix autres individus est réduite à 43,7 cm² lorsque les feuilles sont supplémentées en caséine; mais la quantité d'azote ingérée est cependant plus forte dans ce deuxième lot (10,49 mg. d'azote) que dans le premier lot (8,31 mg. d'azote).

Il nous a été difficile de prendre en considération les formes qualitatives de l'azote; au cours des quelques essais tentés avec des régimes d'acides aminés variés, nous avons envisagé l'hypothèse que certains d'entre eux (ou leur mauvaise proportion) seraient non-appétitifs; en effet dans un lot nourri avec des feuilles jeunes supplémentées par un mélange d'asparagine, d'alanine et de tyrosine, la consommation était réduite au tiers de la normale, ce qui est considérable.

En ce qui concerne les glucides, certains essais montrent, inversement au cas de l'azote, qu'un supplément provoquerait une diminution de ponte, ou plus vraisemblablement un ralentissement de l'ovogénèse (voir fig. 6, évolution morphologique des ovarioles), lorsque l'aliment en serait naturellement riche et qu'il y aurait „surcharge" de glucides.

Ainsi le rapport des indices de fécondité entre deux périodes dont la première comporte la feuille jeune seule et la seconde un supplément est de:

$$\frac{12,6}{4,3} = 2,9 \text{ fois plus faible avec saccharose}$$

$$\frac{17,4}{6,7} = 2,6 \text{ — — avec glucose}$$

$$\frac{15,3}{9,7} = 1,6 \text{ — avec glucose + lécithine}$$

$$\frac{11,1}{9,1} = 1,2 \text{ — avec feuille témoin.}$$

Ce qui semble signifier que, malgré une diminution de fécondité au cours de la seconde période, cette diminution est becaucoup plus forte avec un supplément glucidique mais qu'elle redevient comparable au témoin si l'on ajoute également un supplément lécithinique.

Néanmoins la diversité des résultats obtenus nous oblige à une conclusion prudente sur le rôle modérateur des glucides; nous pensons, qu'en raison de leur destinée et de leur plasticité métaboliques, l'effet i n d i r e c t des glucides alimentaires sur la fécondité du Doryphore dépend essentiellement de l'intensité des besoins énergétiques de l'Insecte.

Constituants phospholipidiques

De nombreuses expériences ont montré l'action inhibitrice de feuilles vieilles ou étiolées sur la ponte de *L. decemlineata.*

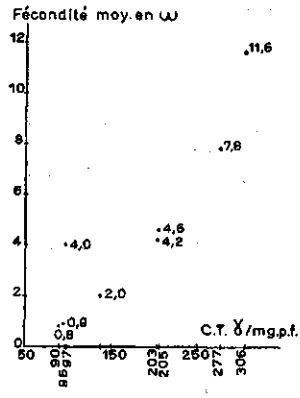

Fig. 5. Relation entre la fécondité moyenne (en oeufs) de *L. decemlineata* et la richesse de l'aliment en choline totale (par mg. de feuille fraîche).

La corrélation la plus significative entre richesse de l'aliment en lipides choliniques et fécondité du Doryphore a été établie à l'occasion d'une étude comparative des qualités nutritives du feuillage de diverses variétés de Pomme de terre (fig. 5).

Par la méthode de supplémentation et au cours de nombreuses expériences, tantôt très significatives et tantôt beaucoup moins, nous confirmons les données de cette corrélation en constatant la tendance très fréquente à l'accroissement de fécondité des femelles alimentées avec du feuillage recouvert d'une émulsion de lécithines[1]).

Nous venons de citer l'effet d'un supplément lécithinique ajouté au supplément glucidique. Dans la même expérience, des lots de femelles nourries sur feuilles âgées („Bintje") pendant une première période de 8 jours, puis de feuilles analogues supplémentées avec diverses émulsions de lécithines

[1]) Nous avons utilisé dans les expériences relatées ici des lécithines d'oeufs du commerce; elles sont mises en émulsion dans un mortier en versant d'abord une goutte d'alcool 70° puis en ajoutant goutte à goutte l'eau ou une solution physiologique en quantité suffisante (MAYER et TERROINE, 1907); lorsque l'émulsion est préparée au mixeur il peut se produire une destruction de certains constituants thermolabiles (GRISON, 1957). Une émulsion à 2 p. 100 de lécithines épandue au pulvérisateur Richardson sur une feuille vieille d'Ackersegen permet d'obtenir un aliment dont la teneur en choline totale pp. 1000 est voisine de 0,400.

pendant les 8 jours suivants, présentent cette tendance à l'acroissement de fécondité comme l'indiquent les rendements ovogénétiques ci-dessous, après correction approchée pour tenir compte de l'augmentation de poids sec due au supplément (Table IV).

TABLE IV

1ère période		*2ème période*	
avec f. âgée seule	4.5 oe/cm²	sans supplément	3.5 oe/cm²
—	5.3 —	avec lécithines 2% (1)	5.8 —
—	4.1 —	avec léc. 2% et $MgCl_2$	5.5 —
—	4.6 —	avec lécithines 8%	5.9 —

Le plus souvent l'indice de fécondité obtenu avec l'alimentation „feuille âgée supplémentée" reste inférieur à celui obtenu sur „feuille jeune", malgré son accroissement par rapport à l'indice sur „feuille âgée seule". En outre un supplément de lécithines sur feuille jeune est rarement efficace.

Le phénomène est mis en évidence avec des lots de 10 femelles nourries d'une manière semblable pendant 6 jours avec des feuilles âgées de variété „Ackersegen" pris pendant une deuxième période de 15 jours avec des aliments différents (Table V).

TABLE V

1ère période		*2ème période*		
ponte totale	rend.ovog.	aliment	ponte totale	rend.ovog.
602 oe.	20 oe/cm²	f.jeune	2500 oe.	67 oe/cm²
532 —	18 —	f.âgée	354 —	31 —
681 —	22 —	— + lécith. 0,5%	775 —	46 —
330 —	18 —	— + lécith. 2,0%	230 —	46 —

Le taux d'accroissement entre les deux périodes obtenu sur f. âgée supplémentée en lécithines (2 et 2,5) est un peu supérieur à celui obtenu avec la même feuille, sans supplément (1,5) mais il est inférieur à celui de la feuille jeune (3,5).

Cela peut être dû: soit au fait que le supplément lécithinique ne correspond pas qualitativement à la ration phosphatidique recherchée par l'organisme de l'Insecte, soit à un effet de masquage de l'action des lipides lécithiniques dû à la présence dans la feuille vieille d'éléments freinateurs tels que certains glucides.

Mais on peut également évoquer l'absence d'oligo-éléments organiques ou minéraux, en particulier des phosphates dont la feuille s'appauvrit au fur et à mesure qu'elle approche de sa maturité (R. FRAISSE, 1952). Une indication, malheureusement insuffisante, sur le rôle des phosphates nous est donnée dans une seule expérience d'une durée de 22 jours mais avec 5 femelles seulement par lot dont l'aliment était constitué par :

A. une émulsion à 5% de lécithines dans l'eau distillée
B. — — dans un glycérolé à 10% de glycérol
C. — — dans un glycérolé à 10% et contenant 3% de phosphate de magnésium et 2% de phosphate bicalcique

Pour établir le rendement ovogénétique, on a corrigé la surface foliaire par un équivalent en poids du supplément:

avec feuille jeune, ponte totale = 606 oeufs; rend.ovog. = 15 oe/cm²
avec f.j. et suppl. A, — = 423 — ; — = 16 —
— — B, — = 394 — ; — = 13 —
— — C, — = 715 — ; — = 45 —

Avec un support feuille âgée la ponte est nulle dans le témoin et très faible dans les essais (rendement de 0,5 oeuf); cependant avec le supplément phosphaté „C" la fécondité est 6 fois plus forte qu'avec les suppléments „A" et „B".

Des émulsions de lécithines à 5% enrichies en chlorhydrate de choline aux doses de 0,1 et 0,2 p. 100 ont été efficaces dans une expérience dont les témoins étaient malheureusement médiocres.

Mais la deuxième émulsion de lécithines-choline incorporée à une poudre de feuilles jeunes de „Bintje" desséchée à 100° C. provoque une reprise de la ponte, lorsque celle-ci a été interrompue pendant un mois par une sous-alimentation sur un aliment peu appétitif constitué par un support normal recouvert seulement de la même poudre de feuilles; nous donnons les fécondités individuelles pendant les périodes successives pour caractériser le phénomène (Table VI).

TABLE VI

périodes	aliment	fécondités individuelles						féc. moy.par fem.
15—24 mai 1948	témoin	109	131	134	171	206	124	146 oeufs
27 mai—18 juin	avec poudre	0	22	42	15	10	10	16 —
19 — 28 juin	d°	0	0	17	0	0	0	3 —
29 juin—21 juil	avec suppl.	173	15	25	42	59	0	52 —

Les poudres de feuilles desséchées de Pomme de terre avaient été préparées pour la mise au point de régimes artificiels (GRISON, 1947a); elles ont été ajoutées avec une charge de bentonite sur des feuilles de Pois, comme support neutre, dans certaines expériences qui ont confirmé l'indication précédente.

Ces expériences, citées ailleurs, font apparaître d'une part que la feuille de Pois supplémentée en poudre de feuille de Pomme de terre constitue une ration à peu près équivalente à celle de la feuille fraîche de Pomme de terre pareillement supplémentée, compte tenu d'une consommation réduite; et d'autre part que l'addition de lécithines à la poudre de Pomme de terre sur feuille de Pois triple ou quadruple la fécondité pour une consommation équivalente.

DISCUSSION ET CONCLUSIONS

Les facteurs biochimiques de la fécondité chez les Phytophages sont peu connus. Les travaux les plus récents et les plus détaillés se rapportent aux Diptères hématophages et spécialement à *Aedes aegypti* L. (LEA, DIMOND, DELONG *et al.* 1955, 1956). Ces auteurs ont déterminé les besoins quantitatifs et qualitatifs d'azote pour assurer la meilleure oviposition: ainsi 8 acides aminés sont indispensables à celle-ci et la ponte est nulle quand un seul de ces 8 acides est absent: arginine, isoleucine, leucine, lysine, phénylalanine, thréonine, tryptophane et

valine. L'absence de l'histidine ou de la méthionine limite le taux de croissance ovarienne mais quelques rares oeufs sont pondus; il en est de même, à un degré moindre, pour la cystine et l'acide glutamique.

Le Diptère phytophage *Ceratitis capitata* Wied exigerait aussi un régime protidique pour assurer une ponte normale (HANNA 1938, 1947). On a pu croire qu'il en était ainsi chez la plupart des Diptères; mais des travaux récents en limitent beaucoup l'importance: d'après RASSO et FRAENKEL (1954) le développement des ovaires de *Phormia regina* Meigen est plus satisfaisant si l'on ajoute à des protéines diverses, outre les sucres, les vitamines du complexe „B" et surtout des sels minéraux notamment du phosphate de potassium; pour ASCHER et LEVINSON (1956) l'enrichissement du régime larvaire en protéines chez *Musca vicina* Macq. est insuffisant pour assurer l'ovogénèse chez l'imago.

Chez l'Orthoptère *Melanoplus mexicanus mexicanus* Sauss, SMITH et NORTHCOTT (1951) obtiennent un accroissement de fécondité en enrichissant en azote le milieu nutritif utilisé pour la croissance du Blé offert aux Insectes. Si l'on ajoute au sucre une ration de pollen et de gelée royale qui fournit de l'azote mais aussi des substances indéterminées, l'augmentation de la ponte des Reines d'*Apis mellifica* L. atteint le double de celle des témoins (CHAUVIN, 1956).

Chez le Doryphore les suppléments protidiques que nous avons apportés à la ration sous forme de caséine semblent indiquer que la fonction reproductrice exige une quantité minimum d'azote au-dessus de laquelle tout supplément est peu efficace.

Les besoins qualitatifs d'azote susceptibles de satisfaire à une ovogénèse normale ne peuvent pas être définis chez les Phytophages avant la mise au point d'un milieu artificiel convenable pour l'Insecte. Dans ce domaine, il convient de souligner la réussite récente de E. S. VANDERZANT (1957) qui a obtenu chez *Pectinophora gossypiella* Saund., pour la première fois, la croissance normale des larves et la reproduction des imagos qui en sont issus sur milieu d'agar chimiquement défini.

Les sucres offerts en suppléments au Doryphore sont remarquablement assimilés par l'Insecte et ils sont en partie stockés sous forme de glycogène dans les oeufs, bien que nous constations souvent une diminution de l'indice de fécondité, ainsi qu'en témoignent les résultats d'analyse suivants (Table VII).

TABLE VII

Aliment	*f. jeunes Bintje*	*f. jeunes + glucose*
quantité d'oeufs pour 20 ♀♀	497 oeufs	307 oeufs
poids total frais des oeufs	304,5 mg	170 mg
poids frais de 1 oeuf	0,61 mg	0,55 mg
glycogène en p. 1000 de p. frais	12,3	17,6
glucose	3,5	4,0

Nous pensons que le Doryphore doit être exigent en glucides pendant la période de maturation en vue du stockage de réserves glycogéniques mais aussi de réserves triglycéridiques ou de lipides énergétiques dans le tissu adipeux du Doryphore avant la diapause (BUSNEL 1939, GRISON et LE BERRE 1953).

Une partie des sucres doit être transformée en graisses comme l'ont bien mis

en évidence FULTON et CHAMBERLIN (1934) chez *Eutettix tenellus* Baker nourri sur un régime exclusivement constitué d'une solution de 1,5% de glucose et de 1,5% de fructose; KOZHANTSHIKOV (1938) a cherché chez plusieurs Lépidoptères, comment les glucides alimentaires étaient transformés en graisses pendant la maturation pour être utilisés dans la formation du vitellus des oeufs.

Pendant la période de reproduction, le Doryphore trouve probablement une ration glucidique suffisante dans son aliment normal, ce qu'expliqueraient les résultats défavorables obtenus avec une ration alimentaire trop enrichie („surchargée") en glucose. Selon nous, on peut admettre que ce sont d'abord les réserves du tissu adipeux qui interviennent dans la fonction ovogénétique: toutes les dissections opérées au cours de la période reproductrice montrent la diminution progressive de ce tissu, pauvre en lipides phosphatidiques.

L'étude du rôle des glucides devrait donc être faite parallèlement à celle du rôle des lipides, sans négliger d'autres constituants essentiels[1]) (SCOGGIN et TAUBER, 1950). J. S. BARKER (1952) a fait varier la composition chimique de *Pisum sativum* L. en modifiant les conditions de culture et, quelles que soient les variations de teneur en azote total et en glucides, il a constaté que les lipides jouaient un rôle actif dans la reproduction de *Macrosiphum pisi* Kalt.

Pour notre part, nous avons établi l'efficacité des lipides phosphatidiques sur la fécondité de *Leptinotarsa decemlineata*; et nous avons contrôlé leur assimilation par l'organisme de l'Insecte en effectuant des „bilans choliniques" rapportés ailleurs. Rappelons cependant les données fondamentales obtenues avec des lots importants de femelles nourries avec aliment normal (f. jeunes „Bintje") et avec le même aliment supplémenté avec une émulsion de lécithines à 5 p. 100 (Table VIII).

TABLE VIII

	Lot avec f. jeunes	*Lot avec suppl. lécith.*
ponte totale pendant 10 jours . .	769 oeufs	903 oeufs
femelles fécondes	19 sur 39	21 sur 31
indice de fécondité	2	3
choline totale en mg :		
— pour 1 gr d'oeuf	1,51 mg	1,40 mg
— pour 100 oeufs	0,945 —	0,860 —
— pour la ponte de 10 jours . .	0,727 —	0,777 —
— par femelle	0,249 —	0,290 —
— par mâle	0,166 —	0,176 —

A l'inverse de ce qui se passe pour les sucres, la teneur en choline de l'unité d'oeuf de Doryphores nourris avec un aliment supplémenté est plus faible, mais l'indice de fécondité est plus élevé et sur la ponte totale la quantité de choline est également plus grande.

L'examen des ovarioles pratiqué tous les 5 jours sur des femelles recevant des

[1]) Ainsi la thiamine, qui favorise la conversion en graisses des sucres alimentaires en excès, est rencontrée en plus grande quantité dans les feuilles jeunes des végétaux que dans les tissus âgés (HURIN, 1944).

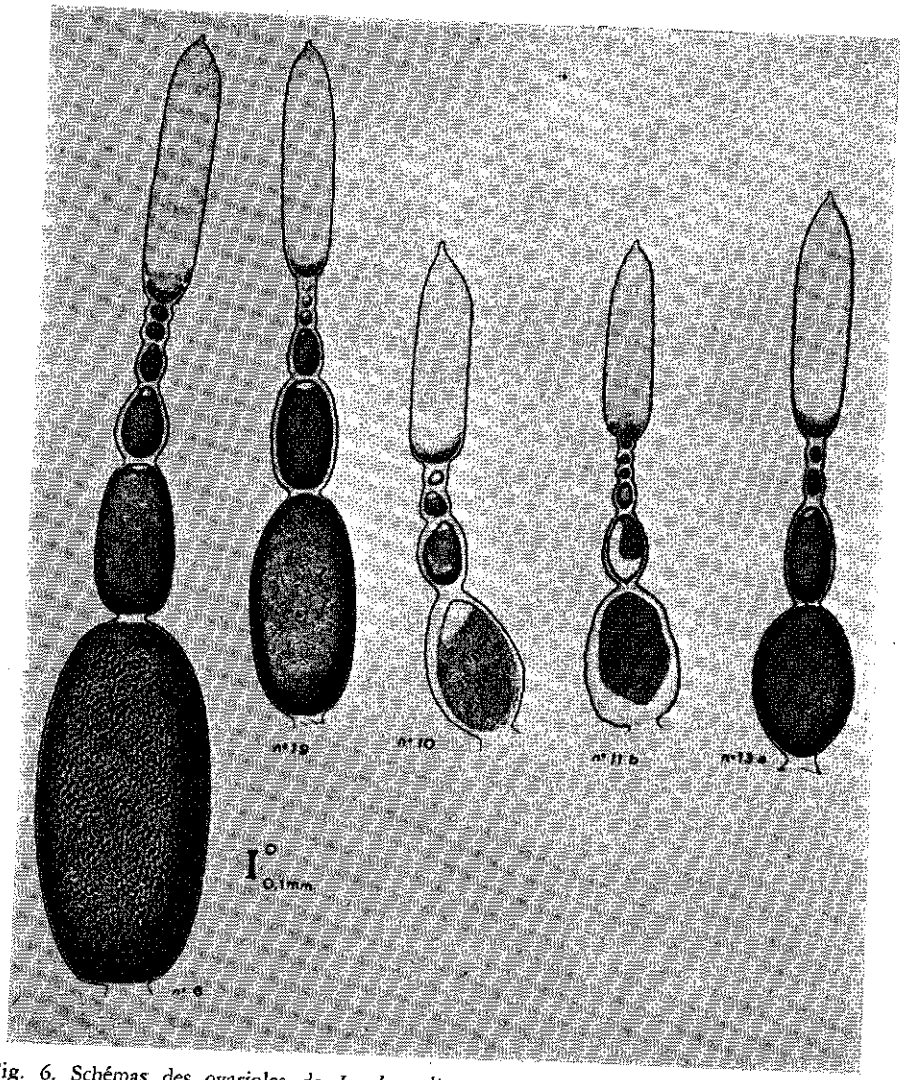

Fig. 6. Schémas des ovarioles de *L. decemlineata* au cours d'une alimentation définie :
— n° 6 après 10 jours sur F. jeune,
— n° 19 — 20 — sur F. jeune + glucose,
— n° 10 — 10 — sur F. âgée,
— n° 11 — 20 — — — —
— n° 13 — 20 — — — — puis 5 jours sur F. âgée + lécithine.

nourritures différentes apporte une nouvelle confirmation du rôle respectif du glucose et des lécithines (fig. 6)[1]).

[1]) Le rôle des stérols aurait mérité d'être pris aussi en considération (de rares essais de supplémentation avec le cholestérol ont été sans résultat). CHAUVIN (1949) a obtenu un meilleur taux de reproduction de *Blattella germanica* L. avec un régime enrichi en stérols à faible dose. Leur rôle a été surtout étudié dans le développement larvaire et la nymphose: HOBSON 1935, FRAENKEL et BLEWETT 1943, GOLBERG et MEILLON 1948, BERGMANN et LEVINSON 1954, RASMUSSEN 1956, etc.

D'autre part l'examen des activités diastasiques a révélé une action lécithinasique très prononcée.

Nous ne savons pas quelle est la fraction assimilable des lipides phosphatidiques. Mais nous accordons une importance particulière à l'opinion émise par PEPPER et HASTINGS (1943) puis par FRAENKEL et BLEWETT (1947) sur le caractère diététique fondamental de l'acide linoléique et sur la nécessité de la présence de cet acide gras dans la plante-hôte pour assurer une fécondité normale chez *Loxostege sticticalis* L. Cet acide est, avec l'acide linolénique et l'acide arachidonique, l'un des précurseurs alimentaires indispensables des lécithines et des phospholipides car il est presque exclusivement trouvé chez le végétal et ne peut être synthétisé par l'organisme animal. VANDERZANT et REISER (1956, 1957) l'incorporent dans le millieu synthétique avec lequel ils obtiennent le développement larvaire puis la ponte des imagos de *Pectinophora gossypiella.*

En conclusion, si les variations du rapport C/N ne présentent aucune corrélation avec les fluctuations de la fécondité de *L. decemlineata,* la teneur de l'aliment normal en azote total et en glucides n'est pas indifférente à ces fluctuations. En particulier l'apport glucidique ne doit pas excéder une certaine justification physiologique du seul point de vue de la fonction reproductrice, sinon une partie de la ration glucidique est dirigée vers les tissus musculaire et adipeux et est susceptible d'accroître le potentiel d'activité motrice de l'Insecte.

Par voie de conséquence indirecte, le rythme des processus ovogénétiques est occasionnellement ralenti puisque le siège d'intense métabolisation des matières est ailleurs: on assisterait en quelque sorte à une „déviation du métabolisme" sans que le rôle des glucides soit pour autant devenu négligeable dans l'accomplissement de la fonction reproductrice chez le Doryphore.

Tandis que, normalement, l'enrichissement et les exigences de l'organisme de *L. decemlineata* en lipides phosphatidiques s'expliqueraient par le rôle de ceux-ci dans le transfert et l'utilisation des glycérides énergétiques, assuré peut-être sous contrôle endocrinien, sans que soit exclue la mobilisation d'une partie des lécithines par la fonction reproductrice et spécialement par la vitellogénèse.

SUMMARY

The fertility of oligophagous plant-eating insects varies, depending both on the species of plant eaten, and on the variety of that plant. We have investigated the effects of certain organic substances in the leaves of the potato *Solanum tuberosum* L. on fertility in the Colorado potato beetle *Leptinotarsa decemlineata* Say. By considering the ratio between the "maintenance ration" for the adult beetle, and the amount eaten during egg production, it is possible to determine the efficiency of egg production.

Causes of variation in the chemical constitution of leaves are reviewed. These include cultural conditions, time of day, stage in development, age of the leaf. Changes in the C/N ratio are less important than changes in the amount of cholin-containing lipids in their effect on the fertility of the Colorado potato beetle.

The effect of nitrogen and carbohydrates was studied mainly by adding different amounts of organic substances to potato leaves. Thus casein increased the rate of egg laying when the leaves were poor in nitrogen. The effect of adding glucose is more difficult to interpret, because of its importance in muscular activity. When glucose is provided in large quantities, some is stored in the ovaries as glycogen and this diminishes during egg production.

The importance of phospho lipides can be shown more exactly; some part of the lecithins are mobilised during reproduction and particularly when yolk is being produced.

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DISCUSSION.

J. DE WILDE : Avez-vous observé une augmentation du pourcentage d'individus en diapause en leur donnant des feuilles sénescentes comme aliment?

P. GRISON : Oui, mais pas systématiquement, car dans nos conditions d'élévage sans photopériode suffisante le pourcentage d'individus en diapause était toujours élevé.

C. ELLENBY : Have you considered the possible influence of photoperiod on your results ? This will vary with the temperature and potato variety. I have found with the potato root eelworm that the number of females is reduced on plants grown under short day conditions, and others have found fewer eggs laid by the root knot eelworm on plants grown on short day conditions. It has also been shown by VAN DEN BRANDE and his colleague that this effect varies with the wave-length of light.

P. GRISON : Les cultures ombrées sont faites sous ombrage permanent et nous obtenons du feuillage étiolé. Je remercie Dr. ELLENBY de ses suggestions et je suis persuadé de l'intérêt d'utiliser le photopériodisme et différentes lumières pour modifier le chimisme végétal. Je n'ai pas considéré les différences de réponse des „variétés" à de telles conditions et cette suggestion est également très intéressante.

A. J. THORSTEINSON : Dr. GRISON has demonstrated that lecithin increases not only *fecundity but also consumption of food.* Does he agree that *the latter is an indication* that lecithin improves the palatability of the food ?

P. GRISON : Les tests de fécondité ne sont pas une démonstration suffisante mais je pense que ce facteur du comportement peut être pris en considération dans le schéma du Prof. DE WILDE et qu'il devrait être étudié selon les méthodes exposées par DE WILDE et Dr. THORSTEINSON.

K. SCHREIBER : I should be grateful if you could tell me, how you have tested the effect of lecithin on the Colorado Beetle.

P. GRISON : J'utilise des lécithines d'oeuf du commerce qui sont émulsionnées dans l'eau distillée après avoir ajouté une goutte d'alcool 70°. L'émulsion est répandue sur la feuille par pulvérisation.

Ph. SEDEE : Did you study possible alterations in the mutual ratio of the fatty acids *during metamorphosis and developping of the eggs* ?

P. GRISON : Je n'ai pas étudié cette question.

SOME PHYSIOLOGICAL ASPECTS OF THE HATCHING MECHANISM OF THE POTATO-ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER

BY

C. ELLENBY

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I am most grateful for this opportunity to participate in this symposium which must, I think, be the first occasion on which plant parasitic nematodes have been placed on an equal footing with their more illustrious, or perhaps I should say, notorious, insect colleagues. Although I have been aware of the similarity of many of our problems, I must say that, speaking on the final day of this symposium, I have been astonished how similar many of them really are.

Since many members of the symposium may not know the potato-root eelworm, I think it might be useful if I said a little about it before dealing with the particular problem I wish to discuss.

Larvae of the potato-root eelworm invade the roots of the potato-plant. Once inside, they move very little and feed on the products of the so called "giant cells" which their presence evidently induces the host plant to form. They go through the usual nematode moults, and, eventually, the mature male leaves the root and returns to the soil. But the last stage female remains behind; her body swells until it ruptures the cortical layers of the root and projects to the outside, but remains embedded and attached at the anterior end. In this position she is fertilized by the male. Her body continues to swell until it is spherical, with a short neck. At first the mature female is white, but it eventually turns brown due to the "tanning" of the body wall by a process very similar to the tanning process of the insect integument. The mature female has now become a cyst filled with several hundred eggs, each containing a coiled larva.

When the plants mature, the roots eventually decay and the cysts fall off into the soil; or they may be dislodged when the plants are lifted. Inside the cyst the eggs are well protected: cysts which have been in the soil for seventeen years may still contain viable eggs.

I have recently been studying the effect of compression on the free larva and also on the intact egg. Single eggs are compressed between glass plates in the simple apparatus shown in Fig. 1. This consists of a cylindrical brass cell cemented to a glass plate which stands on the stage of a dissecting microscope. It is closed at its upper end by a wall consisting of glass in the centre, and of rubber at the periphery where it is cemented to the brass cell. By reducing the pressure in the cell by a pump, the upper wall is pulled in to compress a single eelworm egg on a piece of glass supported at an appropriate height on a hollow metal collar. The egg can be observed continuously and, by controlling an inlet

valve, the compression can be sensitively applied or reduced. The film which you see shows eggs and hatched larvae when the pressure is applied and when the pressure is removed. It shows a number of things which, although, fairly obvious, are nevertheless important to our understanding of the hatching mechanism.

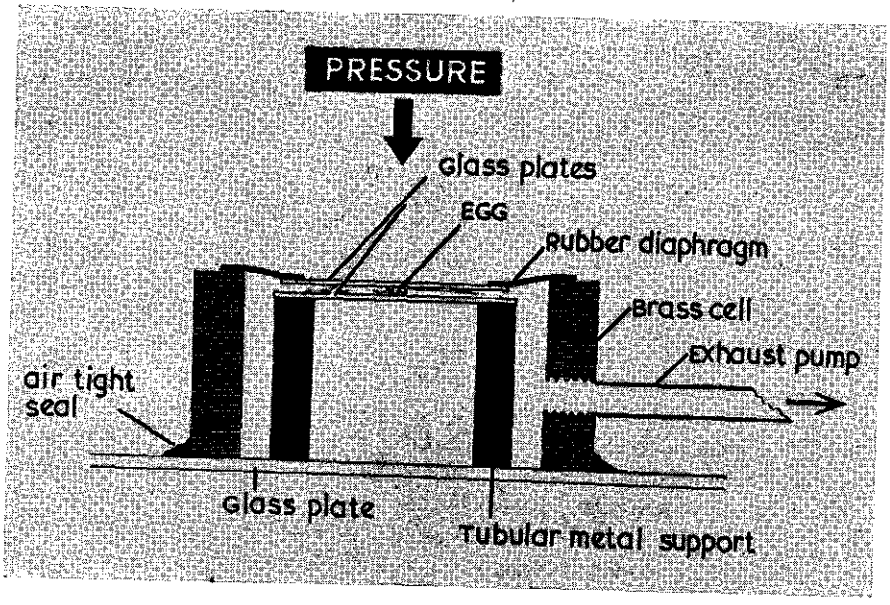

Fig. 1. Compression apparatus. An air inlet (not shown) on the line to the exhaust pump, is controlled by the finger tip and pressure can readily be increased or diminished.

Firstly, it shows that the larva is exceedingly elastic; when compressed, it will extend in length and breadth by more than 50%, and, on removing the pressure, it will return to its original dimensions without apparent harm. Secondly, the intact egg is also elastic, readily returning to its original form on removal of the compression. When an intact egg is compressed, the larva inside changes its position to conform to the new dimensions of the egg; on removal of pressure, it returns to its original position.

If the compression is increased sufficiently, the shell is ruptured and the larva set free. It is very noticeable that the liberated larvae usually begins to move at once. Yet, while they are inside the uncompressed egg, no movement is detectable. It seems clear that no movement takes place inside the intact egg because none is possible: that the larva, due to its own elacticity, is pressing against the shell and prevented from moving.

From all this it appears that the hatching stimulus does not "wake" the larva up, for, most of the eggs in these tests were not "asleep" — as they showed when the eggs were ruptured. The contained larva is pressing against the shell under its own tension — rather like a spring in a box; but the pressure is insufficient to overcome the elastic limit of the shell. The hatching stimulus leads

to an increase in the tension of the larva until this limit is overcome. I hope to show that we may at last have some indication of the possible sources of this increased larval tension.

The nematologist working on the cyst forming eelworms is confronted by all the problems which trouble his entomological colleagues. How the parasite finds its way to the host, how it enters, grows and reproduces. But he is concerned with another problem also, for these animals are stimulated to hatch by substances given off by the host plants. The hatching mechanism is therefore of particular interest to him. Now there are a number of different species of cyst forming nematodes belonging to the genus *Heterodera*. Of these the potato-root eelworm is a particularly specific example, being confined, among cultivated plants, to the potato and tomato. Although the others are, perhaps, not quite so specific, they all show specificity of a sort, being on the whole confined to particular families or groups of related plants. Triffitt showed in 1930[1]) that the potato plant gives off a substance from its roots which has a stimulating effect on the hatching of the potato-root eelworm. We now know that, perhaps with certain exceptions, the different forms are stimulated by substances given off by their own host plants[2]). The position is not quite as simple and straightforward as that, but, speaking generally, it would not be unfair to say that there is, on the whole, a relationship between the hatching of the eelworms and the substances given off by the roots of their host plants. Truly, I think this is a most remarkable example of the evolution of parasite — host specificity.

Most is known about the hatching of the potato-root eelworm, and the nature of its hatching factor has attracted considerable interest. For many years, TODD and his co-workers in Cambridge[3]), and more recently JANZEN[4]), in Groningen have attempted to isolate the factor. But although a good deal is known about its constitution, its exact nature is still unknown; the mechanism of its action is even more mysterious. Attemps to isolate the factor are severely handicapped by assay difficulties, for the only method by which the hatching actor can be detected is the hatching trial which, in its most commonly used form, is of three weeks duration and involves about two million eelworms[5]); and a test of this nature must be carried out at each stage of the attempt at isolation in order to discover which way the "cat has jumped". It seemed to me highly desirable to try to find some preparation other than the eelworm which responds to the hatching factor; not only might this throw some light on the nature of the hatching mechanism itself, but it might also be of direct use as an assay method. Due to the generosity of the Nuffield Foundation, I have been enabled to undertake such an investigation with the help of Mr. A. B. GILBERT; in the remainder of this communication, I would like to describe some very promising results we have obtained.

In 1949 TODD and his colleagues showed[6]) that the hatching factor was probably an unsaturated lactone acid with a molecular weight of about 300. In addition to the lactone ring which it probably contained, a carboxyl group and two "OH" groups were also probably present. It seemed to me not unreasonable to assume that the activity might be associated with the lactone ring: it is well known that the lactones, particularly the unsaturated forms, include some of the most biologically active substances, and, in particular, that the cardiac glycosides

Digitoxin, g-Strophanthin, etc., are among them[7]). Moreover, it has already been shown that some, though not all, of the actions of the cardiac glycosides on the frog heart are also produced by simple unsaturated lactones. [8], [9], [10], [11]). We have now found that the isolated frog heart responds to the hatching factor.

In general, the techniques we have used have been those of GIARMAN[9]). In some trials we have perfused the heart continuously *via* the sinus venosus, using the cannula he describes; more frequently we have, like him, used a modified Straub preparation, cannulating the ventricle *via* the truncus. The former method has undoubted advantages, but about 50 ml. of solution are required for a single test: with the Straub technique, however, the ventricle and cannula form a closed system and a test can be carried out with 1 ml. only. Since it was of overwhelming importance for us to conserve our small supplies of active preparations, the economical Straub preparation was a great help.

A test is carried out as follows. After isolation from the body the heart is first perfused with Ringer's solution. When it has settled to a steady state, the Ringer is replaced by a similar solution, but containing half the Ringer quantity of calcium. A hypodynamic state is induced, the beat being reduced in amplitude; perfusion with a cardiotonic substance in low calcium Ringer leads to recovery. Fig. 2 shows a typical tracing obtained by perfusing with a series of four dilutions of a concen-

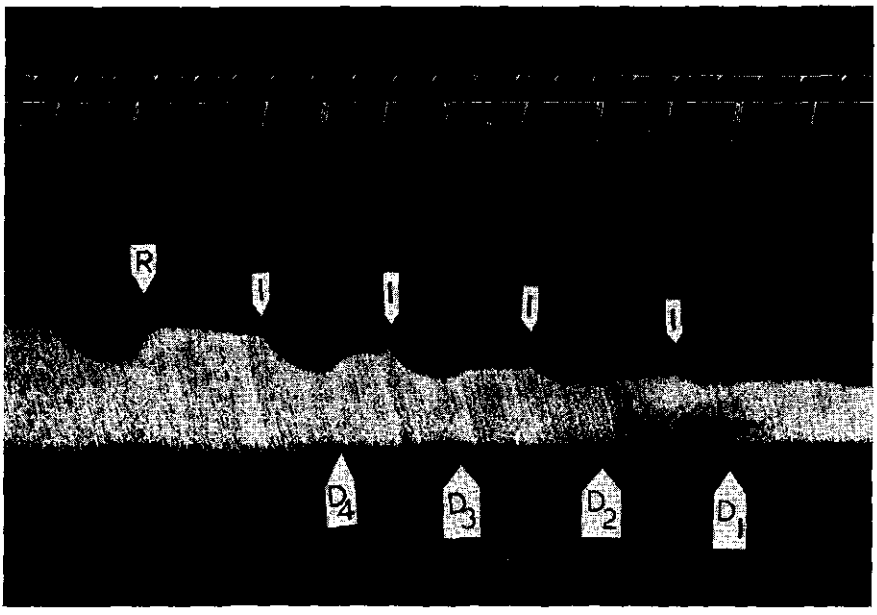

Fig. 2. Isolated frog heart perfused with a series of dilutions of concentrated hatching factor G. 1. Tracing reads from the left with the heart being perfused with Ringer, and then by low calcium Ringer (at signal !) which depressed the beat. Recovery takes place in Ringer (R) and partial recovery in successive twofold dilutions (D4—D1) of the hatching factor in low calcium Ringer, each being followed by low Ca Ringer. Time signal, 30 sec. Straub preparation.

trated preparation of the hatching factor. Since the time signal operates every 30 seconds, it will be seen that the duration of this "assay" with 4 concentrations, was only about 20 minutes. Repeats with other frogs are, of course, necessary, for frogs are variable, but the contrast with the three-week hatching trial is very striking.

Almost all our work has been carried out with either of two concentrated preparations. One, "standard solid", was kindly given to me by Sir ALEXANDER TODD some years ago. For the other preparation, G. 1, we are indebted to Dr. J. JANZEN, of Groningen. With either preparation, the response varies with dilution, within certain limits; a more severe test ,however, is a comparison of the two. The activities have been compared by the minimal concentration required to produce a detectable effect: the standard has its limitations and it would probably have been better to consider the dose necessary to restore the heart completely. However, the necessity to conserve material has again been the deciding factor, and, in the event, comparisons on this basis have shown good agreement.

TABLE 1.

Relative hatching strength and Cardiotonic activity.

Preparation	Optimum Hatching conc. mg./ 100 ml.	Relative Activity, Hatching	Minimal Cardiotonic conc. mg./ 100 ml.	Relative Activity, Cardiotonic
Groningen 1	0.8	5.0	3	6.0
Groningen 2	2.5	1.6	5	3.6
Cambridge "crude solid"	4.0	1.0	1	1.0

The results of the comparisons between these two substances have been set out, in the top and bottom lines of Table. Assessed by the hatching assay, the ratio of their activities is the 5 : 1 in favour of G. 1; by the heart assay the ratio is 6 : 1. The agreement between the assay methods is therefore good. But you will notice that the minimum cardiotonic concentration is four or five times as great as the optimum hatching concentration.

Very recently we have also carried out tests with two further Groningen preparations, G. 2 and G. 3. With these, agreement has not been so good. As shown in the middle line of the Table, G. 2 has a higher cardiotonic activity than its hatching assesment would suggest; but at least it falls into its correct relative position. G. 3 was the weakest preparation we have tested; and a sufficient quantity for tests with only ten hearts was available. In the event, the first run showed a moderate agreement with hatching assay; however, no further positive response was obtained with any of the other hearts. We have no explanation to offer for this as yet; but we have some evidence that the factor in this preparation may have been altered in some way.

As an assay method for the hatching factor, perfusion of the frog's heart has many limitations. The fact that it is far less sensitive is of little importance,

for solutions of root diffusates are readily concentrated[19]). But the frog heart must be perfused with solutions of carefully controlled ion content, and solutions obtained from potted potato plants will be quite unsuitable; but is has already been shown that is is not impossible to overcome this difficulty[4]). Moreover, the Cambride preparation has been assayed successfully by its cardiotonic activity, and this preparation was obtained as a result of the first step in the attempt to isolate the factor, *viz.*, adsorption on charcoal, elution with acetone, and evaporation[3]). A more serious difficulty is the fact that the heart assay is unspecific — clearly the final arbiter of hatching activity must be the eelworm. Nevertheless, we are confident that, used with discretion, assay by perfusion of the hypodynamic frog heart will be of use in attempts to isolate the hatching factor.

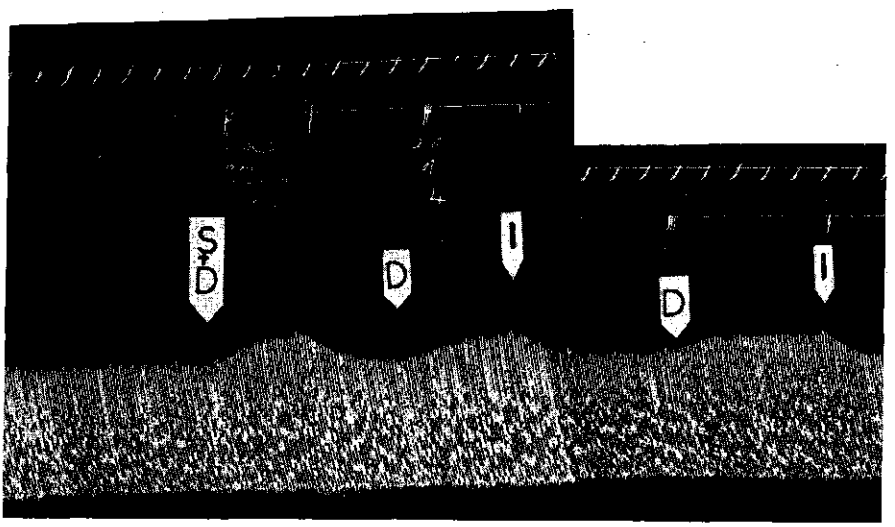

Fig. 3. "Synergism" with g-Strophanthin. Tracing reads from left with heart being perfused with low Ca Ringer. This heart had already been perfused with solutions of hatching factor and g-Strophanthin (S) in low Ca Ringer, and ineffective doses established. Perfusion with a solution containing *both* substances at subminmal dose (S + D) leads to recovery; the effect of the stophanthin persists, for subsequent perfusion with hatching factor alone (D) at subminmal conc., is now effective. Each solution is followed by low Ca Ringer (I). Time signal 20 sec. Straub preparation.

Considering that we are dealing with preparations which are far from pure, the activity of the stronger ones compares very favourable with the more active of the simple lactones. Moreover, as shown in fig. 3, the minimal concentration can be halved by perfusion together, or subsequent, to perfusion with a sub minimal concentration of g-strophanthin. A response at half the minimal concentration has also been obtained by perfusing the heart with the hatching factor plus a subminimal concentration of hydrogen peroxide. This "synergism" with g-strophanthin and potentiation with peroxide are of particular importance. For they indicate that the responses obtained are probably identical with those given by the pure lactones. Moreover, we have found that hydrogen peroxide also

"potentiates" hatching: as shown in Table 2, addition of only 5 mg of peroxide per 100 ml. of a solution of hatching factor more than doubled larval emergence. Peroxide alone was without effect. Larval emergence was low, because a very dilute solution of hatching factor was deliberately chosen; far more larvae emerged in identical drops of more concentrated solutions which, presumably, also indicates that the effect of peroxide could not have been due simply to a shortage of oxygen as such.

The increased cardiac and hatching activity produced by the addition of traces of hydrogen peroxide to solutions of the hatching factor strongly suggests that there are close similarities in the mechanisms involved.

The physiological activity of cardiac glycosides has recently attracted considerable attention since certain of them have been shown to influence Sodium-Potassium transport in erythrocytes[12]), the frog's skin[13]), and isolated rabbit auricles[14]). Digitoxin had been shown to give rise to a peroxide in solution and it has been demonstrated that, in this form, it may influence phosphorylation[15]). Simpler lactones may also have these actions; in fact, it was shown some years

TABLE 2

Increase in activity of hatching factor when accompanied by a trace of hydrogen peroxide.

Concentration of hatching factor, 1/64 optimum.
Concentration of peroxide, when present, 5 μ g./100 ml.

Test solution	Larvae per cyst.	Cyst "hatching", per cent
Water	1.3	40.0
Peroxide alone	1.7	53.3
Hatching factor alone	5.7	56.5
Hatching factor + peroxide	13.4	86.5

ago that they may behave as peroxides[11]), and more recently, it has been shown that certain of them may influence ion transport[20]). We have shown that the hatching factor has certain of the properties of the cardiac glycosides and simpler lactones; if it should be shown that it influences the same fundamental physiological processes, not only will our understanding of the hatching mechanism be deepened and techniques of perhaps greater sensitivity and convenience made available for its detection, but our approach to the question of control may be radically altered.

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SUMMARY OF DISCUSSION

Dr. DEN OUDEN asked whether it might be possible to use the compression apparatus for determining differences between live and dead eggs and larvae.

Dr. ELLENBY pointed out that in the film marked differences were shown between alive and obviously dead eggs. But used for such purposes with not obviously dead eggs, the method would be confronted with the same problem which confonts other methods — to decide whether differences are *really* due to the fact that some of the eggs are dead.

Dr. SCHREIBER noted that, like his own results on resistance to colorado beetle, Dr. ELLENBY's seemed to favour a biophysical rather than a bichemical explanation.

Dr. ELLENBY was not sure that he agreed with the last point. The *ultimate* result would be an increase in physical force, but, assuming that he were correct about the action of the hatching factor, it seemed more likely that the force arose from a biochemical process — the mechanism involved in ion transfer.

Professor DE WILDE asked whether the hatching factor could influence the egg shell.

Dr. ELLENBY thought this unlikely.

Dr. KENNEDY thought it was crucial to the theory to discover whether there was an increase in the turgor of larvae in hatching solutions.

Dr. ELLENBY said that that was one of the reasons for undertaking the compression experiments. But he did not agree that is was necessarily crucial. Although a redistribution of the proportion of ions was not excluded, the generally held view seemed to be that the transfer of Sodium and Potassium takes place ion for ion: this would, by itself, lead to negligable changes in osmotic pressure. But the ion transfer could involve to differences in the polarity of the cell membranes, and this could lead to more transitory changes in tension through muscular activity. But all this was hypothetical since it had not been shown whether the hatching factor did influence ion transfer.

Dr. TAMMES pointed out that Dr. ELLENBY had suggested that the hatching mechanism operated *via* a fundamental physiolical process, yet the potato eelworm was specific. How could the specificity of hatching substances be explained if this were a general principle or substance?

Dr. ELLENBY thought that is was probable that similar substances *were* involved, and that his preliminary observations16 and those of Todd and his co-workers supported this. Specificity often depended on slight differences in molecular structure and perhaps such differences would be found in the hatching substances. The mechanisms should be *expected* to be similar. If they were not to be endowed with magical properties, the substances must work through some process common to the related eelworms, or each must influence a special process. The latter seemed to him much more difficult to envisage.

But all sorts of problems arose from these observations. For example, if they were correct, hatching should be influenced by the ionic background. This they had found to be the case16, and so too had Dr. JANZEN18. If this were also true of related eelworms, as one would expect, the whole question of diffusates of host and non host would need re-examination. Different species of plant might make different demands on the minerals present in the soil and leachings could therefore have different ionic compositions. Low or high emergence in such leachings could therefore be due not so much to weakness of the hatching factor but to differences in the ionic background in which it was functioning.

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RESISTANCE TO THE POTATO ROOT EELWORM IN S. TUBEROSUM SUBSP. ANDIGENA AND ITS IMPORTANCE FOR POTATO BREEDING [1] [2]

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SUMMARY

A survey is given of the way in which a search is made for potatoes resistant to the potato root eelworm *Heterodera rostochiensis* WOLLENWEBER, at the Foundation for Agricultural Plant Breeding, Wageningen. A very high degree of resistance was found in the clone CPC 1673 belonging to the species *Solanum tuberosum* subsp. *andigena*. The inheritance of this resistance is dominant and monofactorial. The nature of the resistance is discussed in detail. For the breeding work the method of repeated backcrossing is used. Valuable seedlings were found in the first backcross generation.

Finally a survey is given of the data on the occurrence of biological races of the potato root eelworm which are able to multiply strongly in the resistant material derived from CPC 1673.

1 INTRODUCTION

Potato sickness is caused by the cyst-forming nematode *Heterodera rostochiensis* WOLLENWEBER. The whole life-cycle of this parasite takes place in the soil. If control of soil parasites is in general not easy, that of the potato root eelworm is hampered severely by the eggs being particularly well-protected by the cyst-wall.

In the Netherlands the spread of the disease is checked by prohibiting the growth of potatoes in infested soil and by the legal regulation that potatoes can be grown at most once in three consecutive years on the same field.

A direct means of control which would be economically justified has not been found as yet. Growing potato varieties resistant to or immune from potato sickness would be the most economical method of control of this disease. This quality of resistance, however, does not occur in the cultivated West European and North American potato varieties (*Solanum tuberosum* subsp. *tuberosum*).

A very high degree of resistance has been found in some varieties of *Solanum tuberosum* subsp. *andigena*, growing in South Peru and Northern Bolivia. This material has become the basis of an extensive breeding programme, aiming at the creation of potato varieties resistant to potato sickness. The following gives some details of this breeding work.

2 THE SEARCH FOR RESISTANCE AND THE RESULTS OBTAINED

A potato plant possesses a certain degree of resistance when no cysts are found on the roots (when grown in infested soil) or at any rate so few that the population of the parasite decreases.

[1] Received for publication November 28, 1957.

[2] Elaborated from the text of a paper read at the Symposium "Plant and Insect" held at Wageningen, The Netherlands, from 28–30 May 1957.

The procedure for testing the resistance at the Foundation for Agricultural Plant Breeding (Wageningen) is as follows:

The seed is sown in a seed-tray filled with soil which is free from living larvae. When the seedlings are approximately 2 cm long they are pricked off into another seed-tray. After some time the seedlings are transplanted in pots of 8 cm diameter. When the seedlings are growing well and the potball is well-rooted, they are ultimately planted into pots of 13–17 cm diameter, filled with heavily infested soil which has been mixed thoroughly beforehand. Then

FIG. 1 TESTING FOR RESISTANCE.

the pots are dug into the soil or into peat dust as far as the brim in order to prevent excessive evaporation.

If tubers are available for testing experiments, they are directly planted in infested soil in the above-mentioned pots of 13–17 cm diameter.

When very precious material is involved, the tubers are first induced to produce roots in little pots filled with healthy soil and later on transplanted into large pots which are filled with infested soil.

The examination for resistance can begin about eight weeks after transplanting in infested soil. After this period cysts, if any, are clearly visible; they are yellow to orange in colour and are clearly conspicuous against the roots and soil particles. For assessing the plants are removed from the pots and the roots visible at the surface of the rootball are inspected for the presence of cysts (Fig. 1). If desired the examination can be repeated later, as the potball can be put back again into the pot with little damage to the plant.

Although this method is a "superficial" one – in as far as only part of the root system is inspected – yet for breeding purposes it has proved of great value since large numbers of seedlings can be tested in a comparatively short

time. And the handling of large numbers is important since the resistance occurs but sporadically in nature.

At Wageningen thousands of plants have been tested in the course of years. Resistance was found in:

a Five clones derived from the Commonwealth Potato Collection (CPC), all of them belonging to the species S. *tuberosum* subsp. *andigena*. An indication of the occurrence of this resistance was first found by ELLENBY (5); the most resistant number is CPC 1673.

b Some clones of S. *vernei* and S. *sucrense* (4,9).

c Some clones of S. *macolae* and S. *famatinae*. Both these species are diploid.

d Five *andigena*-numbers of the collection of South American potatoes (10). at Wageningen. This collection comprises some 900 clones.

It stands to reason that most attention is paid to the resistant *andigena*-material as this material belongs to the same species (S. *tuberosum*) as our cultivated varieties and consequently are easy to cross with.

In the Netherlands so far the line CPC 1673 has been used almost exclusively. The following details are related to this line or to its offspring.

3 THE INHERITANCE OF THE RESISTANCE

To design a justifiable breeding programme, it is necessary first to know the mode of inheritance of the resistance. For this purpose crosses were made between different resistant clones of S. *tuberosum* subsp. *andigena* and between plants of this species and susceptible commercial varieties. The seedlings derived from these crossings were raised and then tested according to the above-mentioned method. It appeared that these seedlings were either entirely free from cysts or attacked just as heavily as the susceptible control-plants. Transitional stages did not occur. On some plants sometimes a small number of cysts (1–5) were found; from special experiments it appeared that these plants had to be classified as resistant ones.

The segregations observed, in resistant and susceptible, could be adequately explained by assuming the resistance to be due to one dominant gene H, inherited in a tetraploid manner.

Resistant plants can then be represented by the following genetical formulae:

H h h h : simplex,
H H h h : duplex,
H H H h : triplex,
H H H H : quadruplex;
a susceptible plant by h h h h (nulliplex).

There appeared to be no difference in the degree of resistance between simplex and duplex seedlings. However, there are great differences in the numbers of resistant offspring of these categories when crossed with a susceptible partner. A simplex resistant plant gives 50 % resistant offspring, a duplex plant about 83 %, triplex and quadruplex plants 100 %.

Some examples of such segregations in resistant and susceptible are tabulated below (Table 1).

Table 1 Results of testing seedlings of the first backcross-generation.

Parentage	Numbers of seedlings tested	Numbers of seedlings		Theor. ratio
		resistant	susceptible	
a) Crossings with simplex resistant seedlings				
A6 [1])– 3 × G 865	10	6	4	1 : 1
A6– 5 × Libertas	82	41	41	1 : 1
A6– 6 × Libertas	30	16	14	1 : 1
A6– 7 × G 874	38	20	18	1 : 1
A6– 8 × G 874	54	29	25	1 : 1
A6– 9 × Libertas	30	15	15	1 : 1
A6–12 × Libertas	28	13	15	1 : 1
A6–14 × G 865	24	15	9	1 : 1
A6–20 × G 865	36	16	20	1 : 1
A6–21 × Furore				1 : 1
A6–26 × G 865	25	11	14	1 : 1
A6–33 × Libertas	34	21	13	1 : 1
A6–37 × Libertas				1 : 1
A6–39 × G 865	35	20	15	1 : 1
A6–41 × G 874	32	18	14	1 : 1
A6–47 × Libertas	16	8	8	1 : 1
A6–48 × G 874	13	7	6	1 : 1
Total	487	256	231	1 : 1
Theoretical		243,5	243,5	5 : 1
b) Crossings with duplex resistant seedlings				
A6–18 × Libertas	19	16	3	5 : 1
A6–34 × G 865	21	17	4	5 : 1
A6–44 × G 874				
Total	40	33	7	5 : 1
Theoretical		33,3	6,7	5 : 1

[1]) A6 : Record × 1673–1.

On backcrossing of resistant F_1-plants with susceptible parents the resistance is maintained to the same degree and this fact is of great importance for potato breeding.

If plants derived from crosses between the resistant clone CPC 1673 and susceptible *tuberosum*-varieties are tested in the way described above, plants will occur with very few cysts apart from the ones which are heavily attacked and the ones which are not at all affected. Genetical examination proved that such plants possess the gene H. Close observations have taught that they possess a somewhat lower degree of resistance than the plants which on testing appear to be entirely free from cysts. These slight differences have a genetical basis.

4 Breeding commercial potatoes for resistance to *Heterodera rostochiensis* W.

From the investigations described above it follows that in breeding potatoes for eelworm resistance no great difficulties are to be expected. The method of repeated backcrossing was used. In this procedure the gene H derived from

a plant with little cultural value (at least in this country) is transferred into the genom of our good potato varieties.

On account of the favourable way of inheritance it is not difficult to raise large numbers of resistant seedlings. However, selecting from this material seedlings with favourable qualities (e.g. short stolons, good yield, convenient size, good starch-quality etc.) is a difficult and time-consuming work, the technical details of which will not be treated in this article. It can be stated that very promising seedlings were found in the first backcross generation already.

5 The nature of the resistance

The roots of the resistant plants as well as those of the susceptible ones produce the "hatching factor", a substance that induces the larvae to leave the cyst and to penetrate into the roots of the plant. It appeared that the concentration of this root-diffusate of some resistant *andigena* × *tuberosum*-seedlings was of the same order as that of the susceptible variety Eigenheimer. The number of larvae invading the roots of resistant plants is certainly not lower than that penetrating the roots of susceptible plants. It is difficult to draw an exact comparison as the numbers of invading larvae are also influenced by the size and structure of the root system. Resistant and susceptible seedlings of the same cross will always show inheritable differences in this respect.

In resistant roots, however, the development of the larvae and especially of the female ones is inhibited. (Fig. 2).

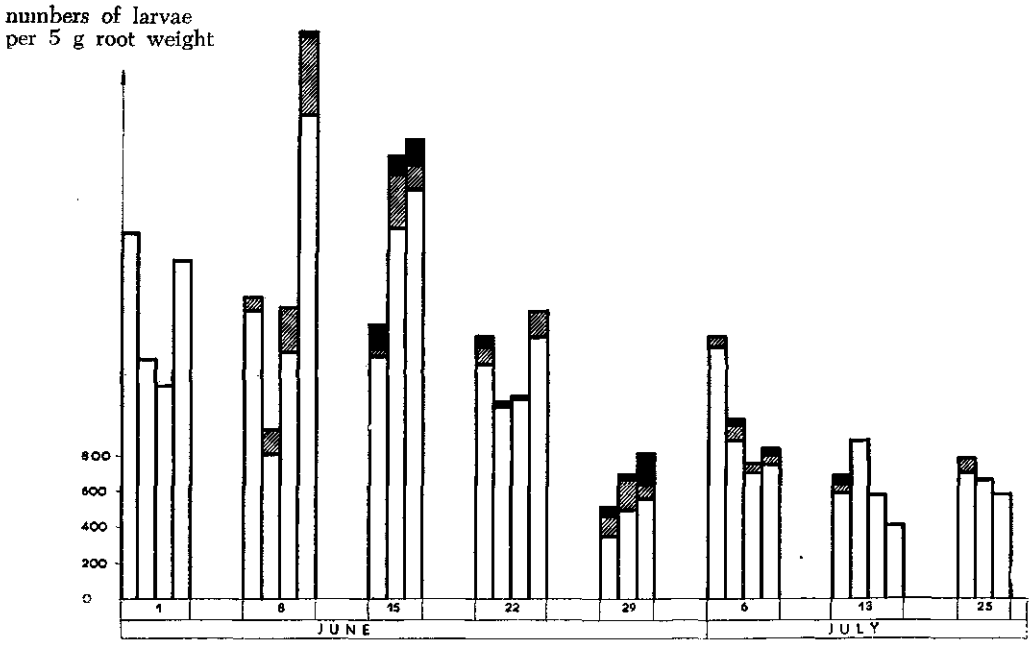

Fig. 2 Numbers of larvae that penetrated into the roots of a resistant *andigena* × *tuberosum* clone and their development in the course of time.

second stage larvae
third stage larvae
higher stages of male larvae

Females reach maturity only sporadically; mature males are fairly frequently observed. This difference in development between the sexes in roots of resistant plants can possibly be explained in the following way. After being about ten days in the roots the larvae start moulting. After this period the males are still in their old skin. It is assumed that they do not take up food any more. The females contrarily moult repeatedly, but their mouth and anus continue to stay in such a position that it is likely that they go on feeding. It is therefore accepted that the females continue to take up food and by that they are much more subjected to unfavourable conditions in the interior of the resistant roots. Nothing is known about the growth-inhibiting factor; may be there is a substance detrimental to the development of the larvae; but there is also a possibility that one or more substances are lacking that are necessary for the development of the larvae.

In order to learn more about the nature of the resistance, resistant plants were grafted on to susceptible ones and the other way round. However, no influence of the scion on the rootstock could be established as concerns the degree of resistance or susceptibility, so that this experiment gave no clearer solution to the problem.

On close examination of the root system of resistant plants mostly a few cysts are found, to be true. But contrary to expectation these cysts do not contain an abnormally small number of eggs. Possibly these females have developed on places of the roots where the physiological conditions determining the resistance are absent or less manifest. One could even suggest that there are islands of tissue in which by a somatic mutation the gene H has changed into h.

From field trials it appeared that the resistant potato varieties are greatly damaged by the invasion of large numbers of larvae into the roots. (Fig. 3). It is highly probable that resistant varieties are equally sensitive to potato sickness as susceptible ones and thus on heavily infested soils are equally affected as susceptible varieties. The great advantage of growing resistant varieties is that they do not enlarge the population of the potato root eelworm. On the contrary, it appeared that the growing of resistant varieties lowered the degree of infestation of the soil (6). For these resistant potatoes exert a nematocidal influence by secreting a hatching substance and by inhibiting the development of the invaded larvae. The reduction of the larvae-population in the soil by growing a resistant potato variety is greater than when immune crops are grown. The reduction which then occurs is to be ascribed to the hatching influence of the moisture in the soil (2).

6 Biological races of the potato root eelworm

At first no indications were found for the existence of biological races of *Heterodera rostochiensis* which would be able to multiply in the resistant material derived from CPC 1673. The possibility, however, that such varieties will be found in the future has been soundly accounted for.

In 1956, Dr. Toxopeus of the Institute of Agricultural Plant Breeding, made a tour to Peru and Bolivia in order to collect new material (11). One of the main objects was to broaden the basis of the breeding work for *Heterodera*-resistance which in the Netherlands is exclusively based on the offspring of one resistant clone.

FIG. 3 PHOTOGRAPH SHOWING THE SENSITIVITY OF THE RESISTANT MATERIAL. IN THE FOREGROUND PLANTS ON A PLOT IN WHICH SOME 40.000 LIVING EGGS PER 200 g AIRDRY SOIL ARE FOUND; IN THE BACKGROUND THE SAME MATERIAL ON A PLOT CONTAINING SOME 5000 LIVING EGGS PER 200 g AIRDRY SOIL.

The first indication of the existence of more aggressive forms of the parasite was found by VAN DER LAAN and the present writer, when they grew resistant plants in pots which were inoculated with cysts derived from Peru (8). On the roots of these plants considerably more cysts developed than on the roots of the same material grown in pots infected with equal numbers of cysts that were isolated from soil of an infested plot in the vicinity of Wageningen.

That these different forms were found in Peru, of all places, is not surprising. For the centre of origin of the potato lies in the North of South-America and Peru forms part of it. The hypothesis is obvious that the potato root eelworm originated in the same region. The genetical variability of the parasite must be greater there than in Western Europe. The fact that the potato root eelworm occurs almost everywhere in Peru supports this hypothesis and it seems that its genetical variability is indeed wider there (1, 11).

A more reliable indication of this variability was gained in 1956. In 1955 a number of resistant clones, derived from CPC 1673, were sent from Wageningen to Peru. They were tested for resistance by IR. S. SIMON of the Estacion Experimental Agricole de la Molina. It appeared that all the seedlings which we had found to be resistant were heavily affected in soil derived from Maco Farm (Prov. of Tarma). From the report of IR. SIMON it can be concluded that the roots of these seedlings were as heavily crowded with cysts as those of our susceptible varieties when grown in highly infested soil of Western Europe (10).

In the meantime similar aggressive forms of the parasite were also found in England and Scotland (3, 7). Although the experiments have not been run long enough to draw decisive conclusions the English workers assume the

occurrence of biological races which are equally aggressive in regard to the resistant varieties as the "common" biological race with respect to our susceptible commercial varieties. Besides there seem to be races that are less aggressive.

It is estimated that the area of infested land on which resistant varieties derived from CPC 1673 and other resistant *andigena*-varieties can be grown effectively should be reduced by 10 per cent on account of the possible occurrence of these biological races. A favourable feature is that the potato root eelworm is a soil-parasite and that it spreads only very slowly.

It is unknown how matters stand in the Netherlands as concerns the occurrence of the aggressive races [3]). The problem is studied and investigated extensively. The existence of aggressive types of the eelworm should be certainly taken into account.

In conclusion it can be said that the problem of potato sickness has not been solved completely by the preliminary successful results of the breeding work. It is reasonable that in the near future a thorough search will be made for initial material that will be also resistant to the new races.

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3) From investigations carried out in the meantime, it has appeared that in the Netherlands, too, aggressive races of the potato root eelworm, which can multiply strongly in resistant material derived from CPC 1673, occur in some places.

SIEVE TUBE SAP.

BY

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INTRODUCTION

The problem of the relation between insects and plants can be approached from two angles, the insect or the plant. Sieve tube or phloem sap is the basic feeding fluid for aphids and coccids and a botanist's view is given of these questions

Botanists are not very successful in obtaining sieve tube sap. Mostly only a few drops flow from a wound and it takes considerable time and work to gather enough fluid for an analysis, with a fair chance included that many substances from the surface of the wound are mixed with it. From many trees it is not possible to get even a few drops of the sap. Aphids and coccids are far more successful, at least in relation to their size. They often get their own weight of of sap in a short time. Also primitive man in the tropics did much better and since ancient times has tapped a sweet sap from the inflorescence of palmtrees which is pure sieve tube sap. It was this sap that was studied by the author.

CLOSING OF VESSELS

There must be some reason why it is so difficult for botanists to obtain the sap. One might even say that plants are well protected from bleeding to death by some kind of closing or clogging of their sieve tubes, when they are cut or damaged. The mechanism of this closing or clogging is not exactly known, but two phenomena can be distinguished. These are:

a. sealing of the wound. The seal is permanent and can be removed by cutting a thin slice from the surface of the wound (TAMMES 1933 for palmsap, fig. 1 and CRAFTS 1939 for cucumber).

   Repeated cutting of the cucumber stem gives some sieve tube sap after each cutting. HUBER supposes that the wound is sealed by coagulation of substances from the sap. GRAFTS observed cloggings of protoplasm on the sieve plates which originate from the explosive and elastic flow after cutting of the vessels. When a piece of a few inches is cut from a cucumber stem clogging is observed on both sides.

b. the tempory closing of the vessels over large stretches which may even extend for several meters. MÜNCH (1930) observed that after cutting and the first elastic ejection of the tubes, no more sap can be obtained over quite a long distance above and below the wound. For red oaks he gave values in summer of 5—6 meters below and up to 1 meter above a fresh wound. After one day the distance decreased to 5 cm above and 10 cm below the wound. This is not

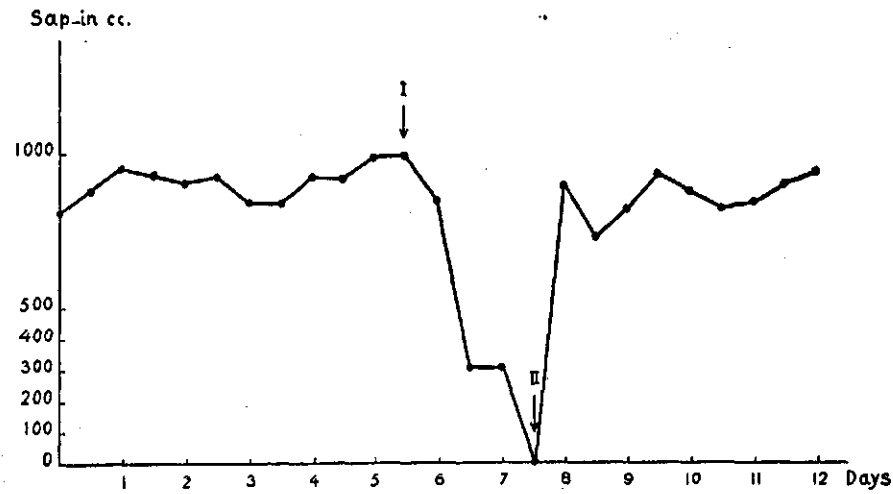

Fig. 1. Flow of sap from a flower-stalk of Arenga. Twice daily a slice was cut from the stump of the stalk. From I to II no slice was cut, which promptly resulted in a decreased flow of the sap. Sap-flow recorded at 6 a.m. and 5 p.m.

related to the rate of flow of the sap, which is much faster. MÜNCH also observed, that bending of a twig or even placing a ladder against a trunk will often cause the sap flow to stop. Restoration of sap flow is a matter of days or hours.

The two phenomena can occur side by side, that is: the temporary closing of the vessels and the sealing of the wound.

Sucking aphids, feeding on sieve tube sap, probably evade these closing mechanisms, or they are counteracted, e.g. by the injection of saliva. KENNEDY and MITTLER (1953) have found, that cut-off aphid stylets gave some sap and continue to do so for 4 days.

PRESSURE OF THE SAP

In vascular plants a translocation from cell to cell takes place in the tissues. But in addition to this, two systems of long distance transport are present, as is well known. The first is the water transport system usually working with a negative pressure, that is by suction. The second is a pipe line with assimilates working with a positive pressure. This pressure in the sieve tubes can be considerable during periods of active flow. With a sugar concentration of about 15% sucrose in the tubes about 10 atmosphere pressure can be calculated when the plants are saturated with water. Direct observations on palms failed because of leaking. However, the author observed about 2 atmospheres of pressure at least. The pipe line runs through tissues with a suction, in fact the whole plant. When an aphid puts its stylet into a plant it can be expected that as long as the stylet follows walls a suction will occur, however, as soon as cells (vacuoles) or the sieve tubes are punctured, a positive pressure will prevail and liquid will be pressed into the stylets. In the passage of intercellular cavities no pressure either

negative or positive will be present. When the suction in the tissues is very high, it may outbalance the positive pressure of the phloem and when a part is cut off, water may be sucked in even through the sieve-tubes. This was observed on cut off leaves from palms, but also on leaves of *Ammophila*. In an experiment the water vessels were closed with gelatin, and a solution with potassium ferrocyanide as a tracer was found to be sucked in by the phloem. Fresh sections were immediately put into alcohol ferrichloride and a beautiful deposit of prussian blue was observed in the sieve tubes over short distances. The method fails when applied to turgescent plants (TAMMES, 1952).

Thus a puncture of a sieve tube in a withering plant may suck in liquid instead of bleeding sap. It is known that aphids leave withered plants. It will be interesting to know whether sap is sucked in from cut off aphid stylets after withering of a plant, thus giving an opportunity to infect plants with a virus containing sap. It is an open question whether during periods of low assimilation, e.g. in the dark or in early spring, the pressure might also become negative, because of the low osmotic concentration of the sap. At least no sap can be tapped after the leaves are shed in autumn and it can be expected, that large differences in rate of flow and in pressure exist during the growing season, which may have its influence on the aphids.

LEONHARD (1940) in studying honey dew of *Lachnus pichtae* on Abies found that the amount of honey dew formed on cut off branches is strongly correlated with the intensity and period of photosynthesis. Shading of a branch (e.g.) decreased the amount of honey dew.

STRUCTURE OF THE PHLOEM PIPE LINE

Anatomically the phloem bundles comprise the sieve tubes, the so called conducting cells and some parenchyma or other cells.

Translocation of sap, as is known, takes place through these sieve tubes. One should, however, never forget that such a tube is a living cell, specialised for transport. Though no nucleus is found in active cells, a protoplasmatic layer is still present along the walls. Active tubes proved difficult to plasmolyze. But new findings by ESAU and collaborators 1955 indicate that even in an active state the tubes can be plasmolysed. Therefore the walls of the tubes must be semi-permeable to a certain degree. It has been supposed that a phloem strand is a bundle of permeable sieve tubes, surrounded by an impermeable sheath. On the strength of observations made by ESAU (1955) one could, however, suppose that each tube is an unit in itself.

Leaking of phloem bundles, caused by carefully injuring of the sheath, either mechanically or by virus has been observed by BENNET and ESAU (1936) and by CRAFTS (1939).

Consequently we do not know whether an aphid which puts its stylet in or near a phloem bundle, will get its sap from the whole bundle or only from one sieve tuve. It is not known either whether phloem bundles or sieve tubes are leaking during or after they are sucked by aphids or coccids.

DIRECTION OF FLOW

Concerning these pipe lines in the plant, called phloem, it is known, that there

are places where fluid is pumped in and other places where it is let out. So the direction of a flow depends on where these places of intake or outlet are situated. In the case of the fruit stalks of the palms the intake was on the pith of the stem where the starch is mobilised and disappears. Usually the stream is from the leaves to the storage organs or young growing parts in summer. The stream can travel against a strong concentration gradient, e.g. LEONARD (1939) has found that sugar beet leaves are completely depleted of their starch and sugars when kept in the dark. Transport takes place to the roots, where some 15% of sucrose is present. ROECKL (1949) found a large increase in osmotic concentration from leaf parenchyma to phloem and an active secretion process is apparent. Virus and growth hormones or other substances are transported in the direction of the flow. The same applies to the systemic insecticides as far as they are transported by sieve tubes or water vessels or both. Downward movement of substances by the water vessels is very rare in plants and in general such a translocation must take place through the phloem. A redistribution is, however, still possible through watervessels, after the downward translocation through the sieve tubes. For control of aphids and coccids it might be important to know whether systemic insecticides are directly transported through the sieve tubes after leaf application.

RATE OF FLOW

In active transport the speed of flow in the phloem is considerable. HUBER (1937) found that concentration waves moving down the stem of red oaks can travel at ± 2 metres per hour. From the amount of sap flowing from the fruit stalk of an *Arenga* palm and the diameter of the phloem bundles a rate of ± 7 metres per hour was calculated (TAMMES, 1952).

Fluorescein and virus are known to be transported a few decimetres per hour. Though transport from cell to cell in the tissues is very slow, in the sieve tubes a very fast translocation of assimilates and other substances occurs as soon as these substances appear in the sieve tubes.

COMPOSITION OF THE SAP

Sugars. In the growing season, a thin sugar sirup is pressed through the sieve tubes of the phloem with the admixture of other substances in small quantities. In palms only sucrose was found by KLUYVER's biological method (TAMMES, 1933). The concentration is rather constant, only when a number of inflorescences is tapped one after another the concentration can decrease considerable e.g. below 10% sugar. Here, the sugar comes from mobilized starch in the stem, a situation which is different from sugars coming from leaves. Here a peak in photosynthesis occurs every day. HUBER observed waves of higher concentration running downwards the stem of oak trees. Sucrose, also in other plants is at least the main but probably also the only sugar in sieve tube sap. This was found e.g. by ZIEGLER in his experiments with paper chromatography. However, after passage through aphids or coccids, changes occur and also fructose, glucose and other sugars are observed in honey dew (DUSPIVA, EWART and METCALF, GRAY and FRAENKEL).

Nitrogen. In palmsap the total amount of nitrogen was 410 mg per liter of sap. MITTLER proved sieve tube sap and honey dew from willows the occurrence of a number amino acids. They were also found in palm sap by DE

GROOT (1953). In palmsap the aminoacids form only a small part of the total amount of nitrogen (about 10%) so that other soluble nitrogen compounds must be present. ZIEGLER (1956) has found that part of the nitrogen were aminoacids, another part was in organic form, but the latter was probably partly due to loss of protoplasm by the quick ejection of the tubes.

Sucking aphids and coccids must use the nitrogen compounds. MITTLER (1953) found the same aminoacids in sap from cut off stylets and honey dew. It was ZIEGLER who quantitively compared the amounts of nitrogen substances in sap and honey dew from Hedera and he observed a large difference in the relation sugar-nitrogen for the sap and the honey dew. In sap there was 23,8 mg per gram of sugar of nitrogen compounds and in honey dew only 0,17 mg. So there is a strong selective adsorption of these compounds. The production of honey dew is possibly not a waste of material but serves the gathering of substances from the sap that are only present in low concentrations.

The other components of the sap can be found in the table, the occurrence of only a trace of calcium is remarkable.

Composition of Sieve tube Sap. (Arenga sacharifera Labill).

Sucrose	About 15%
Other sugars	Absent.
Nitrogen	Total 410 mg/l. Amino acids (glutamic acid, alanine, serine, arginine, methionine and threonine) total ± 40 mg/l. Nitrogen. Rest unknown.
Potash	1200 m/gl. of K. probably in ionic form.
Phosphate	98 mg/l. of P. probably as PO_4 ion.
Magnesium	96 mg/l. of Mg.
Calcium	10 mg/l. of Ca (trace)

SUMMARY

According to physiological phenomena in the plant the following can be expected from the relation between aphids or coccids and sieve tube sap, which is their feeding fluid.

When a stylet is driven into a tissue, suction will occur as long as it passes the walls between the cells. In the intercellular spaces no pressure will be present. As soon, however, as a cell or a sieve tube is punctured a positive pressure will prevail. For sieve tubes of palms this pressure can be calculated at ± 10 atmospheres.

In withering plants the pressure in the cells or sieve tubes will disappear and after puncture fluid may be sucked in, instead of pressed out. It is known that aphids leave withering plants.

It is not precisely known whether the fluid, which is tapped by an aphid comes from a single tube or from a bundle of sieve tubes.

Wounds of sieve tubes are sealed or clogged. Aphids or coccids probably evade or counteract this mechanism.

A rather high sugar concentration occurs in the sap. Various other substances are present but only in small quantities.

A number of aminoacids are present and ZIEGLER (1956) has found that nitrogen compounds were removed for the larger part from the sap of Hedera before it is excreted as honey dew.

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DISCUSSION

Dr. ELLENBY: Would it not be possible to inject some anticoagulants as Heparin into a puncture so to prevent the clogging?

ANSWER: For several purposes, e.g. virus research, we should like to inject substances directly into the sieve tubes. Until now this was not possible. It is possible that the cut-off aphid stylets of KENNEDY - MITTLER give us an opportunity in future.

Dr. KENNEDY: Can you say what causes the sugar, escaping from virus-damaged sieve tubes in the sugar beet petiole, to follow an about straight radial path out toward the epidermis, without deviating laterally in the intercellular spaces. As the aphids stylets often follow a similarly straight path in the opposite direction inward to the sieve tubes, one naturally wonders whether the same guiding forces might be at work.

ANSWER: I do not know where this parallelism comes from. Intercellular cavities have a cuticle and are waterrepellent. A sugar solution will follow the direction of the widest capillaries first.

NAHRUNGSWAHL UND VERMEHRUNGSPOTENTIAL EINIGER SCHÄDLICHEN SCHMETTERLINGSARTEN

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ZUSAMMENFASSUNG

In diesem Referat werden die Resultate unserer Untersuchungen über die Nahrungswahl von vier schädlichen Schmetterlingsarten dargestellt, nämlich der Schwammspinner (*Lymantria dispar* L.), der Ringelspinner (*Malacosoma neustria* L.), der Goldafter (*Euproctis chrysorrhoea* L.) und der amerikanische Bärenspinner (*Hyphantria cunea* Drury). Das Ziel dieser Untersuchungen war, durch Aufzucht mit verschiedenen Futterpflanzen die Rolle der Nahrung bei polyphagen Insekten zu erforschen.

1. Die Nahrung ist ein Faktor, der das Auftreten von Insekten beeinflußt und bei polyphagen Insekten, besonders jenen, die periodisch massenhaft auftreten, eine besondere Rolle spielt.
2. Nahrungsmangel und ungünstige Nahrungsarten wirken auf die Retrogradation, indem sie starke Mortalität der betreffenden Insektenart hervorrufen. Aber auch jene Nahrungsart, welche das Insekt regelmässig und sogar sehr gerne annimmt, kann Ursache einiger rezessiver Eigenschaften sein. Dies ist natürlich für monophage Insekten viel schwerer zu beweisen. Unser Studium über den Einfluss der Nahrungsart auf die polyphagen Insekten zeigt aber, dass die Art oder Qualität der Nahrung in verschiedener Hinsicht einen grossen Einfluss auf das biotische Potential solcher Insekten ausübt.
3. Gemischte Nahrung läßt die Unterschiede im Vermehrungspotential der polyphagen Insekten am wenigsten in Erscheinung treten.
4. Die Nahrungsart der polyphagen Schädlinge hat Einfluss auf die Länge der Entwicklung, Mortalität, Fruchbarkeit, Sexual-Index; mit anderen Worten auf die Dynamik der Population.
5. Die Unterschiede im Einfluss der Nahrungsart auf das Vermehrungspotential der polyphagen Insekten können wir am sichersten feststellen, wenn wir in dieser Richtung eine Selektion durchführen. Zu diesem Behufe füttern wir den polyphagen Schädling während einer gewissen Anzahl von Generationen ständig mit der gleichen Nährpflanzenart, nach Möglichkeit aber unter natürlichen Bedingungen, d.h. bei wechselnder Temperatur und Feuchtigkeit. Die Nährpflanze musst dabei für das Insekt günstig sein.
6. Die Nahrungsart hat nicht auf alle Individuen ein und derselben Brut oder Population den gleichen Einfluss, da ihre Wirkung von den genetischen Eigenschaften der einzelnen Individuen abhängtig ist.
7. Der Einfluss der Qualität der Nahrung zeigt Unterschiede bei Nachkommen, deren Eltern aus verschiedenen Biotopen stammen.
8. Der Nahrungswechsel zeigt seine Wirkung, wenn nicht in der ersten, so mindestens in der zweiten Generation. Wenn diese Änderung in gewisser Hinsicht ungünstig ist, kann sie bei entsprechender Ernährung auch auf das Ende der Gradation bzw. auf die Retrogradition einwirken. Dies tritt beim Schwammspinner ein, wennn der Wind die Raupen aus einer Gegend in die andere verfrachtet.
9. Ungünstige Nahrung kann das Absterben des Schädlings bewirken, sofern er gezwungen wird, solche Nahrung aufzunehmen (z.B. der Schwammspinner die Blätter der Linde). Es gibt übrigens Nahrungsarten, mit denen sich der Schädling zwar sehr gerne ernährt, die aber in grossen Masstab genossen physiologische Schwäche hervorrufen können. Diese offenbart sich in der Abnahme der Eizahl und oft im Ausbruch von Krankheiten.
10. Die physiologische Schwäche braucht sich nicht nur im Auftreten von kleinen und

verkümmerten Individuen zu manifestieren sondern kann auch gewisse innere Störungen erzeugen, denn auch augenscheinlich günstige Nahrung kann im Stoffwechsel der Insekten innere Störungen hervorrufen und Vorbedingungen für Polyederkrankheit, Bakteriose, Mykose oder Pebrine schaffen bzw. den Ausbruch dieser Krankheiten selbst verursachen. Daraus ergibt sich die Frage, welche inneren Faktoren die physiologische Schwäche beeinflussen und welcher Zusammenhang zwischen dem Einfluss der äusseren Faktoren und der Ernährung und den inneren Faktoren besteht.

11. Um den Zusammenhang zwischen der Nahrungsart und der physiologischen Konstitution der Raupen zu klären, haben wir uns entschlossen, chemische Analysen der Nährpflanzen und der voll entwickelten Raupen auszuführen. Dabei ergab sich eine gewisse übereinstimmung zwischen den Eiweißen und Fetten der Futterpflanzen und der Raupen. Doch soll darüber nicht näher berichtet werden, weil diese Untersuchungen noch im Gange sind.
12. Die Gradationskurve mit den Stadien bzw. Phasen der Progradation, der Kulmination und Retrogradation ist allgemein bekannt. Oft kennen wir auch die Ursachen der Abweichungen von der typichen Gradationskurve. Es ergibt sich aber die Frage, was die Retrogradation hervorruft, wenn sich das Insekt unter günstigen Lebensbedingungen befindet. In diesem Falle sind offenbar die inneren Faktoren massgebend.
13. Beim Schwammspinner und beim Ringelspinner endet die Gradation fast regelmässig mit dem Ausbruch der Polyederkrankheit, seltener mit anderen Krankheiten. Der Ausbruch kommt in stärkerem Masse in der Phase der Kulmination zur Geltung.
14. Die Viruskrankheit bzw. die Polyedrie kann nach unserer Meinung und nach Meinung einiger anderer Autoren (JANISCH, ROEGNER, BERGOLD) an die Eigenschaften der Arten, Varietäten oder Rassen der Insekten gebunden sein. Das kommt besonders bei jenen zum Ausdruck, die periodisch Massenvermehrungen aufweisen. Einen viel geringeren Einfluss hat die Polyedrie bei Schädlingen, die regelmässig alljährlich erscheinen. Bei Schädlingen, die periodisch auftreten, führen Polyedrie und andere pathogene Mikroorganismen zum raschen Zusammenbruch der Gradation. Bei Arten, die alljährlich erscheinen, kommen sie als Regulatoren der Populationsdichte zur Wirkung. (Prozessionnspinners).
15. Einige Arten von Schädlingen, wie den Schwamm- und den Ringelspinner, können wir als latentvirotische Arten betrachten, weshalb die Polyedrie bei ihrem Massenauftreten eine entscheidende Rolle spielt.
16. Einzelne Gruppen pathogener Mikroorganismen können charakteristich sein für einzelne Arten von Schädlingen, wie z.B. die parasitischen Pilze für den Goldafter, während bei anderen Insekten im Falle von Übervermehrungen verschiedene Arten von Mikroorganismen gleichsam als Letalfaktoren wirken können.
17. Im Hinblick auf den Ausbruch von Krankheiten bei Insekten sind wir der Meinung, dass bei polyphagen Insekten die Nahrungsart grossen Einfluss auf den selben hat, während bei anderen, namentlich bei monophagen, die Ursache dieser Erscheinung in physiologischer Schwäche und genetischen Eigenschaften der einzelnen Individuen und der Population zu suchen ist.
18. Bei unseren Untersuchungen hat sich gezeigt, das die Klimaverhältnisse, insbesondere die Temperatur und die Feuchtigkeit, einen grossen Einfluss auf den Ausbruch der Krankheiten bei Insekten haben. Schon das Mikroklima spielt dabei eine grosse Rolle, weil bereits ganz kleine Klimaänderungen die Stärke der Raupenmortalität abändern.
19. Die Temperatur und die Feuchtigkeit wirken stark auf den Verbrauch an Nahrungsmenge und auf diese Weise auch auf die physiologische Kondition und den eventuellen Ausbruch von Krankheiten ein.