STYLET PENETRATION ACTIVITIES BY APHIDS

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in het bijzonder de fysiologie der insekten.

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Proefschrift

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erratum

The sequence of Chapters 1 and 2
should be as indicated in the CONTENTS

The page number indication on the
first page of these chapters should
be added by 200

STELLINGEN

I. Er zijn geen aanwijzingen dat bladluizen uitwendige contact-chemoreceptoren bezitten. Dientengevolge vindt voedselplant-selectie bij deze insekten plaats door middel van de pharyngeale smaakreceptoren tijdens styletpenetratie.

- Bromley, A.K. et al. Cell. tiss. Res. 205: 493-511, 1980.
- Wensler, R.J.D. J. Morphol. 143: 349-364, 1974, Cell tiss Res. 181: 409-421, 1977.
- Dit proefschrift.

II. De veronderstelling dat tijdens het patroon 'S' (salivation) alleen speekselsecretie en tijdens 'I' (ingestion) alleen sapopname door bladluizen zou plaats vinden, was een ontoelaatbare simplificatie van reeds bekende gegevens over bladluis-penetratie activiteiten.

McLean, D.L. & M.G. Kinsey, Nature, U.K., 202: 1358-1359, 1964.

III. EPG patronen komen tot stand door activiteiten van de bladluis, en door de positie van de styletpunten in de plant. Aan een positie kan men echter niet ondubbelzinnig een activiteit ontlenen, evenmin als aan een activiteit een positie.

Dit proefschrift.

IV. Tijdens de opname van floeemsap vindt een continue speekselsecretie plaats.

Dit proefschrift.

V. Verminderde acceptabiliteit van planten door bladluizen leidt tot kortere en frequentere styletpenetraties. Het is daarom niet zinvol cultuurgewassen te veredelen op bladluis-resistentie, om daarmee een verminderde aantasting door non-persistente plantevirussen te bereiken.

Klingauf, F., Z. angew. Entomol., 70: 352-358, 1982.
Tjallingii, W.F., Symp. Biol. Hung., 16: 273-285.

VI. Er bestaat geen eenvoudige term om de ruimte aan te duiden tussen de celwand en de protoplast bij plantaardige cellen.

VII. De veronderstelling dat de styletpenetratieweg voornamelijk via de middenlamellen tussen cellen verloopt berust op een misverstand, dat is terug te voeren op het gebruik van het lichtmicroscoop als belangrijkste techniek in het onderzoek naar deze verschijnselen.

- Evert, R.F. et. al., Protoplasma 77: 95-110, 1973.
- Pollard, D.G., Bull. entomol. Res., 62: 631-714, 1973.

VIII. Raster-electronenmicroscopische foto's laten geen conclusies toe t.a.v. de aanwezigheid van poriën in sensillen bij insekten.

- Adams, J.B. & F.W. Fyfe, Can. J. Zool., 48: 1033-1034, 1970.
- Wensler, R.J.D., Cell. tiss. Res., 181: 409-421. 1977.

IX. Voedselplant-acceptatie door fytofage insekten is een proces dat niet is beëindigd nadat de voedselopname is begonnen.

- Taylor, L.R., Entomol. exp. appl. 2: 143-153, 1959.

X. De z.g. tip-recording techniek kan slechts op gebrekkig wijze inzicht verschaffen in de codering van de voedselplantkwaliteit door perifere zintuigen.

- Fujishiro, N. et. al., J. insect Physiol., 30: 317-325, 1984.
- Maes, F.W., Proefschrift R.U. Groningen, 1980.

XI. Het opnemen van maatschappelijke relevantie als een toetsingscriterium voor onderzoeksprojecten biedt geen enkele garantie voor meer toepasbare resulten.

XII. Het instellen van beroepsmogelijkheden tegen ambtelijke beslissingen kan helaas een averechts effect op de zorgvuldigheid van de besluitvorming hebben.

XIII. De kwaliteit en rentabiliteit van het universitaire onderzoek valt niet uit het aantal publikaties per jaar af te leiden.

XIV. Het weigeren van het practicum vergelijkende dierfysiologie door Wageningse biologiestudenten is niet zo zeer een kwestie van gewetensproblemen, dan wel van adolescentieproblemen.

XV. Goed onderwijs wordt minder door het onderwijssysteem bepaald, dan wel door een goede leermeester.

Proefschrift W.F. Tjallingii. Stylet penetration activities by aphids. Wageningen, 13 maart 1985.

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VOORWOORD

Het onderzoek dat als "aardig zijpaadje" in de vorm van een doctoraal onderwerp is begonnen op initiatief van mijn promotor Prof. Dr. L.M. Schoonhoven, is gaande weg uitgegroeid tot een meer uitgebreid project.

Aan het onderzoek heeft nooit een duidelijk vast omschreven doel of projectomschrijving ten grondslag gelegen. Dit was enerzijds een voordeel dat verbonden was aan het werken vanuit de positie van vaste medewerker, anderzijds een nadeel omdat de weg wat meer op de tast gezocht moest worden. Hierbij zijn velen betrokken geweest die ik bij deze gelegenheid daarvoor wil bedanken.

In de eerste plaats mijn promotor, die mij alle vrijheid gegeven heeft mijn eigen weg in het onderzoek te vinden en van wiens vele internationale contacten ik dankbaar gebruik heb mogen maken.

Voorts zou ik wijlen D. Hille Ris Lambers willen bedanken en alle leden van de zogenaamde "kleine club", in het bijzonder Frans Dieleman en Paul Harrewijn, voor hun bijdragen aan de wetenschappelijke discussies die steeds een enorme stimulans voor het onderzoek hebben betekend.

Francis Kimmins wil ik danken voor de plezierige samenwerking t.a.v. hoofdstuk 5

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Voor de assistentie bij de experimenten gaat vooral erkentelijkheid uit naar Birgit Nübel en haar voorgangers Jan Meerman en Bep van der Meyden. Henry van der Valk dank ik voor het isoleren van de planteprotoplasten. Ook hebben vele studenten in de vorm van een doctoraal onderwerp een bijdrage aan dit onderzoek geleverd. Hiervoor dank ik bijzonder Dick, Jan, Jan, Paul en Martin.

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Als last but not least dank ik Thea van Bemmel voor de laatste (type) hand die zij aan de volledige tekst van dit proefschrift heeft gelegd.

INLEIDING

Bladluizen zijn voor hun voedselvoorziening aangewezen op plantesap dat met behulp van uiterst dunne monddelen, de styletten, wordt opgenomen. De styletten dringen door tot in het floeem, waar met name de vloeistof in de zeefvaten het bladluismenu vormt (Pollard, 1973). Dit sap is rijk aan voedingsstoffen en staat bovendien onder een zodanige druk dat de voedselopname waarschijnlijk voornamelijk passief verloopt (Mittler, 1957). De meeste van de ca. 600 in ons land voorkomende bladluissoorten zijn mono- of oligofaag (Blackman, 1974; Hille Ris Lambers, 1979), hetgeen wil zeggen dat slechts één of een beperkt aantal plantesoorten als voedselplant geaccepteerd wordt. Selectie van de voedselplant gebeurt op grond van fysische en chemische eigenschappen. Moericke (1951) vond dat de kleur van de vegetatie (geel-groen) en van de lucht (ultraviolet) een belangrijke rol speelt bij respectievelijk het landings- en het wegvlieggedrag van gevleugelde bladluizen. Bovendien blijken reukzintuigjes op de antennen in staat te zijn om zowel algemene als specifieke plantegeurstoffen waar te nemen (Bromley & Anderson, 1982; Fu-Shun en Visser, 1982). Kennedy et al. (1959) vonden echter dat bij de zwarte bonenluis (*Aphis fabae* (Scop.)) en de koolluis (*Brevicoryne brassicae* (L.)) gelijke aantallen landden op waard- en niet-waardplanten, zodat tijdens het aanvliegen geur en kleur kennelijk niet belangrijk zijn. Wèl was op de niet-waard het aantal wegvliegers veel groter, zodat het netto effect een duidelijke keus voor de waardplant betekende. Bij de voedselplant-selectie lijken op afstand werkende stimuli dus geen belangrijke rol te spelen. Acceptatie of afwijzing vindt pas plaats nadat een individu op een plant geland is.

Het gedrag op een plant kan verdeeld worden in een periode voorafgaande aan de eerste styletpenetratie en de daarop volgende periode die met de eerste styletpenetratie begint. De periode voorafgaande aan de eerste penetratie, die bestaat uit rondlopen met alternerende antennebewegingen, bleek bij *Acyrthosiphon pisum* (Harris) nauwelijks verschillend in duur op waard- en niet-waardplanten (Klingauf, 1970; Wensler, 1962). Echter, vanaf het moment van de eerste styletpenetratie trad er bij deze bladluis een zeer duidelijk verschil op in het gedrag op beide categorieën planten. Het is daarom zeer waarschijnlijk dat de styletpenetratie niet alleen

ertoe dient om toegang tot het floeem te verkrijgen en voedselopname mogelijk te maken, maar tevens,of misschien wel primair, ten doel heeft de kwaliteit van de plant te onderzoeken. Hoe kan styletpenetratie deze rol vervullen? Welke penetratie-activiteiten spelen hierbij een rol, en welke stimuli van de plant zijn daarbij betrokken? Dat zijn de centrale vragen die hier gesteld worden. Natuurlijk zijn bovengenoemde waarnemingen niet als algemeen geldend te beschouwen voor alle bladluissoorten onder iedere testomstandigheid. Er zijn soorten die bij de herkenning van hun voedselplant wèl lichtreflecties betrekken (Moericke, 1969). En er zijn ook voorbeelden, waarbij voorafgaande aan de eerste styletpenetratie, al verschillen in het gedrag optreden, wanneer de bladluis op verschillende plantesoorten wordt geplaatst (Klingauf, 1972). Er zijn echter ook gevallen waarbij pas in een veel later stadium een verschil optreedt tussen waard- en niet-waardplanten (Taylor, 1959). Bij de afbakening van de vraagstelling, die aan dit proefschrift ten grondslag ligt, heeft de veronderstelde rol van dit gedragselement in de voedselselectie echter een belangrijke rol gespeeld. Een aanwijzing voor deze veronderstelling kan ook worden afgeleid uit gegevens over de morfologische structuur van zintuigen op het labium (onderlip of snuit), de styletten, en de pharynx (mondholte).

Een bladluis plaatst de punt van zijn snuit tegen het oppervlak van de plant (Fig. 1a). Vervolgens penetreren de styletten, die in een groef van de snuit liggen, het planteweefsel. Tussen de styletparen liggen twee longitudinale uitsparingen, die het voedsel- en het speekselkanaal vormen, waardoor sap respectievelijk speeksel getransporteerd kan worden gedurende de penetratie. Het opgenomen sap komt eerst in de pharynx terecht (Miles, 1972; Pollard, 1973).

Reeds vele jaren terug heeft men waargenomen dat zich op het uiteinde van het labium een aantal korte sensillen bevindt, waaraan men een smaakfunctie toeschreef (Weber, 1928). Volgens sommige onderzoekers (Adams & Fyfe, 1970) was het aantal van deze sensillen groter bij alate (gevleugelde) bladluizen dan bij de aptere (ongevleugelde) vorm. Wensler (1977) toonde echter aan dat de koolbladluis (*Brevicoryne brassicae* (L.)) steeds 16 van deze sensillen bezit. Ieder zintuigje wordt hier geïnnerveerd door een enkele dendriet met een microtubulaire structuur die karakteristiek is voor mechano-

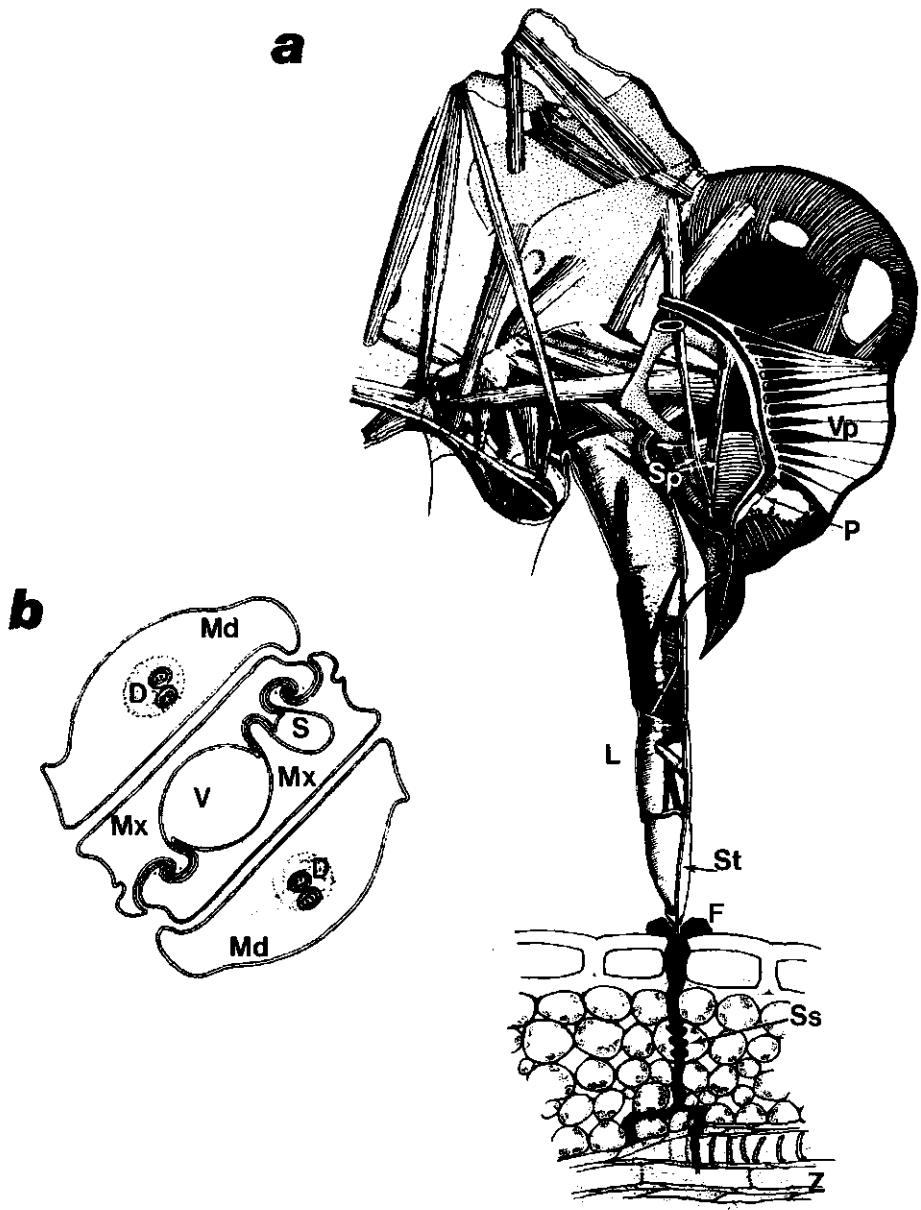

Fig. 1 a Lengte doorsnede door kop- en borststuk van een bladluis tijdens styletpenetratie in een blad (naar Weber, 1928).
b Dwars doorsnede door de styletten (naar Parrish, 1967).

D	dendriet	Md	mandibulaire styletten	S	speekselkanaal	St	styletten
F	flens	Mx	maxillaire styletten	Sp	speekselpompspieren	V	voedselkanaal
L	labium	P	pharyngeaal smaakorgaan	Ss	speekselschede	Vp	voedselpompspieren
						Z	zeefvatcel

receptoren bij insekten (McIver, 1975). Het in hoofdstuk 1 van dit proefschrift beschreven morfologische onderzoek handelt over deze structuren. Ten aanzien van de sensillen van de labiale punt is de conclusie eveneens dat het mechanoreceptoren betreft. Ook wordt, op grond van zijn fijnstructuur, aan een ander zintuig dat in de labiale groef is gelegen, een mechanoreceptor-functie toegeschreven. De tastfunctie van de snuitpuntsensillen sluit goed aan bij de waarneming dat de luis aftastende bewegingen met de snuit op het plantoppervlak uitvoert, alvorens hij tot styletpenetratie overgaat (Ibbotson & Kennedy, 1959). De snuit kan dus niet gebruikt worden om de chemische identiteit van de plant vast te stellen.

De styletten zelf zijn door verscheidene onderzoekers bestudeerd op het bezit van een mogelijke functie als smaakzintuigen. De buitenste (mandibulaire) styletten bezitten ieder een lumen, dat een dendriet bevat met twee microtubuli (Fig. 1b; Parrish, 1967). Hoewel Bradley (1962) heeft verondersteld dat deze dendriet een smaakfunctie toekomt, hebben Anderson en Bradley (1963) geen ondubbelzinnige elektrofysiologische ondersteuning voor deze hypothese kunnen leveren. Wensler (1977) toonde daarentegen aan dat de fijnstructuur van de zintuigzenuwcellen in de styletten alleen kenmerken vertonen die specifiek zijn voor mechanoreceptoren. Aangezien er geen andere structuren op de styletten voorkomen die mogelijkerwijze een chemoreceptieve functie bezitten, zijn ook deze organen uitgesloten van een chemosensorische rol bij de waardplantkeuze. Op grond van deze gegevens moet men dus aannemen dat noch op het labium, noch op de beide styletparen chemische zintuigen voorkomen, die de aard van de plant na uitwendig contact zouden kunnen waarnemen.

Inwendig in de mondholte worden wel gebieden met smaakzintuigen aangetroffen, met name in de epipharynx en de hypopharynx (Wensler & Filshie, 1969; Ponsen, 1972). Gezamenlijk bevatten deze organen ongeveer een honderdtal smaakreceptorcellen. De pharynx is hiermee tot nu toe de enige plaats waar smaakreceptoren zijn aangetoond. Aangezien het functioneren van deze receptoren strikt beperkt is tot de perioden van styletpenetratie onderstreept dit morfologische gegeven de centrale rol die de penetratie-activiteit zeer waarschijnlijk heeft met betrekking tot de voedselplantkeuze.

In de literatuur, zoals bv. in het uitgebreide overzichtsartikel van Pollard (1973), worden styletpenetraties globaal verdeeld in

korte explorerende proefboringen (probe, Probestich) en langdurige penetraties waarbij ook voedselopname uit het floeem tot stand komt. In beide gevallen hebben we primair te maken met een mechanisch proces, waarbij de styletten de plant binnendringen. Deze "mechanische penetratie" wordt bereikt door alternerende bewegingen van de mandibulaire styletten in de penetratierichting, waarbij de beide maxillaire styletten stapsgewijs de wegbereidende mandibulaire styletten volgen (Miles, 1960). Dergelijke bewegingen vormen het hoofdbestanddeel van de binnendringings-activiteit gedurende de gehele styletweg van epidermis, via mesophyl tot in het floeem. Een belangrijke tweede activiteit vormt de speekselsecretie. Hierbij kunnen twee typen secreet worden onderscheiden, namelijk een gelerend type dat na enkele seconden een gel vormt, dat als een schede de styletten omgeeft, en een waterig type (Miles, 1972). Het gelerend speeksel wordt al op de buitenzijde van de epidermis afgescheiden voordat de penetratie begint en vormt de zgn. flens (Fig. 1a; Nault & Gyrisco, 1966). Deze flens dient vermoedelijk voor de verankering van de snuit op het plantoppervlak. Vervolgens wordt iedere keer dat er in het planteweefsel een holte bij de styletpunten ontstaat, deze gevuld met gelerend speeksel, waarna de styletten daar doorheen steken om vervolgens een nieuwe portie af te scheiden, zodat een speekselschede ontstaat. In de lumina van plantecellen is deze speekselschede breed en zijn de afzonderlijke speekselporties als kralen aan een snoer te zien (Fig. 1a). In nauwe ruimtes tussen en in celwanden is de schede dun. Van tijd tot tijd wordt ter afwisseling waterig speeksel afgescheiden dat o.a. pectinase kan bevatten. Deze pectinase zou de middenlamel tussen de plantecellen kunnen oplossen en daarmee een chemische component aan de styletpenetratie toevoegen (McAllan & Adams, 1961). Alleen de binnenste (maxillaire) styletten dringen de zeefvatcellen van het floeem binnen. Hier wordt geen gelerend speeksel afgescheiden (Evert et al., 1973), maar vindt wellicht wel secretie van waterig speeksel plaats, naast voedselopname (Lamb et al., 1967). Gedurende de eerste minuut van een penetratie kan al sapopname via het voedselkanaal worden aangetoond (Hennig, 1968). Via de plant aangeboden fluorescentiestoffen werden bij een dergelijk snel begin van de sapopname tot in de oesophagus aangetroffen. Waarschijnlijk zijn de styletpunten op dit moment nog niet verder dan in de epidermale cellaag doorgedrongen

en betreft het hier een sapbemonstering zoals die mogelijkerwijs ook in het dieperliggende mesophyl plaats vindt. De sapbemonsteringen zouden slechts dienen om de inhoud van de plant in contact te brengen met de pharyngeale smaakorganen. Enerzijds kan het dier daarmee toetsen of het zich op een acceptabele plant bevindt, anderzijds kan het zich op basis van dit monster wellicht oriënteren in het planteweefsel. Voedselopname echter, geschiedt normaliter waarschijnlijk alleen vanuit het floeem.

De meeste gegevens waarop de bovenstaande inzichten in het penetratiegedrag zijn gebaseerd, zijn afkomstig van onderzoek aan plantemateriaal, waarin eerder styletpenetratie had plaatsgevonden. Deze post-experimentele benadering mist de mogelijkheden die de methode van elektrische registratie van het penetratiegedrag biedt. Bij deze methode, die is geïntroduceerd door McLean en Kinsey (1964, 1965), worden de plant en de bladluis ieder met een elektrode verbonden en in een elektrisch circuit opgenomen. Zodra de bladluis met een styletpenetratie begint wordt het circuit gesloten en ontstaan er elektrische signalen die versterkt en geregistreerd kunnen worden. De verschillende fasen in de penetratie-activiteit kunnen hierdoor op de voet worden gevolgd. In hoofdstuk 2 van dit proefschrift worden de elektrische signalen van het penetratiegedrag, die zijn verkregen met een gemodificeerde versie van McLean en Kinsey's methode, beschreven (Fig. 2). Van een aantal signaalpatronen worden ook experimenteel vastgestelde relaties met onderdelen van het penetratiegedrag gepresenteerd (Tabel 1). Dit bleek noodzakelijk te zijn doordat onze verbeterde methode zoveel meer gedetailleerde signalen opleverde, dat de signaalbeschrijvingen van de Amerikaanse onderzoekers en hun conclusies t.a.v. de relaties met onderdelen van het penetratiegedrag (McLean & Kinsey, 1967, 1968) niet meer voldeden. Ook brachten onze methodologische aanpassingen een aantal effecten aan het licht, die bepaalde eigenschappen van plant en bladluis op het signaal uitoefenen.

Om uit te maken welke elementen in de elektrische signalen van de bladluis-plant combinatie afkomstig zijn en welke niet, werden de fysische circuit-eigenschappen nader onderzocht. In hoofdstuk 3 worden deze eigenschappen beschreven en onder meer vergeleken met die van het systeem dat McLean en Kinsey gebruikten. Zo blijkt o.a. dat het signaal twee verschillende fysische oorzaken heeft, nl.

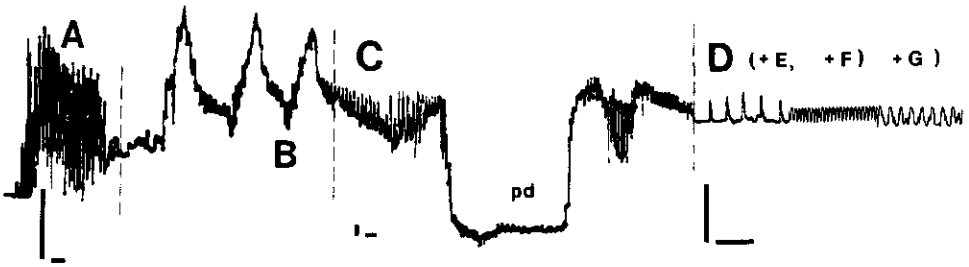

Fig. 2. Het elektrisch penetratie gram. Een overzicht van de belangrijkste signaal patronen: A t/m F. Patroon D komt steeds in combinatie voor met E of F. Tijdens patroon C is een korte (ca. 10 s) 'potential drop' (pd) te zien, die een punctie van een protoplast reflecteert. Tijdschaal, per patroon verschillend afgebeeld, 1 s. Vertikale uitslag, ook per patroon verschillend, 10 mV.

patronen	R	emf	speeksel secretie	sap opname in.	sap opname ex.	andere relatie
A	x		x			
B	x		x			styletweg
C	x		x		x	
pd		x		(x)		(bemonstering)
D+E D+E(c)	x		(x)			
D+E(pd)	x	x	(x)	x		
D+F	x		(x)			(mechanische stylet act.)

Tabel 1. Signaalpatronen met hun belangrijkste fysische component en hun relatie met de belangrijkste penetratie activiteit. x = experimenteel aangetoond, (...) = waarschijnlijk. R = weerstands-(fluctuatie)-component, emf = (fluct.) elektriomotorische kracht component. in., ex., intra- resp. extracellulair.

weerstandsfluctuatie en elektromotorische kracht (emk). De signaalcomponenten die hierdoor veroorzaakt worden, bevatten beide informatie over de penetratie-activiteit. De gevoeligheid van het registratiesysteem voor elk van beide componenten hangt in sterke mate af van de elektrische eigenschappen van de gebruikte voorversterker in relatie tot die van de andere onderdelen van het circuit; bladluis, plant en elektroden. Op basis van deze kennis werd een versterker ontwikkeld die voor emk-componenten een specifieke gevoeligheid bezit. Met behulp van dit instrument bleek het mogelijk meer informatie dan voorheen in het signaal te detecteren met betrekking tot bepaalde elementen in het penetratiegedrag. In de hoofdstukken 4 en 5 wordt beschreven hoe het signaal wordt beïnvloed door de membraanpotentiaal van de plantecel wanneer de styletten een protoplast binnendringen. Het bleek nu mogelijk intra- en extracellulaire penetraties in het signaal van elkaar te onderscheiden. Over dit aspect bestaat in de literatuur nogal wat verwarring. In een gangbare indeling (Pollard, 1973) van plantepenetraties worden twee typen van styletwegen onderscheiden. Het eerste type omvat intercellulaire penetraties waarbij de styletweg door de intercellulaire ruimtes loopt en voorts de middenlamel volgt. Het tweede type penetratie zou zowel inter- als intracellulaire wegen volgen. McAllen en Adams (1961) correleren de intracellulaire styletweg met de gehele of gedeeltelijke afwezigheid van pectinasen in het speeksel van de bladluis. Luizen zonder pectinasen zijn niet in staat de middenlamel op te lossen, zodat een styletweg tussen twee cellen door voor hen afgesloten is. Al deze veronderstellingen berusten echter op de interpretatie van lichtmicroscopische beelden. De optische vertekening in lichtmicroscopische preparaten is als gevolg van de gebruikte coupediktes (5-12 µm) echter dusdanig groot dat de posities van styletten en celwand niet eenduidig vastgesteld kunnen worden. Bovendien zijn door de gebruikte fixatietechnieken de meeste membraanstructuren verdwenen, terwijl voorts het oplossend vermogen te gering is om interpretaties op subcellulair niveau toe te staan. Met behulp van elektronenmicroscopie hebben Evert et al. (1973) aangetoond dat, hoewel de styletweg door het cellumen kan lopen, de styletbundel en de omringende speekselschede dan geheel door het plasmalemma wordt omgeven en dus in feite ten opzichte van de protoplast extracellulair blijft. Ook Rohfritsch (1976)

toonde dit met EM aan bij galvormende bladluizen. Bovendien verschaffen EM foto's een meer realistische weergave van de verhoudingen van styletbundel en celwand. Het moet alleen op basis van die verhoudingen in de meeste gevallen al uitgesloten worden geacht dat de styletweg de middenlamel zou kunnen volgen. In de hoofdstukken 4 en 5 wordt op deze problematiek ingegaan aan de hand van EM onderzoek in combinatie met elektrische registraties.

Zoals uit bovengenoemd EM onderzoek (Evert et al., 1973; Rohfritsch, 1976) al blijkt, loopt de styletweg naar het floeem vermoedelijk vaker door de cellumina dan tot voor kort werd aangenomen. Desondanks verloopt de weg hoofdzakelijk buiten de protoplast om. Uit onze waarnemingen blijkt echter dat de styletten de celmembraan zo nu en dan wel penetreren, hetgeen tijdens de elektrische registraties blijkt uit een potentiaalsprong, die veroorzaakt wordt door de membraanpotentiaal. Deze puncties duren maar 5-20 s en mogelijk wordt de cel meerdere malen aangeprikt. De protoplast lijkt de puncties en de styletpassage met speekselschedevorming te kunnen overleven. Als de styletten in het floeem een zeefvat penetreren wordt ook een potentiaalsprong in het signaal waargenomen, die vaak zeer langdurig kan worden aangehouden.

Elektrische signaalregistraties lijken goede mogelijkheden te bieden om de styletpenetratie, het voedselopnamegedrag en de gedragsreacties van de luis op fysische en chemische factoren in de plant te onderzoeken. Voordat deze techniek voor verdergaand onderzoek gebruikt kan worden dient echter de methodologische vraag te worden beantwoord in hoeverre het natuurlijke gedrag van de proefdieren door de gebruikte proefomstandigheden wordt verstoord. Alleen als deze vertekening bekend is, weten wij met welke mate van betrouwbaarheid de resultaten vertaald kunnen worden naar de natuurlijke situatie. Aan deze problematiek wordt in hoofdstuk 3 aandacht besteed, doordat daar op de mogelijke invloed van elektrische stroom op de voedselopname wordt ingegaan. Vervolgens worden in hoofdstuk 6 een aantal experimentele gegevens besproken, waarbij een reeks parameters van het penetratiegedrag, de levensduur en de reproductiecapaciteit vergeleken worden tussen bladluizen die bevestigd aan een geleidend draadje in een elektrisch registratiecircuit zijn opgenomen en vrij rondlopende bladluizen. Door deze factoren bij twee bladluissoorten, ieder onder waardplant en

niet-waardplant omstandigheden, na te gaan is een indicatie verkregen onder welke restricties deze onderzoeksmethode bruikbare resultaten kan opleveren.

Het in dit proefschrift behandelde onderzoek wil een bijdrage leveren aan een beter inzicht in de verschillende activiteiten die tezamen de styletpenetratie vormen. Tevens wil het een methode leveren waarmee de betekenis van de styletpenetratie voor het proces van voedselherkenning bij bladluizen nader kan worden gepreciseerd. Enerzijds ter vergroting van onze fundamentele kennis over de opvallend nauwe binding die tussen bladluizen en planten kan bestaan, anderzijds om een aanzet te zijn voor meer toegepast onderzoek naar bladluisresistentie bij cultuurgewassen en de overdracht van plantevirussen.

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ELECTRONIC RECORDING OF PENETRATION BEHAVIOUR BY APHIDS

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A simple amplifier is used for electronic recording of aphid penetration behaviour. Several different patterns of electrical signal are described, some of which can be correlated with different aspects of feeding behaviour.

An electronic method of recording aphid penetration behaviour was introduced by McLean and Kinsey (1964). During penetration, aphids attached to a thin lead produce a complex electrical signal (called the "detection signal") consisting of different patterns which presumably reflect different elements of the penetration behaviour. Evidence supporting the correlation of electrical wave-forms with salivation and ingestion is mainly obtained by histological localization of the stylet tips in the plant tissue at the moment of the last performed pattern (McLean & Kinsey, 1967) on the assumption that a particular behavioural activity is performed mainly in a given plant tissue, *e.g.* epidermis, parenchyma, or phloem. Although this assumption may be valid in the case of saliva sheath secretion and continuous ingestion, it seems questionable in other situations such as sap sampling, stylet movements and watery saliva secretion.

The recording method has been modified by different workers. Schaefers (1966) describes a DC variant of the original method (McLean & Kinsey, 1964; McLean & Weigt, 1968) using 60 Hz AC as the voltage applied to the substrate. Brown & Holbrook (1976) describe a modification which is based on the use of a 20 Hz AC voltage. The present study uses Schaefers' variant and aims at a verification of McLean & Kinsey's correlations. Furthermore, it might serve as a starting point for further research on the significance of the detection signal patterns.

METHODS

In our DC-modification, a voltage of 10-30 mV is applied to the leaf or feeding solution (20% sucrose, 0.1% NaCl) by means of a copper gauze or silver wire, respectively. The recording electrode of copper wire (0.2 mm diameter) is attached to the aphid dorsum with conducting silver paint ("Leitsilber", Merck). Both electrodes are connected to the amplifier with a thin and flexible shielded cable.

The experimental set-up is mounted in a Faraday cage.

The amplifier (Fig. 1) consists of a single integrated circuit (CA 3140) connected so as to be non-inverting. A 10 MΩ resistor from the amplifier input to ground keeps the output signal at the baseline when the aphid makes no contacts by penetrating the substrate. The leaf voltage is adjustable by means of a potentiometer between the positive and negative leads from a separate low rim ± 15 V DC power supply unit. Output signals were recorded on tape (Philips Analog-7).

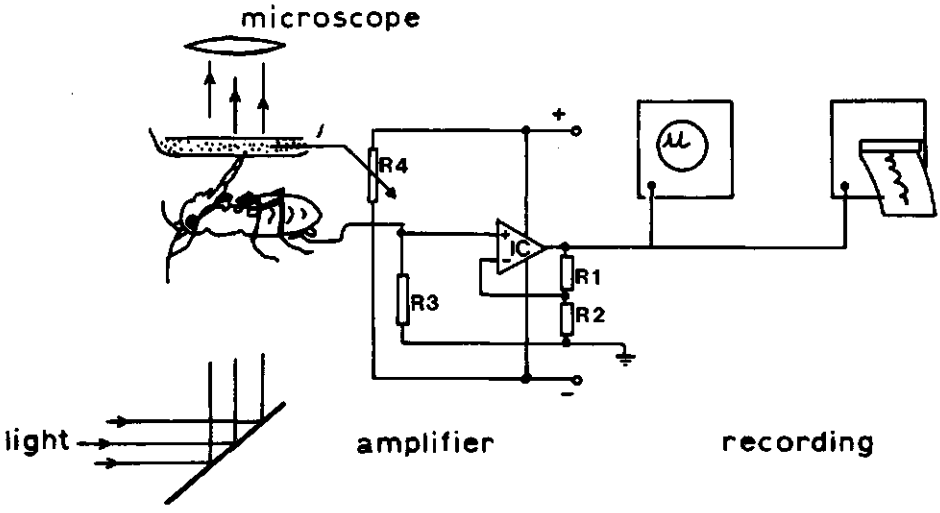

Fig. 1. Diagram of visual observation and electronic recording. — The complete amplifier: integrated circuit CA 3140 (IC), resistors to adjust amplification (R_1, R_2), resistor of 10 MΩ to ground (R_3) and potentiometer to adjust the substrate voltage (R_4). The ± 15V DC power supply is connected to the indicated leads (+, —). Amplification equals $A = R_1/R_2 + 1$. In our set-up 25× and 50× were chosen.

Visual observations of the saliva sheath secretion have been made with a light microscope. An aphid attached to an electrode can penetrate an artificial membrane (Parafilm®) with a sugar solution on the other side. The upper surface of the solution was covered by a cover glass which permits microscopical observations, even with oil-immersion objectives. Detection signals and spoken comments were recorded simultanously on magnetic tape.

Electrophysiological registrations from the muscles of the feeding and salivary pumps were made during the recording of the detection-signals. A pair of capillary microelectrodes near these muscles could record electro-myograms by differential amplification (Grass P-16 amplifier). By using common mode rejection and high pass filtering, interference with the detection-signal was avoided. The aphid was mounted on two-sided tape. The electrodes were positioned by means of a micro-manipulator (Jenoptik). Feeding solutions were offered in a 2 mm diameter glass tube covered by a membrane.

Two types of experiments were done using radioactive ^{32}P (orthophosphate). In the first type the feeding solution was labelled. Using activity of 1 mCi/ml a

minimum ingested volume of 10 pl can be detected in an aphid. We assume that no ^{32}P is excreted during the experiment. The second type of experiment used labelled aphids which produce labelled saliva. In order to label aphids, larvae were kept on cabbage leaves with their petioles immersed in a solution of about 1 mCi/ml ^{32}P. Adults were used after 5 to 10 days. The strength of the label per aphid was roughly estimated by a GM-tube monitor. To avoid surface contamination via contacts of legs and labium and by honey dew excretion, the leaf discs exposed to the aphids for penetration were covered by a Parafilm® membrane. Cerenkow radiation was measured in a liquid scintillation counter (N.C. Mark I).

Some aphids survived a continuous attachment to our experimental set-up for 10 days during which they produced more than 20 larvae. Animals connected to a thin (25 μm diameter) gold wire penetrate somewhat sooner than aphids attached to the more rigid copper wire (0.2 mm diameter).

All experimental insects were adult apterous aphids. *Acyrthosiphon pisum* Harris was used in electrophysiological experiments and *Brevicoryne brassicae* L. in all other tests. Aphids were collected shortly before the experiments from broad bean and Brussels sprouts plants, respectively. Aphids and plants were kept at 15° and 16/8 L.D. photoperiods.

RESULTS

Description of the detection-signal patterns

The amplitude of the detection-signals is not constant but depends on the resistance between the dorsal electrode and the aphid. The abundant wax secretion by *Brevicoryne* presumably causes a gradual increase in resistance resulting in a gradual decrease of the amplitude of all patterns. Therefore the relative amplitudes are used in addition to frequency in order to characterize the different patterns (Fig. 2). The detection-signal is nearly always positive. The following provisional description may require additional details when more causal relations eventually show up.

Pattern A (Fig. 2a, b) is the first of all patterns performed. It has a short duration of maximally 10-20 sec and sometimes interrupts other patterns. Its amplitude may vary considerably and may exceed those of other patterns. Its frequency ranges from 5-15 Hz.

Pattern B (Fig. 2a) consists mainly of large slow waves. The amplitude may initially be as high as the largest A-amplitude but it decreases gradually in the course of time. A single wave has a duration of about 2 sec. The waves occur with a frequency of 0.1—0.3 Hz. The leading edge frequently shows a similar frequency as pattern A, although its amplitude is smaller. This alternating voltage pattern is superimposed on a fairly constant voltage level of about a quarter of the maximum value. The duration of the pattern may be very variable. The pattern occurs in the beginning of each penetration, may be repeated, and is occasionally found with one or a few waves at the end of the penetration activity.

Pattern C follows B in time, and is a complex pattern (Fig. 2a, c, d, e). Some

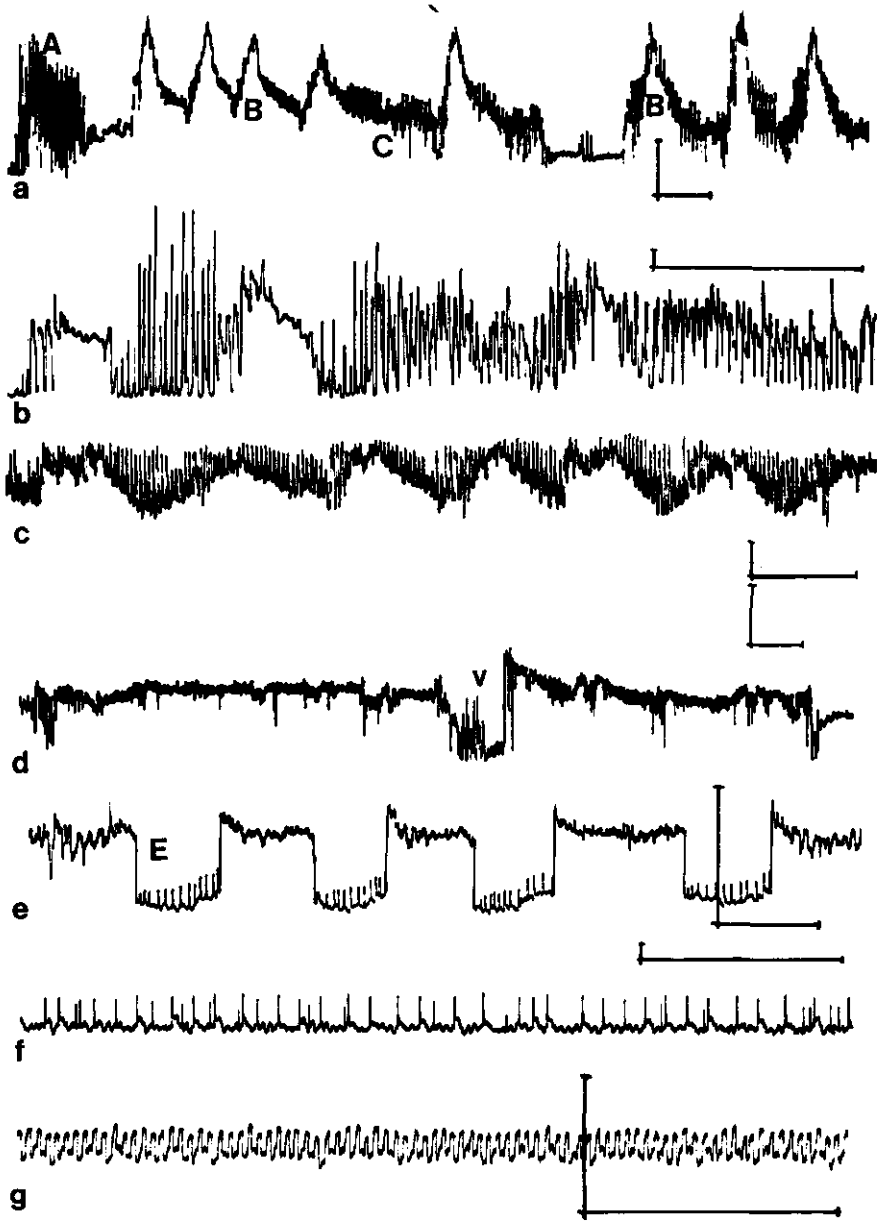

Fig. 2. Some examples of different patterns. Bars equal 10 mV, vertically, and 5 sec (g : 1 sec) horizontally. —a) Pattern A (A) and B. Between large B-waves (B), pattern C (C) starts already. Note A-like frequencies on leading edge last four B-waves. —b) Pattern A, with some indistinct B-waves, on larger time scale. —c) Pattern C with regular B-like rhythmical component. Interfering higher frequency (5 Hz) resembles A. —d) Pattern C with voltage drop and return (V). Note negatively directed pulses on higher plateau seem to change to positive direction during voltage drop. —e) Pattern C with a number of successive voltage drops during which E pulses are displayed (E). —f) Pattern D and E. —g) Pattern D and F. Frequency of 10-11 Hz and pulse width about 50 msec.

elements resemble other patterns. A very regular repetition of small waves at the same frequency as the B-waves is observed, which are preceded by a frequency resembling pattern A, but with reduced amplitude. An abrupt drop (and return) of the voltage level to a second lower level (Fig. 2d) or a kind of oscillation between these levels is a characteristic feature. Pulses with a negative going phase at the upper level may change into positive signals after a voltage drop to the reduced voltage plateau. The mean amplitudes of pattern C are lower than those of patterns A or B. The "upper" voltage level of pattern C approximately equals that of B. Pattern E is frequently super-imposed on C and sometimes it is restricted to short periods (0.5-2 sec) of a voltage drop (Fig. 2e). The duration of the C pattern is very variable and may be much longer than A or B. Pattern C can be interrupted by A, B or D.

Pattern D (Fig. 2f, g) is a steady voltage at a level of about the same value as B or C, or somewhat lower. A very slow fluctuation (< 0.05 Hz) is sometimes seen in the beginning. Long periods of this pattern are often seen in coincidence with pattern E.

Pattern E occurs exclusively superimposed on C and D (Fig. 2e, f). Regular positive pulses of about 20 msec have a more or less square wave form with a frequency of 1-3 Hz. Although the shape is rather constant some deviations may occur. The amplitude is independent of the voltage of C or D. A gradual decrease to below the noise level is often seen as well as the reverse phenomenon.

Pattern F (Fig. 2g) could be described as a remarkable variant of pattern E as well. It has similar small pulses but their duration is about 50 msec while they occur in a higher frequency of 12-20 Hz. Its occurrence is mainly restricted to pattern D. Although E and F have been observed in the same individual aphid at different times there seems to exist no gradual transition. *Acyrthosiphon* showed this pattern more frequently than *Brevicoryne*. Pattern F may last for several hours.

The duration of patterns A, B and C is hard to determine since they overlap or have similar elements. Also a quiet period during C may be misinterpreted as pattern D.

No clear differences in the detection signals have been found between insects on plants or on feeding solutions.

Correlation of electrical activity and behaviour

Visual observations (Fig. 1, left) of the saliva sheath secretion revealed a close correlation of the single B-wave with the formation of a blob of saliva at the end of the sheath. After secretion of such a blob a swinging movement of the whole sheath is observed. During these movements the stylets penetrate the blob and the sheath therefore is composed of a series of blobs stuck together. The swinging movements seem to coincide with the A-like frequency during the leading edge of the B-wave.

Electrophysiological recordings of the cibarial pump muscle activity during pattern D and E show a periodicity in the myograms. This periodicity is not correlated with the E pulses (Fig. 3). Similar myograms due to saliva pump muscle activity, however, were found to coincide with the E-pattern pulses.

The amount of ingested ^{32}P labelled solution appears to be strongly correlated with the duration of pattern D. A lower but significant correlation is also found with the total duration of patterns A, B and C (Fig. 4). Furthermore, the secretion of radioactive saliva appears to be strongly correlated with the total duration of ABC whereas no significant correlation is found with pattern D (including E and F) (Fig. 5). The amount of saliva is given is as relative value, obtained by dividing the radioactivity of the secreted saliva by the radioactivity of the whole aphid as measured before the experiment.

The correlation of ABC with ingestion is not due to the statistical correlation of ABC with D (Spearman $r = 0.4$, $P < 0.001$), but is based on causal relations of ABC with ingestion. This is demonstrated by the fact that aphids which did not display D in addition to ABC showed significant ingestion (Table I). During salivation this problem plays no role since the correlation of ABC with D is low ($r = 0.03$, $P = 0.4$).

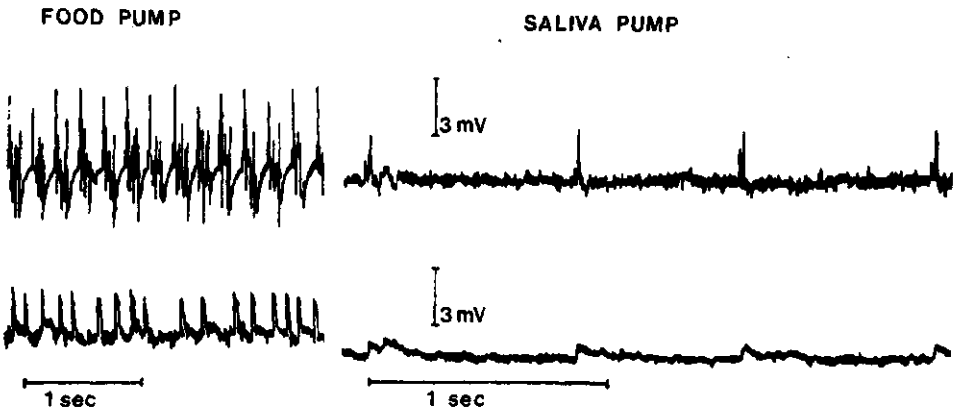

Fig. 3. Electrophysiological recordings. Upper trace represents myograms, lower trace represents detection signal. Closer observation shows lack of correlation of upper with lower trace (left), and (right) good correlation of upper with lower trace. Frequency of E pulses rather high and irregular (left), occurring mostly in beginning of pattern D display.

CONCLUSIONS

An objective way of classifying the different patterns of the detection signal as suggested above by the description of patterns A to F, seems preferable to a classification into I (ingestion) and S (salivation) patterns, as has been proposed by McLean & Kinsey (1967), especially since ingestion and salivation do not seem to be restricted to these patterns. For instance, ingestion has been shown to occur during A, B and C. Pattern A shows similar properties to McLean & Kinsey's pattern "S". We were unable, however, to demonstrate a correlation of this pattern with salivation or ingestion activities. There is a slight indication that a short waveform of frequency similar to A, that alternates large B-waves, coincides with stylet movements. The large B-waves themselves are correlated with saliva sheath

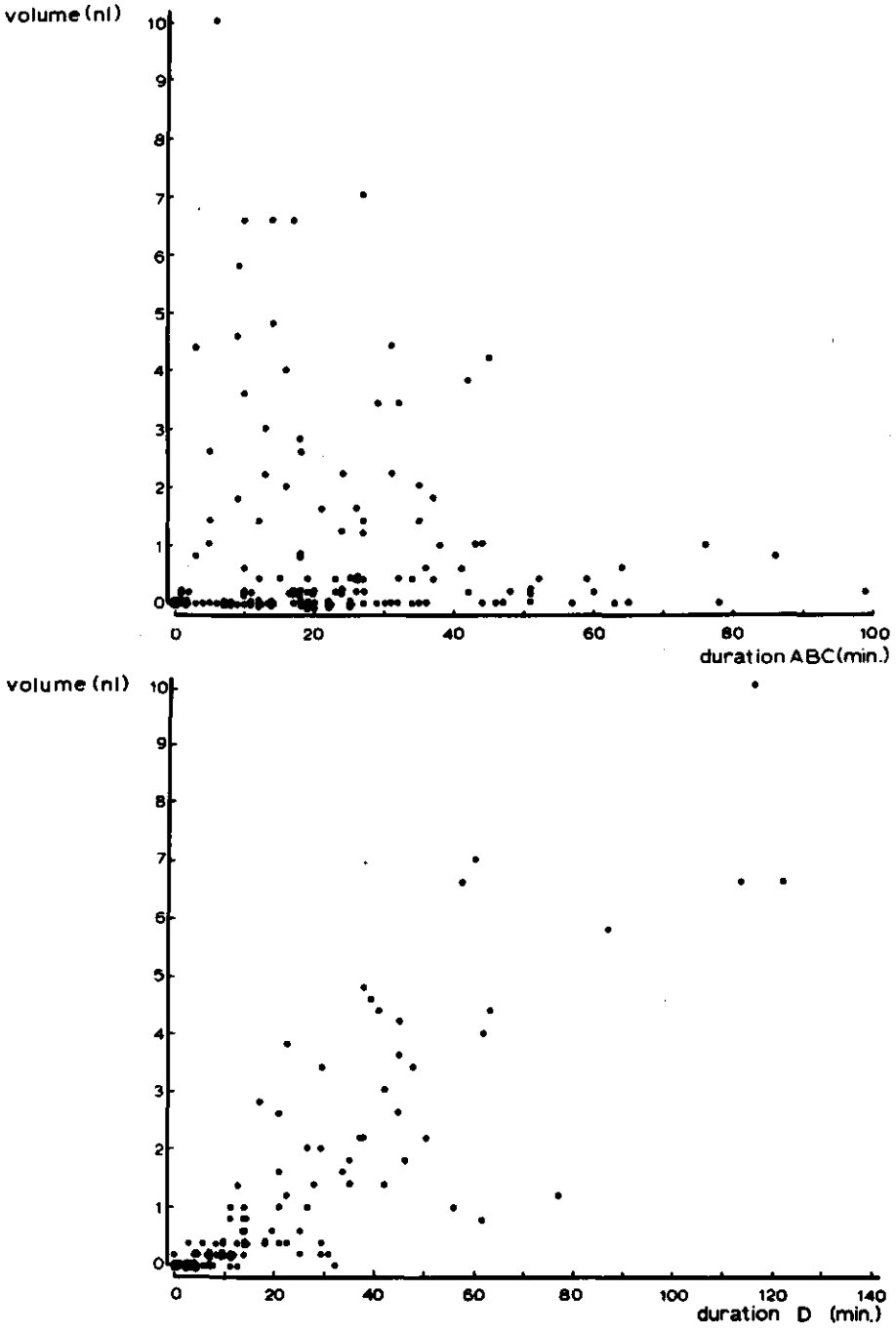

Fig. 4. Scattergrams of ingested volume with duration of patterns ABC and D, respectively. Spearman's correlation coefficient of Volume with ABC, $r=0.42$, $P<0.001$, $N=169$; with D, $r=0.90$, $P<0.001$, $N=169$.

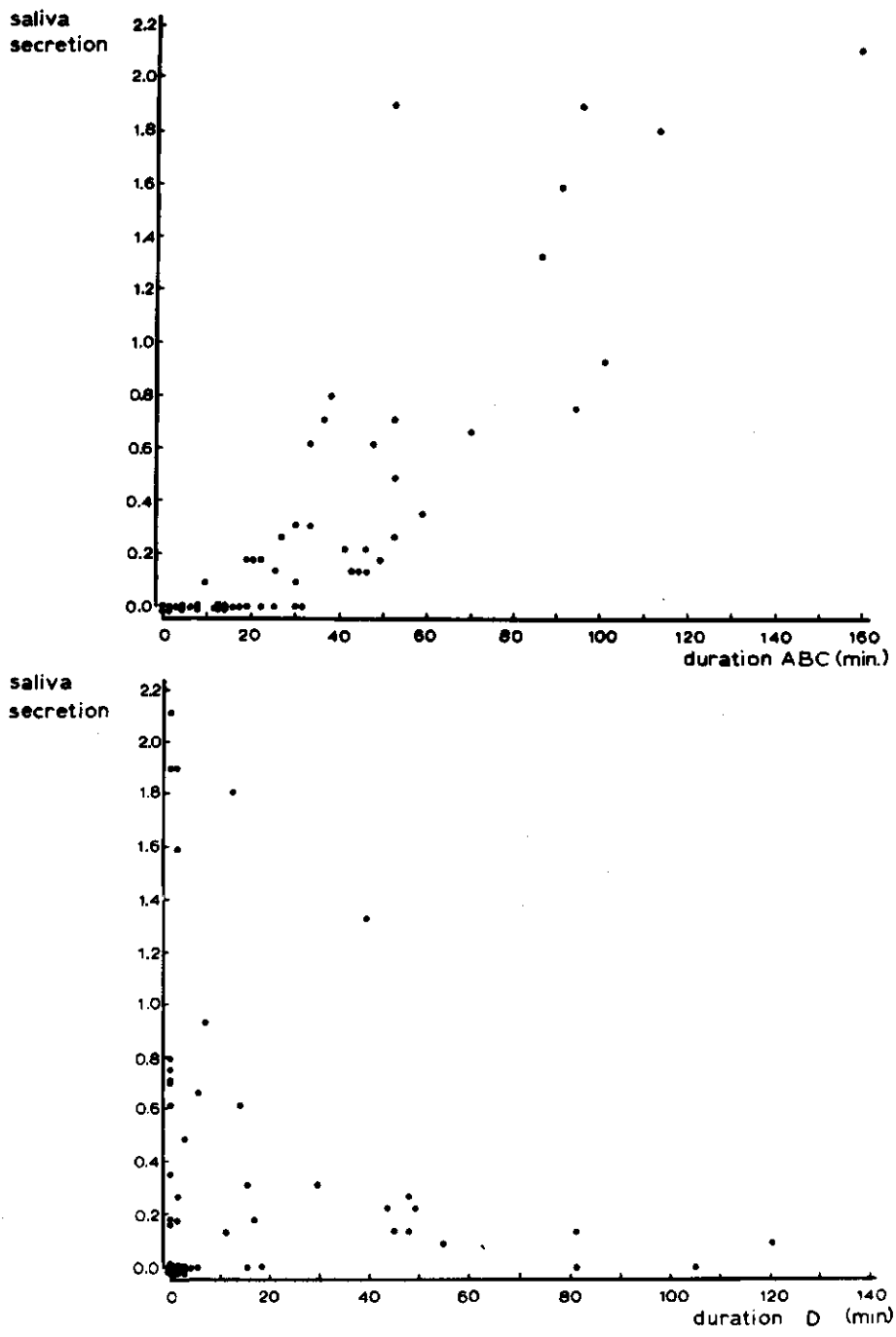

Fig. 5. Scattergrams of saliva secretion with duration of patterns ABC and D respectively. Spearman's correlation coefficients: Salivation with ABC, T=0.87, P<0.001, N=54; with D, r=0.07, P=0.316, N=54.

TABLE I

Ingested volumes in relation to the duration of the patterns

	Number	Ingestion (nl)	Duration of the pattern (min)
No penetration	23	— *	—
Pattern ABC only	20	0.47	381.1
Pattern D in addition to ABC	126	142.40	2121.2†

* No contaminations
† D

formation in such a way that one wave reflects the secretion of one sheath element. With regard to their shape, the B-waves seem to be equivalent to McLean & Kinsey's pattern "X". Our pattern C is a complex of various patterns. Some elements may be related to A and B. Simultaneous occurrence with pattern D is not detectable since both patterns have a voltage plateau. Although transitions from C to D are easily recognized, temporal separation of A, B and C appears difficult. Whether or not certain parts of A, B or C are responsible for ingestion, as determined with labelled substrates, has not yet been examined. Pattern D represents the main part of ingestion and is equivalent to McLean & Kinsey's pattern "I". Pattern E reflects saliva pump activity. Apparently a continuous salivation may occur during food intake. There was an absence of correlation between pattern D, during which salivation (pattern E) frequently occurs, and the secreted radioactivity. A possible explanation may be that this saliva is of the watery type (Miles, 1959) which is ingested immediately after secretion. Our applied voltage did not interfere strongly with the aphid physiology (as suggested by McLean & Weigt, 1968), since survival and reproduction in an attached situation were reasonable good.

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RÉSUMÉ

ENREGISTRER ÉLECTRONIQUEMENT LE COMPORTEMENT DE LA PÉNÉTRATION CHEZ LES PUCERONS

Le comportement de la pénétration chez les pucerons (Homoptera) est enregistré électroniquement par un amplificateur simple. Cette méthode fut introduite par McLean & Kinsey (1964). Les ondes différentes du signal électrique sont descrites à nouveau et classifiées de A à F. Des expériences avec isotopes radio-actives ont mis en évidence une corrélation significative d'ingestion avec l'onde D ainsi qu'avec A, B en C ensemble. Il apparaît que la sécretion de la salive est seulement en corrélation avec les ondes A, B et C ensemble et non avec D. Cependent en même temps que l'onde D, l'onde E apparait et il a été mis en évidence par expériences électrophysiologiques que cette dernière represente l'activité de la pompe à salive. Il semble que la salive radio-active produite pendant D est ingerée directement

après excrétion. Des observations visuelles démontrent une corrélation de l'onde B avec la formation fourreau salivaire. Aucune recherche n'a encore été faite sur les relations entre les ondes A, C et F et le comportement.

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MECHANORECEPTORS OF THE APHID LABIUM

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Morphological evidence is provided for a mechanoreceptive function of the labial tip receptors, as well as of two newly described receptors in the stylet groove. Electrophysiological tip recording techniques could not demonstrate a neural response due to chemical stimulation of the tip sensilla, the larger labial hairs or the stylet tips. It is concluded that there are only mechanoreceptors present on the aphid mouthparts.

A sensory function has been ascribed to the small sensilla on the labial tip in aphids since the time of Weber (1928). Indications of a chemoreceptive function analogous to other Hemiptera (Schoonhoven & Henstra, 1973) were obtained by Wensler (1962), but recent work has proved that this suggestion was premature; morphological evidence for an exclusive mechanoreceptive function is shown (Wensler, 1977). The single dendrite which innervates each sensillum, shows a tubular body structure typical of arthropod mechanoreceptors (Thurm, 1964; McIver, 1975).

Although it is evident from behavioural work that "probing" activity plays a major role in host-plant selection (Wensler, 1962), it is also apparent that prior to probing different plant materials may affect aphid behaviour (Klingauf, 1972; Tjallingii, 1976). Whether or not these plant influences act via gustatory, olfactory, visual, or even mechanoreceptors is uncertain.

The aim of the present study is to make an inventory of the sensilla of the aphid mouthparts as far as they play a possible role in host-plant selection. Some new details of the tip sensilla are given as well as a morphological description of two other receptors located in the stylet groove. In addition, the results of some electrophysiological experiments with chemical stimulation are discussed briefly.

MATERIALS AND METHODS

Alate and apterous virginoparous *Brevicoryne brassicae* L. were fixed for 5 hr at 5° in Karnovsky's fixative in cacodylate buffer pH 7.2. For orientation purposes the dissected labia were embedded in 1% agar in the same buffer. A post fixation with 2% OsO_4 in s-collidine buffer pH 7.2 for 16 hr was followed by dehydration in a graded series of acetone solutions. The labia were subsequently embedded in a

Styrene-Methylmethacrylate mixture 11:9. Thin sections were obtained with an Ultratom III (LKB) and stained with uranylacetate for 30 minutes and lead-citrated for 10 minutes. Micrographs were made with a Siemens Elmiskop-1 and Elmiskop-101.

Prepared sections of apterous adult *Nasonovia ribis-nigri* Mosley were placed at my disposal by F. L. Dieleman, Department of Entomology, Agricultural University, Wageningen.

Aphids for scanning electron microscopy were washed briefly in xylene, fixed for 2 hr in glutaraldehyde, washed and critical-point dried. They were gold-coated and mounted with silver paint for examination in a Jeol, JSM-U3 scanning EM.

RESULTS

Cuticular components.

Several cuticular components render the labial apex a compact and rigid structure. Most components within the cavity of the labium are continuous with the external labial wall or the inner stylet groove wall. The ventral half of the external labial wall is thickened to 3.5 μm at about 12.5 μm from the tip. In general the stylet groove is circular in cross-section with a folded dorsal slit (Fig. 2). A ventral slit which tapers proximally is also present at the tip. Two L-shaped longitudinal bars arise from the thickened stylet groove wall and extend over the whole length of the labial segment (Fig. 2 c-g). The bars close the stylet groove dorsally.

The distal labial wall which supports the sixteen sensilla, numbered 1—8 on each side (Fig. 1) is relatively thin (0.2 μm). Each sensillum is innervated by a single neuron and does not show any pore other than the moulting pore described below. The sensillum is placed eccentrically over an inner cuticular tube, 1.5 μm in diameter and about 10 μm long, with a wall of 0.3 μm thick. Electron-light, endocuticular material separates the labial wall from the distal end of the cuticular tubes. The tubes are proximally associated with the electron dense cuticle of the labial wall (receptors 1, 2 and 3) or the stylet groove wall (receptors 4, 5 & 6), while the tubes of receptors 7 & 8 join to form a doublet (Fig. 2b, c) with no other connection to the labial wall than the endocuticle. The lumen of the cuticular tubes is in open connection with an extracellular fluid filled cavity or distal sinus, as well as with a haemolymph space of the entire labial tip.

Distally, where it forms the ventral slit the stylet groove contains two flat projections (Fig. 2b) which increase in diameter proximally. No pores are present in the projections that are filled with light cuticular material with a somewhat layered or fibrillar structure. Proximally the projections fuse first with the groove wall and then with each other, partitioning the ventral slit. The bowl-shaped structure of the fused projections (Fig. 2c) gradually decreases in size. In this region the tapered scolopale tips of two stylet groove receptors are shown. A U-shaped tube containing the groove receptor dendrites arises from a median ventral mass of lighter cuticular material (Fig. 2b) and is closed more proximally over a limited distance (Fig. 2f). The tube is about 3 μm in diameter and 8 μm long. Distally the

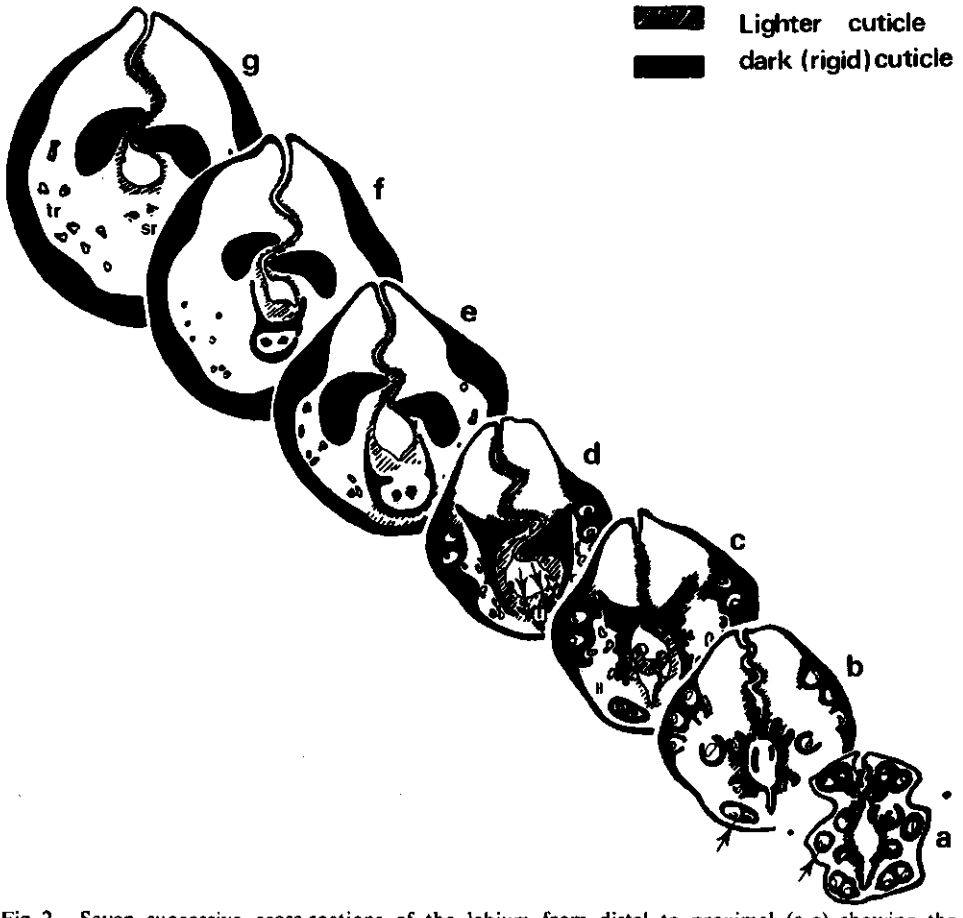

Fig. 2. Seven successive cross-sections of the labium from distal to proximal (a-g) showing the cuticular components. Stylets removed. Only the dendrites of tip-and stylet groove receptors indicated. See Fig. 1. for numbering of sensilla. a) Separation between cuticular tube (arrow) and labial wall and occurrence of ventral slit of stylet groove. b) Cuticular tubes fuse with locally thickened walls of outer labium and stylet groove, except numbers 7 and 8 (arrow). Note cuticular projections in stylet groove. c) Two L-shaped bars close stylet groove. Note bowl-shaped structure of fused cuticular projections which close off ventral slit. "H" indicates location of haemolymph space. d) Appearance of flattened stylet groove receptor scolopales (arrows), ventral to disappearing bowl-shaped structure. In median ventral light cuticular mass, beginning of U-shaped cuticular tube (u) is seen. e) Completed L-shaped bars and U-shaped tube. f) Closed part of U-shaped tube. g) Proximal to U-shaped tube at 12.5 μm from the tip. Dendrites of tip receptor (tr), and stylet groove receptors, (sr).

tube wall separates the distal cavity of the groove receptors and the haemolymph space mentioned above.

Sensory neurons

The dendrite of each labial tip receptor is connected to the base of the sensillum. The tapered scolopale termination is inserted at the moulting pore which shows a

plug of electron dense material, 0.2 μm in diameter (Fig. 4a). At the base of the sensillum the scolopale is surrounded by electron dense material which nearly fills the lumen locally. The distal dendrite is 0.5 μm in diameter and contains about 100 microtubules forming a tubular body. More proximally the number of microtubules is reduced to about 50. A dilated and infolded region of the dendrite and the scolopale occurs at a distance of 12.5 μm to 16 μm from the tip. The dendrite

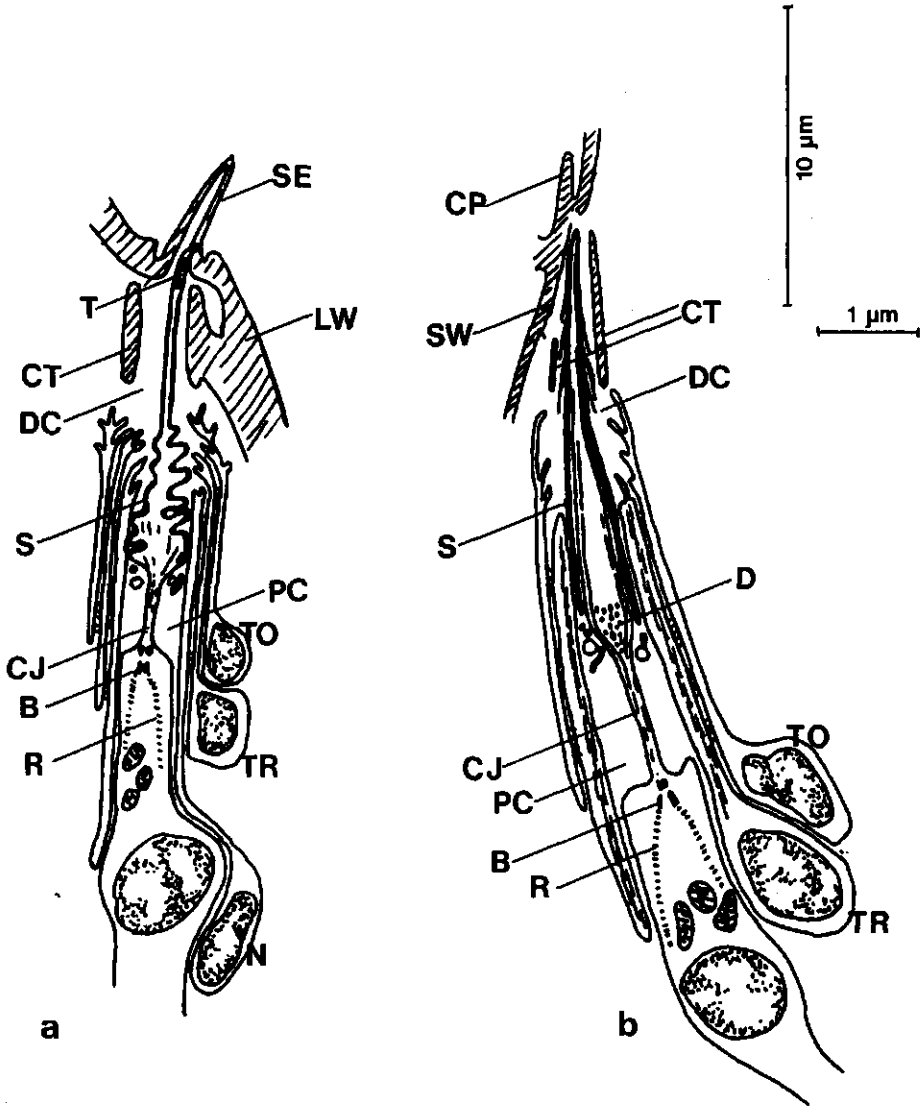

Fig. 3. Diagram of (a) labial tip sensillum and (b) stylet groove receptor. B, basal bodies; CJ, ciliary junction; CP, cuticular projection; CT, cuticular tube; D, dotted electron dense material; DC distal cavity; LW, labial wall; N, neurilemma cell; PC, proximal cavity; R, ciliary rootlets; S, scolopale; SE, sensillum; SW, stylet groove wall; TO, tormogen cell; TR, trichogen cell.

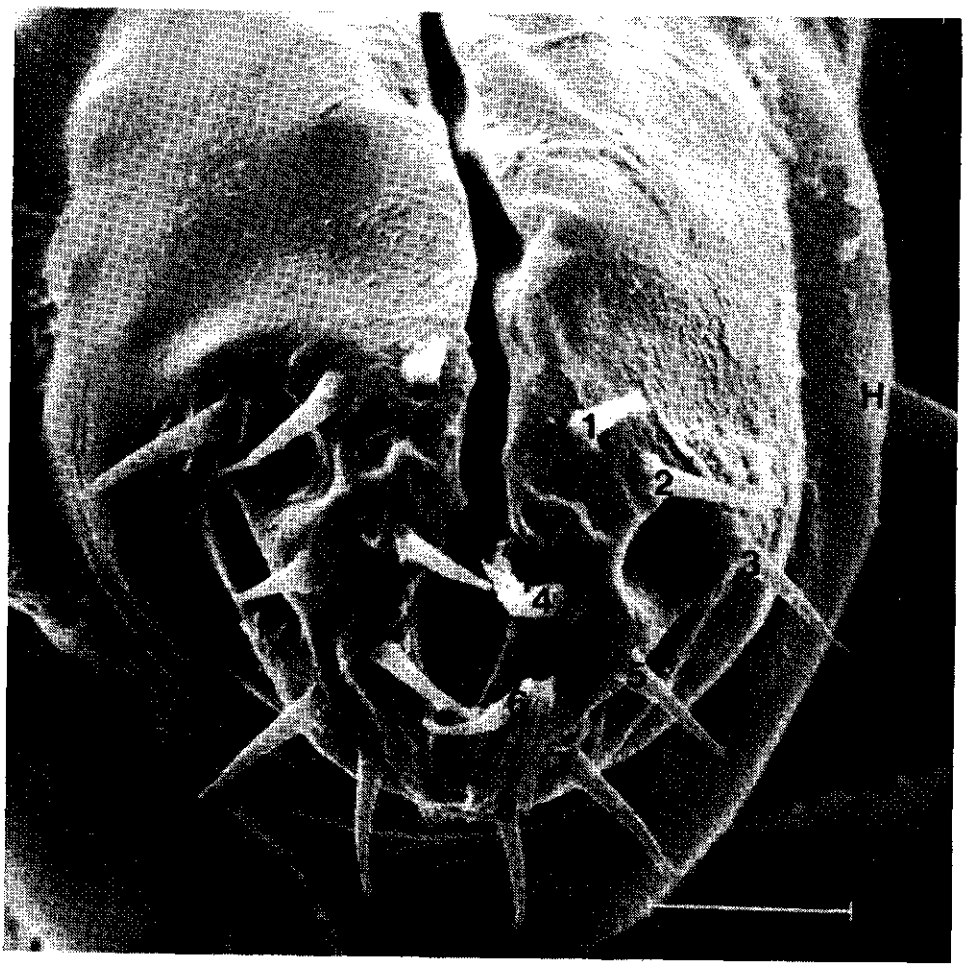

Fig. 1. Scanning electronmicrograph of labial tip with bilaterally symmetrically arranged sensilla, numbered 1 to 8. At the right the base of one of the more proximal protruding hairs (H) is seen. Bar 5 µm.

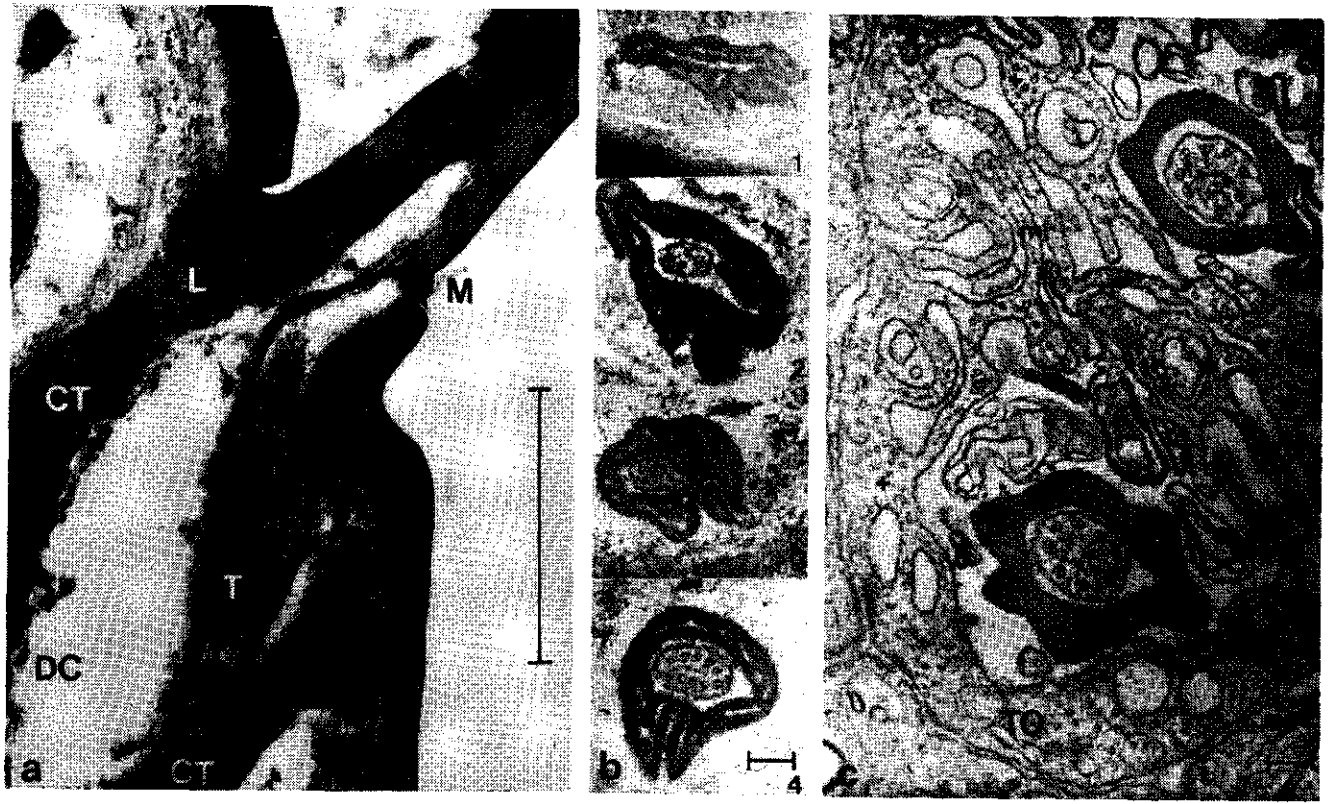

Fig. 4 a Labial tip receptor of *Masonovia ribis-nigri* Mosley. Note dendrite termination at electron dense plug of moulting pore. CT, cuticular tube; DC, distal cavity; L, lighter electron dense cuticular material; M, moulting pore; T, tubular body of dendrite. b, c) Transverse sections of stylet groove receptor dendrite with increasing number of microtubules from distal tip in proximal direction (b 1-4). The scolopale, S, demonstrates branched and infolded longitudinal ridges (b) reduced more proximally (c). Note ciliary arrangements of microtubules in this rather distal region (c). The tormogen cell, TO, loosely envelopes the dendrite with microvillar projections, m, and numerous microtubules (+). There is no trichogen cell in this region. Bars equal 0.1 μm.

Fig. 5. Dendrites of stylet groove receptors. a, b). Longitudinal sections of constricted dendrite region or ciliary junction. Scolopale (s) terminates (t) near constriction where electron dense dots are seen in center (D). Proximal cavity (PC) surrounds dendrite. Where dendrite dilates to proximal segment (PS) microtubules join in two basal bodies (B) with ciliary rootlets (R). Trichogen cell (TR) contains rigid fibrils (arrow). c). Cross-section of two (second can be seen left) dendrites near constriction. Dots of electron dense material (D) seen in dendrite as well as microtubule doublets (d) Bar 0.5 μm. d) Constricted dendrite with ciliary arranged microtubules in proximal cavity (PC). Two enveloping sheath cells seen (TO, tormogen and TR, trichogen cell) with septate junctions (+). Trichogen cell contains rigid fibrils (arrow). Bars equal 0.5 μm.

diameter varies from 0.75 to 1.2 μm. Proximally the three adjacent sheath cells lie close to the dendrite and intercellular spaces are reduced (Fig. 3a). A constriction of the dendrite to 0.3 μm occurs in the ciliary region where no scolopale is observed. The microtubules form a 9 × 2 + 0 arrangement and an intercellular space between dendrite and sheath cells forms the proximal sinus of about 0.75 μm in diameter. Two basal bodies are located in the inner segment of the dendrite and ciliary rootlets extend in the direction of the cell body that is at a distance of about 40 μm from the tip.

The stylet groove receptor dendrites terminate in the light cuticular material ventral to the stylet groove and adjacent to the cuticular projections in the groove. The dendrites dilate gradually from 0.2 μm apically to 0.7 μm near the proximal constriction (Fig. 3b) which has a diameter of 0.2 μm itself. This constricted region seems homologous to the ciliary junction region of the tip receptor dendrite. Single microtubules increase in number (Fig. 4b) to about 10 at the proximal end of the inner cuticular U-shaped tube. In this region doublets are formed in ciliary 9 × 2 + 0 arrangement (Fig. 5d), however, some micrographs suggest one central microtubule, but this is not consistent. At the dendrite constriction, dotted concentrations of electron dense material arise which extend proximally (Fig. 5a, c). The doublet microtubules seem continuous over a distance of about 10 μm distal to the basal bodies. Two basal bodies and ciliary rootlets extend in the dilated inner segment of the dendrite which contains numerous mitochondria (Fig. 5b). The cell body is located at about 50 μm from the labial tip, somewhat more proximally than the tip receptor cell bodies.

The distally flattened scolopales become gradually circular in cross-section and display branched and infolded longitudinal outer ridges (Fig. 4b). Proximally the ridges are reduced in number and length. Nevertheless, some thinner parts of the branched ridges are connected to the scolopale distally and do extend in the proximal direction. The scolopale diameter increases from 0.3 μm at their termination to 0.75 μm near the constriction of the dendrite.

Sheath cells

There are three sheath cells wrapped around each tip receptor dendrite. Microvilli of these cells extend into the outer extracellular cavity up to the proximal termination of the cuticular tubes. In this region the haemolymph space is in open connection with the distal cavity but more proximally the cavity is closed off by the sheath cells. The sheath cells contain numerous single microtubules and adjacent membranes are joined by abundant septate junctions. The cell bodies of the tormogen cell and the trichogen cell are located distally to the neuron soma and the cell body of the neurilemma cell is located more proximally.

The stylet groove receptor dendrites are each enveloped by two sheath cells. Microvillar projections of the tormogen cell extend up to the proximal end of the cuticular tube. The trichogen cell contains five to seven longitudinal fibrils of electron dense material, 0.1—0.3 μm in diameter. The trichogen cell and its supporting fibrils (about 8 μm long) enclose the extracellular proximal sinus at the

region of the contricted dendrite (Fig. 5b). In this cavity a few vesicles and tubules occur, probably originating in the trichogen cell. Both sheath cell bodies are found distal to the nerve cell body.

DISCUSSION

The present description of the labial tip receptors shows discrepancies with Wensler's study (1977). The most important difference concerns the method of attachment of the dendrite scolopale in the ecdysial canal or moulting pore of the sensilllum as has been described for other hemimetabolous insects (Gnatzy & Schmidt, 1971). Furthermore, evidence is presented with regard to the functional unity of the inner cuticular parts of the labial wall, except for the distal region. This region on which the sensilla arise, shows flexible features. No differences between species or morphs have been observed. Behavioural studies have shown that the palpating movements of the labium are generally performed during small scale exploration of the plant surface. The tip sensilla, the more proximal larger labial hairs, also innervated by a single dendrite with a tubular body, and the individual stylets all failed to give any neural response to stimulation, by general or specific host plant constituents in experiments using the electro-physiological tip recording technique (Tjallingii, unpubl.).

The stylet groove receptors are probably mechanoreceptors. They may play a role in transmitting information on the position and movements of the stylets in the labial tip. Noteworthy are the dimensions of the rigid elements of the receptor structure such as the scolopale and the trichogen cell fibrils. The functional relation between these structures and the cuticular projections in the stylet groove is difficult to understand.

Aphid mandibular stylets each contain two dendrites with a pair of microtubules (Parrish, 1967; Forbes, 1969). No pores, however, have been demonstrated, nor have unequivocal electrophysiological responses to chemical stimulation been obtained by other workers (Anderson & Bradley, 1963).

These facts in addition to the present results seem to allow us to state conclusively that aphid mouthparts are devoid of external chemoreceptors.

I thank Mr. F. L. Dieleman and other members of the "kleine club" for criticism; Mr. J. Groenewegen and Dik Lohuis for instruction with the electron microscope; Professor L. M. Schoonhoven for guidance and criticism.

RÉSUMÉ

MECANO-RECEPTEURS DU LABIUM DES APHIDES

Morphologiquement on a mis en évidence que les récepteurs du labium ainsi que ceux du fourreau des stylets décrits à nouveau ont une fonction mécano-réceptive. On n'a pu non plus montrer par la méthode électrophysiologique l'existence d'une fonction chimioréceptive au niveau des grands poils du labium et des stylets. On conclue qu'il n'existe au niveau des pièces buccales des aphides que des récepteurs mécanoréceptifs.

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CHAPTER 3

ELECTRICAL NATURE OF RECORDED SIGNALS DURING STYLET PENETRATION BY APHIDS

ABSTRACT

Electrical penetration graph, EPG, is a term suggested for the method of electrically recording of stylet penetration behaviour in aphids. The EPG appears to have two components with different electrical origin; i.e., resistance and electromotive force (emf). Fluctuating aphid resistance in series with the amplifier input resistor causes a fluctuating voltage due to dividing of a DC potential in the primary circuit. This circuit potential is composed of a supplied potential and potentials from the preparation such as half cell potentials at the electrode surfaces. The emf component consists of fluctuating potentials generated within the preparation itself. The value of the amplifier input resistance is rather critical; a high value in proportion to the aphid resistance favours the emf component, and a low value emphasizes the resistance component. Although resistance fluctuation forms the largest component of the signal, some parts of EPG patterns stem from emf. The DC method provides a more detailed signal than an AC system. The current through the aphids appears very low and is not likely to influence feeding, or other biological functions of the aphid.

INTRODUCTION

Twenty years ago a method for electrically recording of aphid penetration was introduced (McLean & Kinsey, 1964). In this method a thin wire is attached to the insect abdomen. As soon as the aphid stylets penetrate the substrate, the electrical circuit is completed and a signal can be recorded.

Subsequent papers (McLean & Kinsey, 1965, 1967) demonstrate a correlation of signal patterns with the location of the stylet tips in the plant tissue. Considering the current ideas about stylet position in relation to penetration behaviour (as reviewed by Miles (1968) and Pollard (1973)) McLean & Kinsey (1967) concluded that there was a 'definitive correlation of electronically recorded wave forms with aphid probing activities'.

Using a modified recording method we showed that at least six different patterns exist (Chapter 2). Some of these occur simultaneously and are superimposed. With radio isotope experiments it was confirmed that some patterns are more correlated with saliva secretion or with ingestion than others, but it seemed unlikely that aphids would produce exclusively either a salivation or an ingestion pattern. In the course of our research, and by comparing methods, we became aware of a number of questions about the electrical properties of the aphid and their relations to the input specifications of the amplifiers used. McLean & Weigt (1968) discussed some of them but several aspects remained obscure. Presumably this is one of the main reasons why this promising technique (Miles, 1972; Campbell *et al.*, 1982) has not been used more extensively. Another reason may be our limited knowledge of the penetration behaviour itself. We do not know, for example, whether penetrating stylets pass between or through the cells, cell walls, protoplasts, or vacuoles. Neither do we know which stimuli at these places, are important to and perceived by the penetrating aphid, nor precisely when sap samples are taken and from which parts of the plant tissue. Also, the composition of the saliva and its function from moment to moment is not known. Research on the electrical recording of stylet penetration may extend our knowledge of this complicated behaviour. In this paper, which presents a description of the electrical properties of the plant-aphid preparation in

relation to the amplifier specifications, we will try to contribute to an improvement of the electrical recording technique. Moreover, a comparison is made between DC and AC systems.

MATERIALS AND METHODS

The aphids used in the experiments include *Brevicoryne brassicae* (L.), *Myzus persicae* (Sulzer), *Acyrthosiphon pisum* (Harris), *Megoura viciae* (Buckton) and some other species (Chapter 4). Apterous virginoparous adults were used predominantly, but sometimes alates or juveniles were also used. Aphids were reared in the laboratory at about 15°C and 16 h light per day, or collected in the field.

Here we introduce the term 'electrical penetration graph' (EPG). This term indicates that the recorded graph is a result of an overall electrical signal caused by stylet penetration activities. It may be recorded from aphids or other penetrating insects. Other terms, definitions or descriptions which have been suggested in the literature we consider either too limited (electrically recording of ingestion and salivation; McLean & Kinsey, 1964) or too general (Electronic Monitoring System; Holbrook, 1978).

Primary circuit. In our DC system the EPG is recorded only if the aphid completes the electrical circuit with its stylets. This primary circuit (Fig. 1) is composed of a number of elements, of which the electrical properties are the subject of this study. An adjustable DC voltage source supplies an electrical potential to the substrate. The feeding substrate may be either a parafilm sachet containing an artificial diet or solution, or a potted plant, or a part of a plant in water. The substrate electrode is located in the diet, the potting soil, or the water. The aphid is attached to a thin (20-50 µm) metal wire by means of conducting silver paint. This wire acts as the aphid electrode, and is connected to the DC amplifier (gain = 25x or 50x). The last element is the input resistor of the amplifier. The input resistor is grounded, thus completing the circuit. The amplifier described previously (Chapter 2) was modified in two ways. The input resistor was changed from $10^7\Omega$ to $10^8\Omega$ and the adjustability of the voltage supply was extended to ± 600 mV.

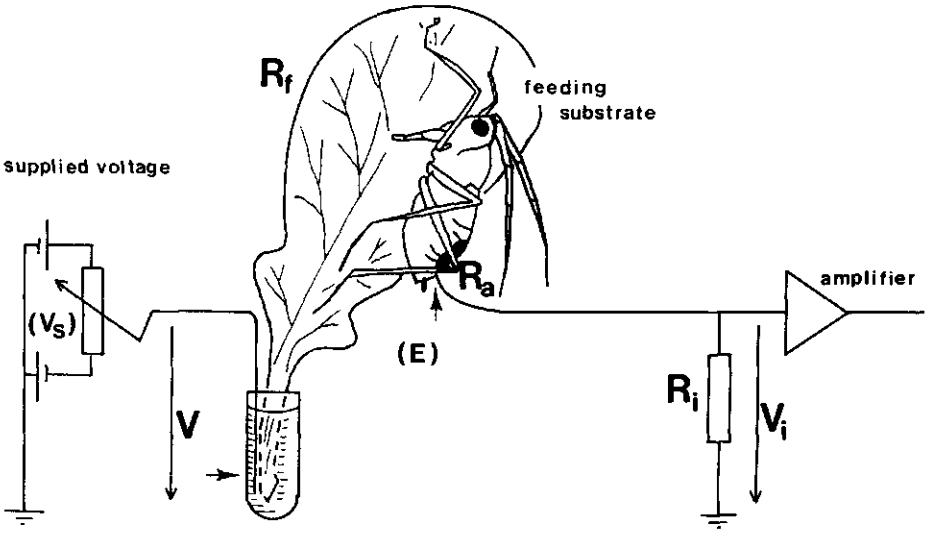

Fig. 1. Primary circuit for recording an electrical penetration graph (EPG) during stylet penetration in an excised leaf. Amplifier (25 or 50x) output to recording devices. (V_s), adjustable potential (± 600 mV); (E), preparation potential (mainly electrode potentials, small arrows); V, circuit potential ($V = V_s + E$); V_i, signal potential (i.e., voltage across R_i); R_i, input resistor; R_a, aphid resistance; R_f, resistance of feeding substrate. Large arrows, voltage across arrow base and ground.

Resistance measurement. We assume that the aphid represents a major resistance (R_a) in the primary circuit, additionally to the input resistor of the amplifier (R_i). Since the feeding substrate resistance (R_f) does not generally exceed 300-400 kΩ (c.f., McLean & Weigt, 1968), we can neglect this relative low resistance for the

sake of simplicity. The signal potential (V_i) accross R_i can be described then according to Ohms law as

$$V_i = V \frac{R_i}{R_i + R_a} \quad (1)$$

where V is the summed potential due to all electrical potentials in the primary circuit. These potentials are: the supplied potential (V_s) and the sum of other stationary potentials from sources in the preparation, such as electrode potentials (Strong, 1970). If these preparation potentials are represented by 'E' (Fig. 1) then,

$$V = V_s + E \quad (2)$$

Hence, the magnitude of V can be controlled by adjustment of V_s. The relations of equation (1) and (2) were used in two different experimental procudures to determine aphid resistance.

In the first method a square pulse of 50 mV (ΔV_s) is superimposed on the supplied potential (V_s) during EPG recording. V_i will then increase with an amount ΔV_i, which is in proportion to R_a:

$$V_i + \Delta V_i = (V + \Delta V_s) \frac{R_i}{R_i + R_a} \quad (3)$$

Subtracting of (1) from (3) provides R_a as:

$$R_a = \left(\frac{\Delta V_s}{\Delta V_i} - 1\right) R_i \quad (4)$$

in which R_a is now independent of V and can be calculated when ΔV_i is measured on a graph or on the oscilloscope. However, measurement of ΔV_i is not easy, since the signal potential fluctuates continuously. On the other hand, the frequencies of these fluctuations are rather low, and certainly lower than the rise time of the square pulse edges. Therefore the potential changes in the EPG caused by the pulse edges are clearly shown and form a reliable estimator of ΔV_i. Then R_a can be obtained according to (4).

In the second method V_s needs to be adjusted to a potential which is equal to E but with an opposite sign, thus resulting in a zero volts circuit potential according to (2) ($V = 0 = V_s + E$). Generally E is unknown and consequently V is unknown. When V_s compensates for E and a square pulse is applied in addition, V has either a value of zero volts or that of the square pulse amplitude. Since V is constant during the pulse, the changes of V_i, which can be observed in graphs (see Fig. 4a), reflect the changes in R_a according to (1). Thus, this method enables calculation of R_a for each measured value of V_i during the entire pulse, not at the pulse edges only. This second method is less convenient than the first, because V_s is difficult to adjust properly, due to the 'residual amplitude' (see Results).

From different values of R_a obtained during a particular EPG pattern, a resistance fluctuation ratio of an aphid can be derived in terms of its minimum and maximum as

$$\frac{R_a \text{ max} - R_a \text{ min}}{R_a \text{max}} \times 100\% \qquad (5)$$

This ratio can be calculated for each individual EPG pattern.

Measurement of potentials from preparation sources. In the foregoing we assumed that V_i is caused by the effect of a changing aphid *resistance* on a constant potential V. The potentials from the preparation, E, which contribute to V are rather constant in the course of time in this assumption. However, preliminary observations urged us to suppose an additional origin of V due to fluctuating sources of biopotentials in the preparation. These sources can be indicated as *electromotive forces* (emf). To measure these, an amplifier with a high input resistance is needed. Such an 'emf amplifier' was developed with an input resistance of $10^{11}\Omega$ as compared to $10^{8}\Omega$ in our ordinary amplifier. The emf amplifier will fairly accurately record continuous (DC) potentials as well as fluctuating (AC) potentials due to emf sources from the plant and aphid.

The input resistance, R_i, of the ordinary amplifier has about the same value ($10^8\Omega$) as the mean value of R_a. Using the ordinary amplifier V_i will reflect not more than half of the potential V according to equation (1). In measurements with the emf amplifier the observed signal potential, V_i, becomes approximately equal to V ($R_a = 10^8\Omega$ and $R_i = 10^{11}\Omega$ in equation (1)). Then the signal potential will be rather independent of fluctuations in R_a. Even when R_a increases to $10^{10}\Omega$, V_i maintains a value of 91% of V (Table 1). In Table 1 the magnitude of V_i as percent of V is used as the accuracy of measurement of source potentials encountered by the penetrating aphid. The higher the value in the columns (min. or max.) is, the more accurate the measurement will be. On the other hand, if aphid resistance fluctuations contain important information, the responsiveness to them of each amplifier should

R_i | R_a →	$10^8\Omega$	$10^{10}\Omega$	
$10^7\ \Omega$	9 %	0.1%	98.9%
$10^8\ \Omega$	50 %	1 %	98 %
$10^{11}\Omega$	99.9%	91 %	8.9%
	(max.)	(min.)	
	Magnitude of V_i ($= \frac{V_i}{V}$ 100%)		Fluct. ratio of magn. ($= \frac{(\text{max})-(\text{min})}{(\text{max})}$ 100%)

Table. 1. Responsiveness of three amplifiers to resistance and emf fluctuations of the preparation. Magnitude of V_i as percent of V reflects responsiveness to emf component, fluctuation ratio *reflects responsiveness to resistance component*. V_i, signal potential; V, circuit potential; R_i, input resistances of the amplifiers; R_a, aphid resistances.

be given when different amplifiers are compared. In Table 1 the fluctuation ratio of the V_i magnitude, caused by R_a fluctuation, indicates such. This ratio can be derived similarly for the minimal (min.) and maximal (max.) magnitude of V as in equation (5) for R_a. The higher this value, the better the resistance fluctuation is reflected in the signal. With these two parameters, the magnitude of V_i and its fluctuation ratio, three different amplifiers are compared. The R_i values belong to our original amplifier ($10^7\Omega$, Chapter 2), to the ordinary ($10^8\Omega$) and emf amplifier ($10^{11}\Omega$), whereas the R_a values (10^8 and $10^{10}\Omega$) can be considered as minimal and maximal R_a values.

The signals of the two amplifiers can easily be compared by arrangement of a flexibly wired $10^8\Omega$ resistor between two sockets on the outside of the emf amplifier. One socket is connected to the amplifier input and the other is grounded. When the resistor is plugged in its value determines R_i, and the specifications are identical with the ordinary amplifier. By removing it, the emf amplifier is restored. By alternately inserting and removing it during a penetration the respective graphs can be recorded also alternately. Besides the high input resistance made of a plastic foil, the emf amplifier contains a circuit to compensate for the input current owing to the applied Ic-Op-amp (AD 515). This circuit allows a zero volts base line to be recorded when the aphid does not penetrate (open input). In the ordinary amplifier the $10^8\Omega$ input resistor forms just a sufficient conductor to ground the open input, which prevents a biassed zero reference level. Higher resistances than this cannot be applied without input current compensation. Otherwise, the amplifier output will give the voltage of the power supply (± 15 V).

Capacitance measurement. For the calculation of the aphid capacitance (C_a) it is necessary to know the input capacitance of the amplifier (C_i). When C_i is known, C_a can be obtained. Both capacitances were derived from experiments. From these experiments also the best fitting arrangement of capacitances and resistances in the primary circuit could be derived.

C_i represents a stray capacitance which can be thought in parallel with R_i (Fig. 6). It can be measured directly with a commercially available capacitance meter, but it has also been estimated experimentally. In this procedure a $10^8\Omega$ series resistor (R_s) is connected between the amplifier input and the square wave generator. The distortion of the square pulse which is due to the circuit properties is amplified and displayed on an oscilloscope, where its features are measured. The value of V_i for this circuit as a function of time is given by:

$$V_i\ (t) = V_{sq} \frac{R_i}{R_i + R_s}\ (1 - e^{-t/\tau}) \qquad (6)$$

in which t is time, V_{sq} the square pulse amplitude, e the base of the natural logarithm, and τ is a time constant which provides the relation to C_i as:

$$\tau = \frac{R_i R_s}{R_i + R_s} C_i$$

When the parameters V_i and t are measured on the oscilloscope, C_i can be calculated for a given V_{sq}. Both methods give values of about 6 pF for C_i.

Similarly V_i as a function of time can be formulated for almost any possible arrangement of the aphid and plant capacitances and resistances. Thus, hypothetical electrical equivalents can be tested by comparing the observed and calculated time courses. Consequently, the best fitting arrangement can be selected and with its equation, C_a can be obtained for a given C_i. However, the equations for these relations are rather complicated and a full discussion of them would go beyond the scope of this paper.

RESULTS

Resistance. When V_s changes from positive to negative, V_i does likewise. This is demonstrated by connecting the substrate electrode to a triangle wave source at a low repetition rate (Fig. 2). When V_s is maintained at a steady positive or negative level, V_i maintains its positive or negative values correspondingly (Fig. 3). However, a comparison of the signals shows that the positive wave form is not an exact mirror image of its negative counterpart.

Fig. 2. Signal potential (V_i, upper trace) following triangular changes of V_s (lower trace) with 10 s period and ± 200 mV amplitude. Potential drop (between arrows) maintains its own negative sign. Vert. bar: upper trace, 100 mV; lower trace, 500 mV. Time scale, 1 s. Pattern C, *Brevicoryne*.

Aphid resistances of about $10^9\Omega$ are obtained (Fig. 4 and 5). Within pattern C a fluctuation from 4.10^8 to $10^{10}\Omega$ is observed (Fig. 4a). The fluctuation ratio in this example of pattern C, obtained according to equation (5), is therefore 96%. For pattern D+E a ratio of 12% has been calculated, while pattern A and B have ratios similar to C or even higher.

Besides these rapid fluctuations a slow increase of the aphid resistance is found in the course of the experiment. In Fig. 5 this increase is given for two different aphids during 41.5 h following attachment to the dorsal electrode. The initial values may differ considerably, but over the course of time the change appears very similar.

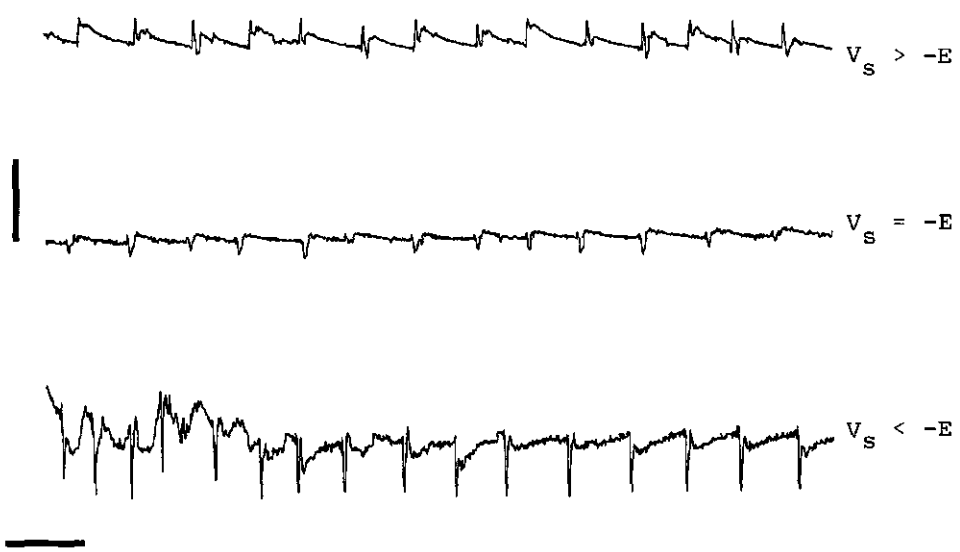

Fig. 3. Pattern D+E recorded with three different V_s adjustment positions. V_s > -E, upper trace. V_s < -E, lower trace. V_s = -E, i.e., compensating for E, middle trace (residual amplitude). Vert. bar, 10 mV. Time scale, 1 s.

Preparation sources. A considerable signal potential is observed when the supplied potential is adjusted to zero volts or the preparation is grounded directly without using a potential supply. Since V_s is zero volts then, V equals E according to equation (2). Further adjustment can reduce the observed potential almost completely. Then V_i is zero volts as a consequence of V being zero volts (equation (1)), and V_s equals -E according to equation (2). The value of V_s required to achieve this reduction may be positive or negative, and generally lies between -300 and +300 mV, which indicates that the preparation potential, E, lies between these values. In potted plants however, when a rod electrode is inserted into the soil, this potential may be up to ± 800 mV. When the emf amplifier is

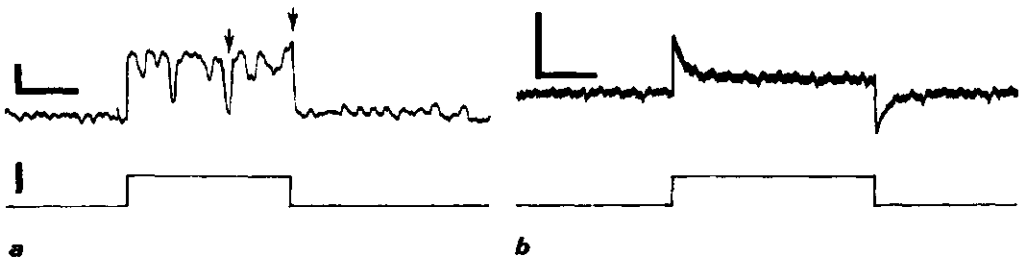

Fig. 4. Signal responses (upper traces) to 50 mV square pulse (lower traces). Vert. bar: 5 mV (upper) 50 mV (lower). Time scales, 1 s.
a. Response during pattern C when V_s = -E. Right arrow, min. R_a ($4.10^8\Omega$), left arrow, max. R_a ($10^{10}\Omega$).
b. Response during pattern D when V_s > -E. Aphid with bad silver paint contact showing result of high pass filtering.

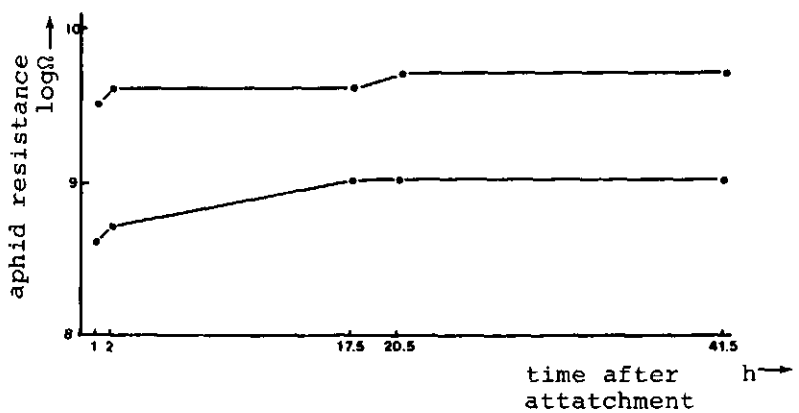

Fig. 5. Increase of aphid resistance in the course of time in two individuals, presumably due to wax secretion under the silver paint.

applied and V_s is adjusted to zero volts, the signal potential, V_i, accurately reflects the E values, which were found in agreement with those above. In the course of time (hours) this value sometimes changes continuously (drift) in either a positive or negative direction. When the ordinary amplifier is used this drift causes a change in signal potential and sign comparable to half a period in Fig. 2 but much slower.

A complete reduction of the signal by adjustment of V_s appears to be impossible. The low residual amplitude (Fig. 3, middle trace) will increase with further attempts at positive or negative adjustment. When the residual amplitude is recorded alternately with the ordinary and the emf amplifier the signals appear similar. When the two signals are compared at other V_s adjustment positions they appear less similar. Though generally the wave forms from the ordinary and the emf amplifier have many features in common, some patterns or pattern elements differ more or less, whereas in a few remarkable cases there is even no resemblance at all. The potential drop during pattern C is an example of such an element (Chapter 4 and 5). In contrast with the ordinary amplifier, adjustment of V_s changes nothing in the signal but the DC level when the emf amplifier is applied.

Capacitance. Values of 1 to 2 pF for the aphid capacitance, C_a, have been obtained from the measurements. Though the aphid resistance may change considerably (see resistance), the capacitance fluctuations remain rather small.

On the basis of the agreement between the calculated and the observed distortion of a square pulse, the best fitting arrangement of resistances and capacitances in the primary circuit was selected. In this arrangement R_a is in parallel with C_a (Fig. 6).

DISCUSSION AND CONCLUSIONS

The direct effect of the supplied potential (V_s) adjustment on the signal indicates that resistance fluctuation of the aphid represents a major component of the EPG in the given primary circuit. On the other hand, the existence of a certain residual amplitude, which cannot be reduced by further V_s adjustment, demonstrates that there

must also be another signal source. Otherwise V_i should be zero since V is zero volts (eq. (1)). This additional minor component is referred to as the emf component and is apparently generated by the preparation itself. Although McLean and Kinsey (1964) suggest the possibility of such an emf source, more recent work (McLean & Weigt, 1968), refers only to the effect of resistance fluctuation. Our results indicate that the emf component of the signal is due to a fluctuating source potential which is part of V and therefore contributes to V_i.

The fluctuation ratio of the aphid resistance is different for each pattern, which might indicate that the resistance is determined by different mechanisms. Valve mechanisms which can open and close the food or saliva canal, fluctuating concentrations of electrolytes in the food or saliva, and stylets entering into more or less conductive elements of the plant tissue, can be thought as possible causes of aphid resistance fluctuation. Each pattern may either be caused by one of these mechanisms, or by a combination of mechanisms. Combinations can be thought to act simultaneously or subsequently. Similarly one may speculate on the biophysical causes of the emf component. Presumably this emf is mainly caused by streaming potentials from fluids passing through the two stylet capillary canals. Such potentials depend on affinity differences of the capillary wall to charged particles and ions. They are also determined by the capillary diameter, the electrolyte concentration, the fluid velocity, etc. (Kortüm & Bockris, 1951). The emf component may also be caused by biopotentials from plant or aphid, such as membrane potentials from plant cells (Chapter 4 and 5) or electrical potentials from the gut, heart, brain, or muscles. However, it is not likely that the latter potentials influence the EPG significantly, unless they originate close to one of the extremities of the stylet canals. More remote sources are more or less short-circuited. Besides, the amplifier gain is too low (50x) to show most of these potentials.

The contribution of the resistance and the emf component to the signal potential (V_i) depends very much on the value of R_i in the primary circuit (Table 1). The magnitude of V_i in relation to V reflects the contribution of the emf component to the signal, and its fluctuation ratio reflects the resistance component. Using a

$10^8\Omega$ input resistor seems a reasonable compromise in which both components are recorded. Generally most phenomena studied in electrophysiology are due to emf. The input resistance required for the amplifiers used needs to be very high to avoid the effects of passing significant electrical currents through the preparation, and to avoid influence of resistances inherent in the preparation itself. However, in our method the preparation resistance fluctuation provides essential information that should not be overlooked.

The signal potential visible when V_s is adjusted at zero volts or grounded proves that the supposed preparation potential E is real. The higher values of E that were found in potted plants, are presumably due to electrochemical interferences with specific soil components at the electrode interface. Thus, electrode potentials may be the main causes of E. Although such potentials interfere with the signals generally recorded with DC amplifiers, they can either be avoided by applying non-polarisable electrodes (Strong, 1970) or they can be compensated for. Since it is impossible to avoid them in our EPG system we are bound to compensation. During normal EPG recording V_s adjustment is generally maintained at a potential of about (-E + 100) mV. When compensated for, the residual amplitude is the emf component as concluded above. Thus, with the ordinary amplifier the emf component can be recorded but since the signal is small (c.f., Fig. 3, middle trace), it needs further amplification. In adjustment positions of V_s other than compensating the potential E, the resistance component is recorded in addition and generally overrules the small emf component. The emf component is not disappeared, but is superimposed on the resistance component. Since the emf component in the example of Fig. 3 (middle trace) shows small negative pulses, its amplitude is added to the pulse amplitude of the negatively adjusted signal (lower trace), which is, as a result, somewhat greater that the pulse amplitude of the positively adjusted signal, from which it is subtracted. Although the effect is small in this example, it may be distinct in other patterns or when the emf component is proportionally greater. This may explain why the appearance of the positive wave form is not an exact mirror image of the negative wave form.

According to Ohm's law a current flows in the primary circuit when it is completed. Since we have measured the potentials and

calculated the resistances, we may estimate the maximum current passing through the aphid. Assuming an aphid resistance of $10^8\Omega$ and a voltage across the insect of 250 mV, which corresponds with a circuit potential of 500 mV, the current will be 2.5 nA. In other cases, a value calculated for current passing through an aphid was 3.7 nA (McLean & Weigt, 1968), through a mosquito 6 nA (Kashin & Wakely, 1965), and 70 nA through the blood sucking bug *Rhodnius* (Smith & Friend, 1970). Although this is a very small current, it represents a considerable current density in the small stylet canal. Taking a surface area of 1 and 1000 μm^2 for the aphid and the bug, the resulting current density is 250 mA/cm^2 and 0.7 mA/cm^2 respectively. For the aphid this value is comparable to that applied in electrophoresis. However, it seems unlikely that the movement of charged moieties or the electroendoosmotic effects on fluids (Kortüm & Bockris, 1951) in the stylet canals will seriously alter aphid feeding or salivation. The effects seem to be small in proportion to the fluid velocity in the stylet canals. A velocity of 7 mm/s was calculated from a diet uptake in *Myzus persicae* of 7 pl/s (unpublised data), which corresponds with the rate of diet uptake found by Mittler (1970). Electrophoretic effects of ions or proteins in other parts of the aphid or plant will be negligible, since the area exposed to current passage is much larger, and so the density will be much smaller.

Although the calculated capacitance of the aphid (C_a) is small, 1 to 2 pF, combination with the high values of R_a and R_i may create frequency filtering within the bandwidth of the EPG (0-75 Hz). If, as an example, the input capacitance (C_i) of 6 pF and a C_a value of 2 pF are combined with a R_i of $10^8\Omega$ and a R_a of $75.10^8\Omega$, an attenuation difference between a signal frequency of 50 Hz and DC (=0 Hz) of 12.7 dB can be calculated. This means that a 50 Hz frequency is recorded more than 4x better than a DC potential. When R_a is $5.10^8\Omega$ the attenuation difference is only 0.9 dB. Thus, a high R_a value limits the low frequency responses of the primary circuit. For a square pulse the consequences are shown in Fig. 4b. The edges pass the circuit considerably better than the pulse height. Because of this relation, an increase of R_a in the course of time (Fig. 5) may lead to a distortion of the EPG. Although the aphid capacitance is not constant, there are no indications that it changes much. If

the capacitance changes during a penetration from 1 to 2 pF, the attenuation difference between DC and 50 Hz will change from 9 dB to the 12.7 dB mentioned. This indicates that the aphid-capacitance changes limit the frequency responses of the primary circuit less than do aphid-resistance changes.

On the basis of the foregoing calculations and measurements we may give now a simple electrical equivalent for the penetrating aphid in the primary circuit (Fig. 6). Electrical contact between the stylet tip and the leaf is represented by a switch (s). The variable resistance of the penetrating aphid (R_a) is the main resistance and is in parallel with the variable aphid capacitance (C_a). Several sources of potentials are present, including two half cell potentials, one at each electrode (E); a fluctuating plant source (e), i.e., the emf component of plant origin; and a fluctuating aphid source (e), i.e., the emf component from the aphid. Apart from the amplifier input capacitance (C_i), the other components have been presented in Fig. 1.

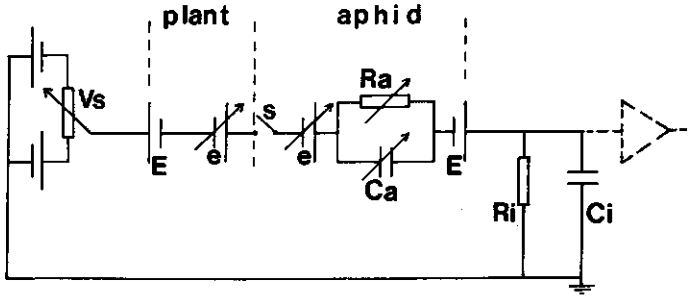

Fig. 6. Simple electronic equivalent of penetrating aphid in primary circuit. V_s, supplied potential; E, electrode potentials; e, fluctuating emf; s, switch; R_a, aphid resistance; C_a, aphid capacitance; R_i, input resistance; C_i, input capacitance. Dotted lines, electrode/plant/aphid/electrode interfaces. Dotted triangle, amplifier.

It is difficult to compare the ordinary amplifier with the AC system, introduced by McLean and Kinsey, since several specifications of the AC amplifier and the recording devices used were not reported (McLean & Weigt, 1968). Nevertheless, a few remarks can be made. The primary circuit of the AC system differs only in two aspects from the DC circuit: a different supplied potential, i.e., a 60 Hz carrier wave, and a lower input resistance of only 47 kΩ. It is not certain whether the latter is a pure resistance or an impedance since audio amplifiers often have a capacitor coupled input to block DC potentials. If we assume that it is an input resistance, only a very small fraction (V_i, about 0.05%) of the supplied 60 Hz potential (V_s) enters the amplifier input according to equation (1). As a consequence either the amplitude of V_s or the amplifier gain needs to be much larger than in our system. In the AC system the amplitude of the carrier wave follows the changes of R_a; i.e., amplitude modulation. Further on in the system the amplitude is rectified and filtered (low rim) te get a smooth signal. The consequence of this procedure is that information of frequencies above 40-50 Hz is rejected or at least attenuated.

For the emf component the responsiveness of the AC system is different. This component does not alter the carrier wave amplitude, but it is superimposed on it. Since the audio amplifier has a bandwidth of 10 Hz to 20 kHz (McLean, 1977) the information below 10 Hz is rejected. Thus for the emf component the AC system forms a band-pass filter from about 10-40 Hz. Since the EPG provides significant amplitudes up to a frequency of 75 Hz with the main amplitudes up to 25 Hz (unpublished filter experiments), only the middle frequencies of the emf component and the lower frequencies of the resistance component pass through the AC system. By applying a lower carrier wave frequency (20 Hz, Brown & Holbrook, 1976) a considerable part of the resistance component frequency is also rejected, while a higher frequency (e.g., $\geqslant$ 100 Hz) obviously favours the higher signal frequencies (Campbell *et al.*, 1982). An advantage of the AC system seems to be that DC potentials and drift from the preparation do not interfere with the signal, but a disadvantage is that other DC information is also rejected by the system. Thus, an essential difference with the DC system is that the AC system is not responsive to a slow emf component. Another disadvantage of an

AC system, particularly at 50-60 Hz, is that it may interfere with the nerves and muscles more than a DC system (Katz, 1966).

Obviously, the bandwidth of the devices used for amplification, rectification, and recording may cause considerable distortion of the signal if they are not in accordance with the produced frequencies. An ordinary audio tape recorder, like an audio amplifier, is unsuitable for frequencies below 20 Hz. Instead, an FM tape recorder should be used. Most flatbed chart recorders have a bandwidth of only 5 Hz (DC-5 Hz), whereas it should have a bandwidth of 50 Hz or more.

It may be concluded that the EPG is the resultant of interactions of the electrical properties of the penetrating aphid and the circuit of which it is a part. The input resistance of the amplifier is a very important element of this circuit. Its value determines the ratio of the contribution of emf and resistance to the signal. An understanding of the electrical properties of the preparation and the instrumentation is essential for a correct interpretation of the graphs.

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CHAPTER 4

Membrane potentials as an indication for plant cell penetration by aphid stylets

ABSTRACT

Electrical penetration graphs (EPGs) of aphids on plants demonstrate distinct periods of lowered potential level: the potential drop (pd). Such pds are produced frequently during the stylet pathway to the phloem. Experimental evidence supports the hypothesis that the pd corresponds with a stylet puncture of a (epidermal or mesophyll) cell membrane. The intracellular potentials of -100 to -180 mV are thought to be measured thereby with the stylets acting as microelectrodes. The short pd of 5-15 s can be described as a sequence of three distinct phases. Besides the short pd, occurring during pattern B and C, long pds are produced during the complete pattern D+E. Pattern D+E may occur on the potential level of the pd (abbr.: D+E(pd)) and on the potential level of the preceding pattern C (abbr.: D+E(c)). Pattern D+E(pd) seems to be related to sieve element penetration, at least in a number of cases. Both pds, short and long, are produced on host and non-host plants, on susceptible and resistant cultivars, using several aphid species.

INTRODUCTION

Aphid penetration behaviour can be recorded elecrically by making the insect and the plant part of an electrical circuit (McLean & Kinsey, 1964; Chapter 2 and 3). This recorded signal, the electrical penetration graph (EPG), has been described in terms of amplitude and frequency as a sequence of six different patterns, A to F (Chapter 2). The EPG appears to have two different electrical origins: resistance fluctuation, and electromotive force (emf) (Chapter 3). The most remarkable emf pattern fragment is the potential drop (pd). Short potential drops frequently occur during pattern C, a pattern that has been correlated with stylet sheath formation and other penetration activities between epidermal and phloem tissue. Salivation and some ingestion have been demonstrated both to occur during this pattern. Long potential drops may occur during the succeeding composite pattern D+E. Pattern D has been correlated mainly with ingestion. Although this pattern is called 'ingestion' by workers using AC devices (McLean & Kinsey, 1967), there is evidence for a rather continuous salivation, presumably watery saliva (Miles, 1972), when pattern E is produced simultaneously (Chapter 2).

Experimental results leading to a hypothesis for the origin of the potential drop are reported in the present article and a description of the observed wave forms under different electrical conditions is provided.

MATERIALS AND METHODS

Brevicoryne brassicae (L.) on Brussels sprouts was the main aphid-host plant combination used in this study. Additionally, some other combinations were studied: *Acyrthosiphon pisum* (Harris) and *Megoura viciae* (Bukton) on broad beans, *Macrosiphum euforbiae* (Thomas) on potato, *Myzus persicae* (Sulzer) on cabbage, *Nasonovia ribisnigri* (Mosley) on lettuce, *Rhopalosiphum maidis* (Fitch) on barley, *Eriosoma lanigerum* (Hausmann) on apple, and *Tuberolachnus salignus* (Gmelin) on willow. EPGs of *B. brassicae* and *M. persicae* were also recorded on artificial diets and 20% sucrose solutions containing 0.1% NaCl. Recordings were also obtained from *B. brassicae* penetrating 21% sucrose solutions containing protoplasts which were isolated from cabbage leaves.

To obtain these protoplasts the lower epidermis was peeled from leaves with fine forceps. The leaf areas in which this was successful were cut out and placed on top of an 11% mannitol solution in Petri dishes. This preplasmolitic solution was replaced after about 20 min by an enzyme solution containing 1% cellulase and 0.1% Macerozyme® (pectinase) at pH 5.4 in which the leaf pieces were incubated overnight at 25°C. Next the pieces were stirred gently to release the protoplasts, and the larger leaf remnants were removed. The remaining suspension was centrifuged at about 80 g for 5 min. Pellets were resuspended in 21% sucrose and as a result of further centrifugation at about 80 g for 10 min the protoplasts floated on top of the solution. With a Pasteur pipette a few droplets of this floating layer were transmitted to a parafilm membrane stretched over a small (20x20 mm) perspex frame. After insertion of a thin gold wire electrode in this fluid a cover glass was placed on top to allow microscopical observation of the protoplasts and of the aphid stylets. The aphid was brought into contact with the membrane from below after attachment with silver paint to a thin (50 µm) copper wire, firm enough to support the insect. For plant penetrations the more flexible (25 µm) gold wire was used. Our ordinary amplifier (Chapter 2) was used with an increased input resistance ($10^8\Omega$). The emf components of the EPG were recorded separately with a high input resistance ($10^{11}\Omega$) emf amplifier, which allowed more accurate recordings of the potentials arising in the aphid and the plant tissues (Chapter 3). Further details of the resistance and emf component of the signal could be studied by changing the potential of the primary circuit during recording with the ordinary amplifier. This could be achieved by adjustments of the potential supply to the plant or diet, referred to as the supplied potential. The magnitude and sign of the resistance component alter with the supplied potential, whereas the emf component does not change (Chapter 3). EPGs were recorded with a FM-tape recorder (Racal 7DS) and a chart recorder (Watanabe, WTR 771) with a 75 Hz bandwidth.

RESULTS

Short potential drops during pattern C in plant penetrations are

characterized by a sudden drop of the signal potential, a continuation of this potential for 5-15 s, and a rapid return to the original potential level (Fig. 1 and 2). The duration of 41 pds in three given penetrations ranged from 4.5-14.6 s with a mean of 6.9 s (SD = 2.1). The first pd may occur as soon as 10 s after the beginning of a penetration during pattern B, but generally they are observed for the first time during pattern C after about one minute. The intervals between two pds differ considerably in length in most penetrations. For instance, in two penetrations not shown here, the mean duration of 86 intervals was 52 s (SD = 46) within the range of 8 to 250 s. Occasionally, however, (5 observations out of 58) pds occur at a higher frequency and more regularly (Fig. 1.2). The frequencies measured in these penetrations were in the range of one pd every 25-35 s and the periods covered by these regularly repeated pds lasted from 5 to 30 min.

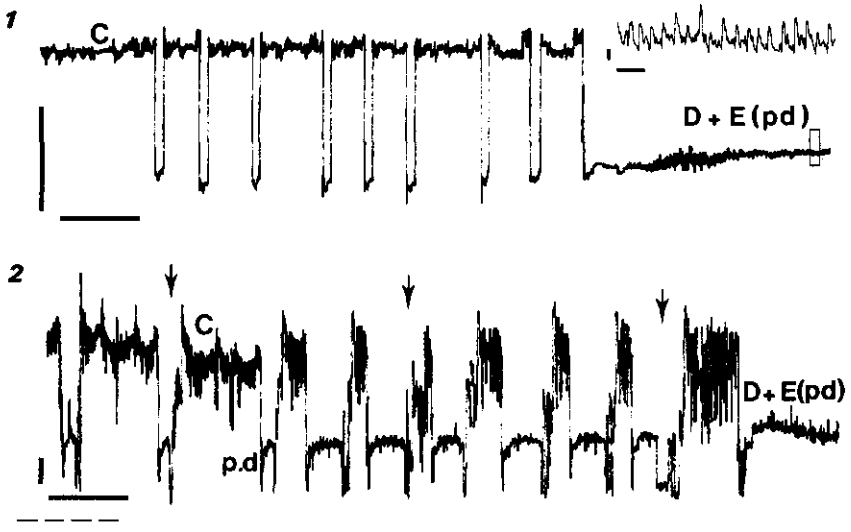

Fig. 1. Short potential drops (pd) during pattern C (C) followed by long pd with D+E pattern (D+E(pd)). *1.* Accurate potentials in emf graph with detail from framed period. Vert. scale, 100 mV, detail 10 mV. Time scale, 1 min, detail 10 s. *2.* Graph made with ordinary amplifier with three pds followed by six regularly repeated pds. Arrows: 50 mV square pulses for resistance calculation purpose (Chapter 3). Note low potential of phase I and III. Vert. scale, 10 mV. Time scale, 1 min. Dotted line: zero ref. level.

The appearance of the short pd, when recorded with the ordinary amplifier, depends on the sign and magnitude of the supplied potential (Fig. 2.*1* and 2.*2*). When adjusted positively, the whole signal, including the lower part of the pd, can be recorded with a positive sign (Fig. 2.*1*). Negative adjustment leads to a negative sign. Figure 3 provides a reconstructed average of many recorded short pds in these two extreme adjustment positions, which enables a further description of the wave forms in three distinct phases.

The first phase (I) includes the descending edge of the pd. The second phase (II) forms the period of a maintained low potential level, referred to as the pd-level. During the third phase (III) the signal returns to the original potential level of pattern C, referred to as the c-level. Figures 2.*1* and 2.*2* show also that during phase I and III the signal potential is small in relation to the zero reference level (absolute potential value). In Fig. 3 this is indicated, including the rapidly fluctuating interferences which may occur during these phases (dotted lines). In Fig. 1.*2* the low values of phase I and III are distinctly pointing downwards. On the other hand, emf graphs (Fig. 2.*3*) do not show a clear phase I and III. Though immediately before the pd starts and shortly after its termination a small potential increase can be noticed. Small peaks, produced with increasing intervals, during phase II (Fig. 2) demonstrate a similar sign (pointing downwards) in the emf graph and the negatively adjusted signal. Their sign in the positively adjusted graph is opposite (pointing upwards) and the amplitude is smaller.

Only emf graphs show a reproducible value of the pd level during phase II within one penetration (Fig. 1.*1*). From different aphids and plants the pd-level values ranged from -100 to -180 mV. The ordinary amplifier records only a fraction of this value and sometimes the pd-level can hardly be distinguished from the c-level (c.f., Fig. 2.*1*). During positive adjustment of the supplied potential the level is even somewhat elevated in relation to the lowest parts of phase I and III, but during negative adjustment the pd level is distinct.

Occasionally a deviating pattern of associated wave forms may be found during phase II. Most deviations can be considered as a more extended or incomplete phase. In *B. brassicae* the decreasing fre-

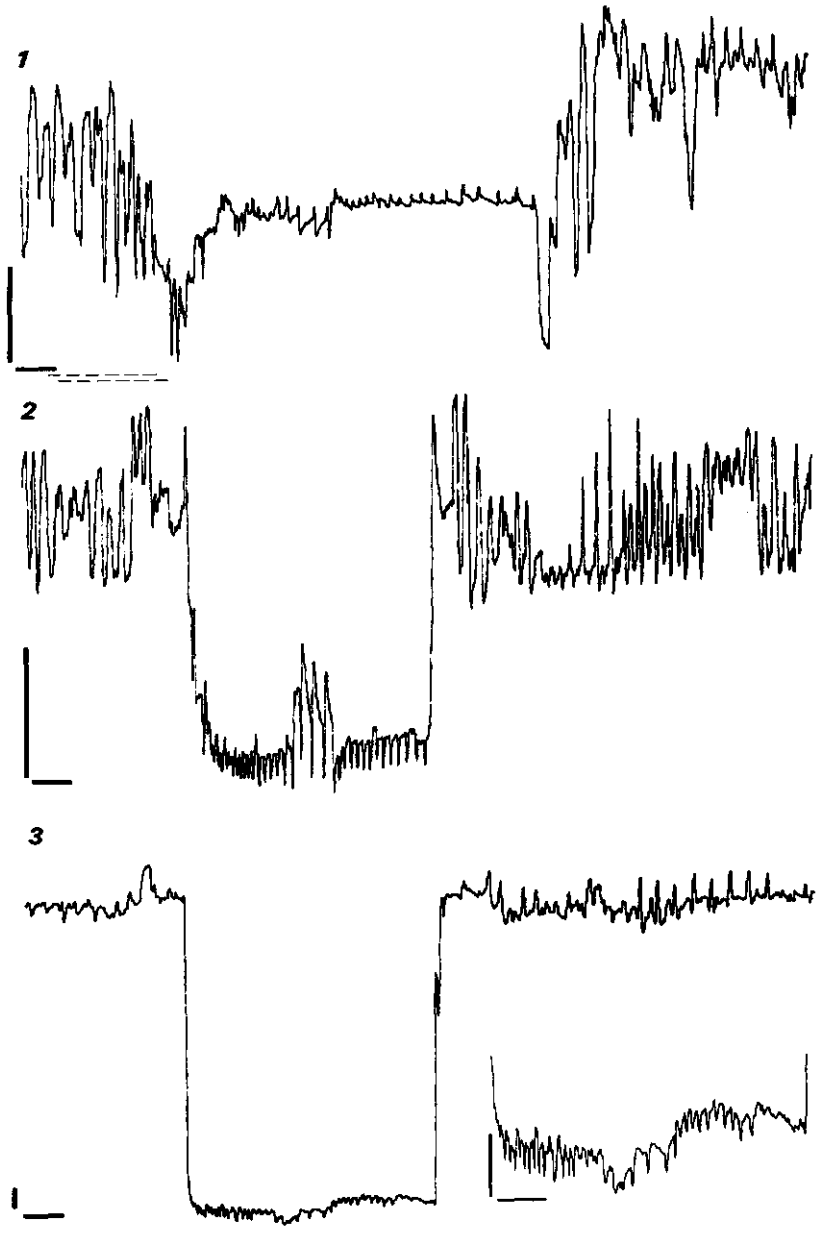

Fig. 2. Short pd recorded with different amplifiersand supplied potential adjustments during one single penetration of *B. brassicae* on cabbage.
1. Ordinary amplifier, supplied potential very positive.
2. Ordinary amplifier, supplied potential very negative.
3. Emf-amplifier. At the right: detail of phase II.
Vert. scale, 10 mV. Time scale, 1 s.
Dotted lines: zero ref. level (lower line belongs to *2.*).

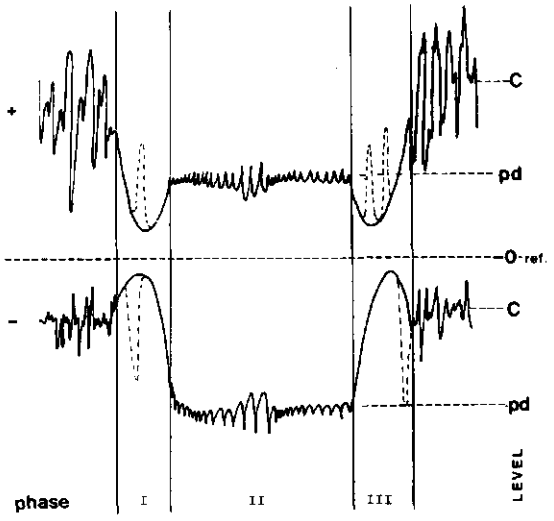

Fig. 3. Reconstructed average of pd signals with positive and negative adjustment of supplied potential. Three phases of assosiated wave forms are recognised. Sign depends on adjustment: I and III opposite, II same sign. C, c-level; pd, pd-level; 0-ref., zero reference level. Time scale as in Fig.2.

quency of small peaks seems to repeat itself a second time (Fig. 2).

EPGs obtained from aphids on artificial diets or pure sucrose solutions behind parafilm® membranes never show the pd phenomenon. However, these graphs show periods of similar duration and with similar associated wave forms as phase II in graphs from plant penetrations (Fig. 4). The potential level changes only with a value

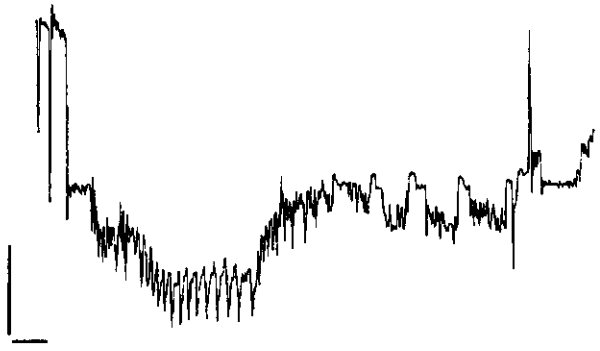

Fig. 4. Diet penetration recorded with emf amplifier. Similar wave forms as associated with pd on plants. Potential change about 20 mV. Vert. scale, 10 mV. Time scale, 1 s.

of about 20 mV. When aphids penetrate a sucrose solution containing isolated plant protoplasts the results are different. In this situation pds do occur, but they are encountered only once or twice at the beginning of the penetration. Microscopic observations of the stylets during these penetrations show that the stylet bundle and the surrounding salivary sheath make lateral movements once the sheath has reached a certain length. It could be seen that the stylet bundle pushes, by such movements, the floating protoplasts aside, rather than penetrating them.

Long potential drops, recorded during plant penetrations, have been found to coincide with the complete pattern D accompanied by pattern E (Fig. 1, D+E(pd)). Initially phase I and II and their associated wave forms are produced, but soon the regular E-pulses can be seen, although at first the pulse is irregular. Thus pattern D+E may occur on pd-level and on c-level as well. During the same penetration both levels may be observed, but always separated from each other by periods of a different pattern. No direct transitions of one potential level to the other have been recorded. The level is maintained during the full length of the D+E pattern, which may continue for one hour or longer. Long pds are not produced on diets or protoplasts suspensions. In contrast with the short pd, the long pd thus remains restricted to plant penetrations.

Both types of pd have been recorded from all aphid species tested on their respective host plants. In *B. brassicae* both types were also observed on the non-host plant *Tropaeolum majus*, whereas in *N. ribisnigri* they were observed on susceptible as well as on resistant lettuce cultivars (see also Mentink *et al.*, 1984). Some differences have been observed between the wave forms associated with phase II, produced by different aphids species, but these differences have not been investigated yet. It cannot be determined from our experiments whether these differences are due to variance within one species or are caused by differences between species.

DISCUSSION AND CONCLUSIONS

The similarity between membrane potentials of plant cells (Higinbotham, 1973) and the pd-level values measured in our experiments suggests that the pd results from an intracellular puncture

by the stylet tips of the aphid. The results from penetrations into diets and protoplast suspensions support this hypothesis. The food and saliva canals provide suitable electrical conductors when filled with fluids, and the stylet walls form a surrounding insulating layer. This situation strongly resembles the experimental technique used to record transmembrane potentials with glass micro-electrodes (Strong, 1970). In this way the stylet canals serve as salt bridges connecting the interior of the plant cell via the aphid body and the dorsal electrode to the amplifier input.

Short pds presumably arise when epidermal or parenchyma cells are punctured, since they appear as early as 10 s after the initiation of a probe. When the stylets pierce the plasma membrane the intracellular potential is recorded, and shows as the leading edge of the pd. The end of the pd, i.e. the return to the c-level, may be caused either by withdrawal of the stylet tip from the protoplast, or by piercing through the membrane at the opposite side of the cell, or by death of the cell. Because of the large number of pds during a penetration, the latter two possibilities would imply more intracellular stylet pathways or necrotic cells respectively, than is known from the literature (Pollard, 1973) and therefore can be discarded. Also, the number of cells along the stylet pathway between epidermis and phloem does not seem to correspond with the number of pds found, neither does a time of 5 to 15 seconds seem to be sufficient for the stylets to cover the distance from wall to wall across a cell (Bradley, 1952). On the other hand, the pd duration and the dimensions of plasmodesmata are too small in relation to the stylets, to explain the pds as signals from stylets crossing these structures. The most likely explanation, therefore, is that a cell is punctured several times. In contrast with our physiological evidence for intracellular penetration, EM-graphs, of stylet pathways in *Picea* and *Hordeum* do not show any evidence of intracellular presence of the stylet bundle in the parenchyma protoplasts (Rohfritsch, 1976, 1977; Evert *et al.*, 1973). Although stylets are shown inside the cell lumen, they remain outside the plasmalemma. An explanation for this paradox might be that after a disruption by the stylets the membrane is able to restore the punctured area. If during the puncture no gelling sheath material is left inside the protoplast, no detectable traces will remain

inside the protoplast. Stylet tips have been demonstrated inside a sieve element protoplast (Evert *et al.*, 1973), a situation that could very well give rise to the long pds. The suggestion that long pds represent an insertion of the stylets into a sieve element is supported indirectly by recent experiments, which demonstrated sap exudation from amputated stylets to occur only when they were cut during a long pd (Mentink *et al.*, 1984). However, it should be mentioned that some of these D+E(pd) amputations provided no exudation. If we exclude experimental imperfections, this observation could indicate that not every long pd is related to sieve element penetration.

Much more difficult to explain is the D+E(c) pattern. Our present knowledge suggests that it might be related to continued extracellular ingestion. There is no evidence in any case, to exclude this or other variants of pattern D from ingestion.

Since the wave forms associated with phase II occur also in EPGs from diets, they must originate from the aphid and not from the plant or insect-plant interaction. The different features in figure 2.*1*-2.*3* demonstrate that each of the three phases has resistance components in addition to emf components (Chapter 3). The low potential value during phase I and III (Fig. 1.*2* and 2.*1*), is apparently mainly caused by increased resistance, as may be concluded from its dependency on the supplied potential sign. The emf graph shows only a slight increase during these phases, which supports this conclusion. The two rapid pd edges which maintain their polarity in Fig. 2.*1* and 2.*2*, and also the rapid deflections (dotted lines, Fig. 3), have an emf nature. The resistance component might arise from a reduced conductivity at the stylet tip due to close contact with the membrane. The rapid emf deflections between the pd and c-level, are presumably caused by changes of the position of the stylet tips prior to, and following on the intracellular contact. In this case intra- and extracellular potentials are encountered in succession. The opposite sign of the small peaks during phase II in Fig. 2.*1* and 2.*2* indicates a resistance component, but Fig. 2.*3* shows that also an emf component is involved. When the sign of both components is the same, the amplitudes are added, when they are opposite, they are subtracted. This explains the amplitude difference between Fig. 2.*1* and 2.*2*. When the

magnitude of the supplied potential is high (positive or negative) the contribution of the resistance component may mask the emf components, like the pd level in Fig. 2.*1*. However, the graphs shown here represent extreme cases. Usually the supplied potential is adjusted positively at a lower value.

Distortion of the recorded signals may occur when the silver paint contact on the aphid dorsum is insufficient. The contact may then act as a high pass filter for the EPG frequencies. This results for the pd in pronounced edges, especially the leading edge which is steeper, and a hyperbolic return during phase II due to the time constant of the filter. The pd-level of phase II may disappear completely. A similar effect occurs in AC devices as applied by McLean and Kinsey (McLean & Kinsey, 1964; McLean & Weigt, 1968). Their pattern X represents presumably a rather distorted pd. The frequency of our regularly repeated pds during some periods in the EPG (Fig. 1.*3*) corresponds closely with the frequency as calculated from their graphs: once every 21-29 s in *A. pisum* at 15° and 20°C (McLean & Kinsey, 1968). Our observations on the other hand, do not support the suggestion of these and other authors, that the periods of regularly repeated X-waves, or pds, are very common (McLean & Kinsey, 1968; Nault & Styer, 1972). The observation that X-waves are produced only on plants (McLean & Kinsey, 1967), again corresponds with an important feature of the pds. Also the observation that the stylets only reach the phloem when the 'ingestion pattern' (= our pattern D) was immediately preceded by an X-wave (Montllor *et al.*, 1983), seems to fit with the long pd (i.e.: D+E(pd)) we have observed. The leading edge of the pd will give rise to an X-wave in the recording of Montllor *et al.* (1983), while the pd-level remains undetected due to the characteristics of their intrumentation. In view of this, the supposed similarity between the X-wave and our B-waves (Chapter 2) cannot be maintained any longer.

The different appearances of the pd can be explained by the magnitude of DC potentials in the primary circuit which are determined by adjustment of the supplied potential, and by variable electrode potentials (Chapter 3). Additionally, distortions due to poor electrode contacts may occur. Therefore it is concluded that the phenomenon is the same in all these different graphs. The exact relationships of the peaks and wave forms associated with phase II to

penetration activities is still unknown and the present results provide no evidence whether sap sampling, salivation, stylet movement or any other activity plays a role during these intracellular punctures.

The pd represents apparently a very common event in plant penetration of all aphid-plant combinations investigated. Some species, like *E. lanigerum*, *R. maidis* and *M. euforbiae* were investigated because of their supposed predominantly intra- or extracellular stylet pathway (McAllan & Adams, 1961; Pollard, 1973). It may be noted however, that no deviating number or duration of the pds has been found in these species. This fact emphasizes the problem of reconciling EM and electrophysiological studies on intra- and extracellular aphid-plant interactions as discussed above.

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CHAPTER 5

Ultrastructure of sieve element penetration by aphid stylets during electrical recording

F.M. Kimmins and W.F. Tjallingii

ABSTRACT

The probing activities of *Brevicoryne brassicae* (L.) on a host plant *Brassica oleracea* (L.) were electronically monitored using a DC device. Two electrical penetration graphs are described, including a pattern, D+E, at two different potential levels; the normal level of pattern C, D+E(c), and the potential drop level, D+E(pd). Stylets were amputated during these patterns, sap exudation from the stumps was scored, and the penetrated leaf area with the remnants of the stylets were processed for EM. Sap was not produced from stylets cut during D+E(c). About 70% of stylets cut during the D+E(pd) pattern produced sap and in micrographs, stylet tips had pierced the sieve element plasmalemma and were found inside the cell lumen. This supports the earlier hypothesis that the D+E(pd) pattern is related to intracellular sieve element penetration. The use of both, D+E(c) and D+E(pd) as ingestion signals is discussed.

INTRODUCTION

Aphid penetration activities in plants, including stylet movements, salivation and ingestion, cannot be observed directly. The electronic monitoring systems of McLean and Kinsey (1967) and Tjallingii (Chapter 2) can, however, provide immediate information about penetration activities, by producing waveform patterns or an electrical penetration graph (EPG). In these systems the aphid is part of an electrical circuit; as the stylets penetrate the plant, the circuit is completed and changing voltages are amplified and recorded. The waveform consists of two components of different physical origin (Chapter 3). A resistance component, caused by conductivity fluctuations in or near the food and saliva canal, and an electromotive force (emf) component which arises from potential sources within the aphid or plant itself. Both components contribute to the EPG in a changing ratio specific for each pattern. For the EPG to be of value, however, in understanding the mechanism of probing, it must be correlated with stylet penetration activity.

Specific patterns of the EPG have been correlated with salivation and ingestion of aphids probing into leaf disks and artificial diets (Chapter 2). Three patterns, 'A', 'B' and 'C' were correlated principally with salivation and pattern 'D' with ingestion, but some ingestion occurred during patterns A, B and C. Also, during pattern D+E, saliva pump muscle activity was correlated with the pulses of the E pattern.

The potential drop, a sudden change of approximately -100 to -180 mV in the signal voltage has been studied recently (Chapter 4) and short potential drops of 5-20 s duration appeared during pattern C, while longer potential drops were recorded which contained the D+E pattern (D+E(pd)). Pattern D+E also appeared without the potential drop, that is on the same potential level as pattern C (D+E(c)). Potential drop signals were not recorded when aphids penetrated artificial diets, but they were found when aphids penetrated diets containing isolated plant protoplasts. Since the membrane potential of plant cells has been measured as -100 to -200 mV (Higinbotham, 1973) it is suggested in Chapter 4 that the aphid stylets act as a micro-electrode, and that a potential drop signal represents membrane penetration. Thus the long potential drop

containing the D+E pattern (D+E(pd)) could reflect membrane penetration (pd), ingestion (D) and, possibly, salivation (E). In a preliminary study (Mentink et al., 1984) stylets were amputated during the D+E(pd) pattern and other patterns. Sap exudation from the stylet stump was observed only during D+E(pd) which indicates that the stylet tips were inside a sieve element cell (Mittler, 1957).

For further justification of this interpretation we have combined EPG recording with stylet cutting and electron microscopy. The stylet cutter (Unwin, 1978) severs the stylets rapidly during an EPG pattern and provides a marker for sieve element penetration in the form of sap exudate at the severed stump (Peel, 1975). Electron microscopy allows observations of the cell membranes and the precise location of the stylet tips in the plant tissue. In this paper the EPGs of two aphids are described in detail and micrographs of the stylet tip position are provided. Another paper will describe the pathway taken by the stylets through the mesophyll tissue.

MATERIALS AND METHODS

Aphids and Culture Methods. A culture of *Brevicoryne brassicae* (L.) was reared on *Brassica oleracea* L., Brussels sprouts, at 15-17°C, 16 h photoperiod and relative humidity 70% ± 5%. For all experiments, adult apterous aphids, fed on a lower leaf of a 7 week *B. oleracea* plant grown in a 15 cm pot, were used.

Electronic monitoring and stylet cutting. Stylet penetration was recorded using an amplifier with a high input resistor ($10^{11}\Omega$) in addition to input current compensation, referred to as the emf amplifier in Chapter 3 and 4. This amplifier selectively records electromotive force components of the EPG. A gold wire (2 cm long and 25 µm diameter) was fixed with silver paint to the aphid dorsum and connected to the amplifier. Plant, aphid and amplifier were placed in a Faraday cage. The EPG was recorded on a chart recorder (0-75 Hz) and tape recorder (FM).

The D+E(pd) pattern is of particular interest, so aphids were allowed to probe until this pattern was observed. Stylets were then cut using a high frequency microcautery (Unwin, 1978). After cutting

the presence or absence of the sap exudate at the severed stump was scored. Stylets were also cut during the D+E(c) pattern.

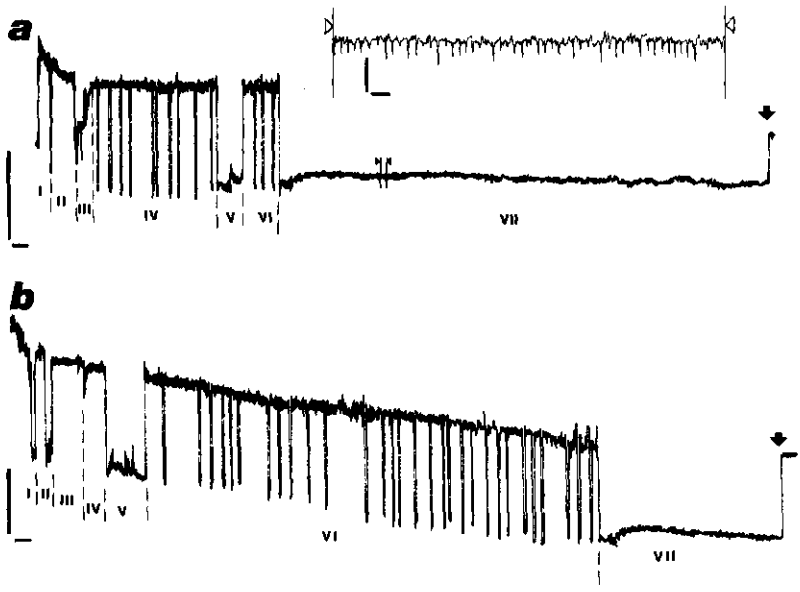
Electron Microscopy. After the stylet was cut, the whole leaf was removed from the plant and immersed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer. The portion of leaf containing the stylet was cut away whilst in the fixative and left for 3 hours. The material was post-fixed for 1 hour in 1% OsO_4, dehydrated and embedded in Epon-Araldite (Luft, 1961). Sections were cut on a LKB IV microtome, stained with uranyl acetate and lead citrate and viewed with a Philips 301 M electron microscope.

RESULTS

The complete EPGs recorded until the stylets were cut during pattern D+E(pd) are shown for two aphids (*a* and *b*) in Fig. 1a and 1b. The EPGs have been divided into different phases which are explained in the Figures. The details of patterns A, B and C are obscured in these condensed graphs, but the potential drops are apparent. The stylets were cut after 30 min of pattern D+E(pd) for aphid *a* and 11 min for aphid *b*. A drop of fluid appeared at the top of the severed stylets after 1-5 s.

The EPG of aphid *a* had a total duration of 47 min. The first 15 min (Fig. 1a, phases i-vi) contained patterns A, B and C and 14 potential drops with a mean duration of 5-6 s. Two longer potential drops were also recorded during this EPG; one had an irregular appearance and lasted for approximately 1 min before returning gradually to the c-level of the graph (Fig. 1a, phase iii), whereas the second contained a D+E(pd) pattern and lasted for 1 min 21 s (Fig. 1a, phase v).

The EPG of aphid *b* had a total duration of 49 min. In the first 36 min (Fig. 1b, phases i-vi) the initial A, B and C sequence was followed by pattern D+E(c) (Fig. 1b, phase iii), which lasted for 1 min 42 s. Pattern C was then interrupted by a D+E(pd) for 2 min 28 s (Fig. 1b, phase v). The final C pattern was recorded for 28 min and contained 30 potential drops with a mean duration of 7 s. The whole EPG shows a steady decline of the DC potential. A similar change occurs in the initial phase of the graph from



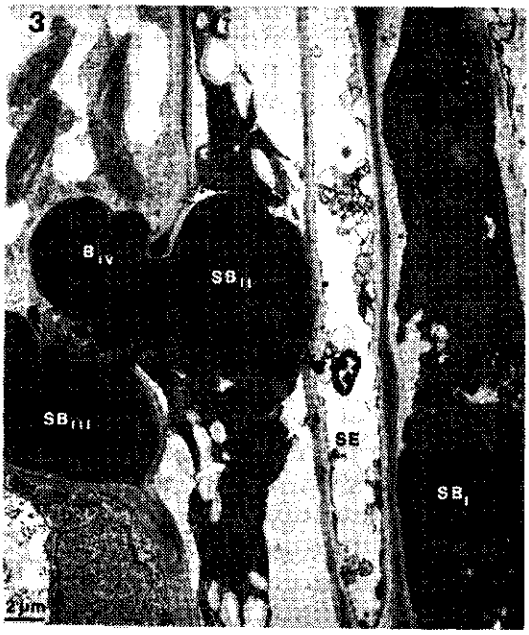
aphid *a*

phase	pattern	duration min	s
i	A,B, 1 pd	0	51
ii	B,C	1	29
iii	pd	0	57
iv	C, 10 pds	8	10
v	D+E(pd)	1	21
vi	C, 3 pds	2	21
vii	D+E(pd)	30	4
	total	45	13

aphid *b*

phase	pattern	duration min	s
i	A,B, 1 pd	1	41
ii	C, 1 pd	1	0
iii	D+E(c)	1	42
iv	C	1	39
v	D+E(pd)	2	28
vi	C, 30 pds	28	12
vii	D+E(pd)	11	23
	total	48	5

Fig. 1. Sequential EPG pattern phases of two aphids. Stylets were cut (arrow) during D+E(pd). a. Aphid *a*, inset with detail of D+E(pd). b. Aphid *b*. Time scales 1 min, inset 1 s; vertical bars 100 mV, inset 10 mV.



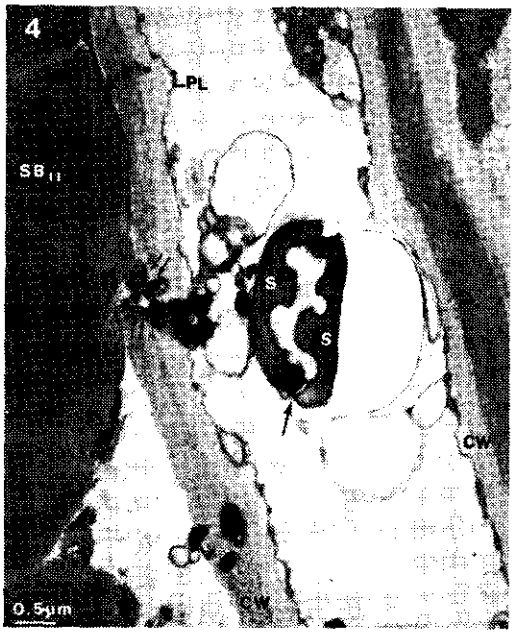


Plate 1. Transverse section of maxillary stylet tips in a sieve element cell (*SE*). Plasmalemma indicated by arrow. Surrounding cells include companion cell (CC) and phloem parenchyma cell (PP). Aphid *a*.

Plate 2. Detail of plate 1. Arrows indicate membraneous material around the stylets (*S*). Darkly stained areas present in the cytoplasm and cellwall (CW). Aphid *a*.

Plate 3. Transverse section of maxillary stylet tips in a sieve element cell (SE). Four salivary sheath branches (SB_i-SB_{iv}) are shown in adjacent cells. SB_i and SB_{ii} have entered cells and the cytoplasm shows disruption and shrinkage, SB_{iii} and SB_{iv} lie between a cell wall and the plasmalemma. Aphid *b*.

Plate 4. Detail of plate 3. Single arrow indicates membraneous material around the stylet tips. Paired arrows show the broken sieve element wall. Aphid *b*.

aphid *a* (Fig. 1a, phases i-iii).

Stylets of 18 aphids were amputated during recordings of pattern D+E(pd). The severed stumps of aphids *a* and *b* and of 11 others exuded sap whereas the remaining 5 did not. The duration of D+E(pd) before stylet cutting varied from 2 min 18 s to 32 min 12 s with the stylet stumps which exuded sap, and from 3 min 6 s to 7 min 30 s with stumps which did not. The duration of short, not-continued D+E(pd) patterns, similar to those shown in Fig. 1a and 1b (phase v) was measured in EPGs of different aphids and ranged from 1 min to 5 min 12 s with a mean of 2 min 24 s (n=15).

Electron Micrographs. The stylet tips of aphid *a* and *b* were located in sieve element cells. Plates 1 to 4 show transverse sections of the maxillary stylets which form the tip of the stylet bundle. Membraneous material is present in and around the stylets (Plates 2 and 4, arrows). The nucleus and tonoplast are absent and although cytoplasmic material is present, few organelles can be identified. The plasmalemma (pl) is shown clearly in Plates 1 and 4. The maxillary stylets of aphid *a* lie close to this membrane and the continuity of the plasmalemma appears to be disrupted on the left hand side of the stylets (Plate 2). In addition, areas of damage can be seen in both sieve element cells. Plate 2 shows two darkly stained areas, one between the stylets and the cell wall and the other in the cell wall. In Plate 4, the sieve element wall is broken and in this area dark material is present, which differs in appearance from the wall material and the saliva. Adjacent cells contain gelled saliva associated with earlier side branches of the salivary sheath (Plates 3 and 4). The walls and cytoplasm of these cells has been disrupted.

DISCUSSION AND CONCLUSIONS

The ultrastructure of the sieve element cells in which the stylet tips of two aphids penetrated is similar to sieve elements described in other dicotyledons (Parthasarathy, 1975).

The positions of the stylet tips inside the lumen of the sieve element strongly supports the hypothesis that the D+E(pd) pattern (or the long pd), is related to intracellular sieve element penetration (Chapter 4). Only the maxillary stylets have been found in

the sieve elements. This corresponds to the description of stylet penetration into sieve elements given by Pollard (1973), and also to the EM graphs of stylet tips of *Rhopalosiphum maidis* in *Hordeum* sieve elements (Evert, *et al.*, 1973). No sheath material was present inside the punctured sieve elements. Although in Chapter 2 the E pulses are related to saliva pump muscle activity, neither those radioactive tracer experiments nor the present EM graphs indicate saliva secretion during pattern D+E. This suggests that if the supposed salivation occurs, the saliva is presumably of the watery type (Miles, 1972), and is ingested instantly through the food canal together with the plant sap. According to this hypothesis hardly any watery saliva would enter the sieve elements during the D+E(pd) pattern.

The dark material associated with the damaged areas of the cell walls, near the stylet tips, is not callose, a substance produced by cells in wound responses because it has a distinctive electron-translucent appearance in micrographs (Eschrich, 1975). The membraneous material around the tips could include remnants of the plasmalemma, though it is probably identical to the membrane which surrounds the stylets. Such a membrane has been demonstrated to remain after the chitinous stylets have been formed in *Rhopalosiphum maidis* (Parrish, 1967).

The question remains, how specific is the D+E(pd) pattern for sieve element penetration? The EPGs contained short periods of this pattern. With aphid *a*, a single sheath was traced through the mesophyll to the sieve element. Thus it is unlikely that the short D+E(pd) patterns in Fig. 1a, phases iii and v represent sieve element penetration. The salivary sheath of aphid *b* was branched, but again, only one sieve element was pierced and the short D+E(pd) pattern (Fig. 1b, phase v) occurs early in the EPG so it may correspond to penetration of a mesophyll cell. Also sap exudation did not occur from all 18 stylets which were cut during D+E(pd). It is possible that this was due to technical failures. The stylets may have been partially withdrawn or the microcautery may have sealed the severed ends of the stylets. If these are excluded, the absence of sap might also indicate that sieve elements were not penetrated in these 5 occasions. These D+E(pd) patterns could be the short, not-continued type, examples of which are shown in Fig. 1a and b

(phases v). Only one of them, cut after 7 min 30 s, was beyond the range of not-continued D+E(pd) patterns measured in other EPGs. In contrast all stylets cut after more than 7 min 30 s of D+E(pd) yielded sap. From these results we may conclude that a D+E(pd) pattern only indicates intracellular sieve element penetration when it is continued for more than about 8 minutes. Working with the set up of McLean and Kinsey, Montllor *et al.* (1982) chose on empirical grounds a 15 min period of the 'ingestion' pattern ('I'), after an 'X'-wave, as an indication for committed phloem ingestion in *Schizaphis graminum* in sorghum. The sequence 'I' after an 'X'-wave, is presumably identical to our D+E(pd) pattern (Chapter 4). Our experiments provide no direct evidence for ingestion during the D+E(pd) pattern. But it is likely that passive ingestion occurs due to the pressure of the phloem sap. Also the earlier found correlation of pattern D with fluid uptake (Chapter 2) supports ingestion. However, it remains obscure whether during D+E(pd) ingestion is continuous and at the same rate as the observed exudation from severed stylets.

When stylets were cut during D+E(c) no sap was produced. Light microscopy of the stylets indicated that the stylet tips were not in phloem tissues (Mentink *et al.*, 1984) and the absence of a potential drop suggests that the tips are in an extracellular position. If the D+E pattern is indicative for intracellular (pd) or extracellular (c) ingestion, the questions arise: what is available in the extracellular spaces for ingestion and does the aphid use the material for feeding or chemical cues? Hennig (1968), for instance, demonstrated ingestion prior to phloem feeding in *Aphis fabae*. Since there are no chemoreceptors present on the aphid labium or the stylets (Wensler, 1974, 1977; and Chapter 1), gustation of plant sap during stylet penetration can occur only by the pharyngeal organ (Wensler & Filshie, 1969) after ingestion. For gustation only a small amount of fluid is required; not more than the volume of the food canal from the pharynx to the stylet tips. Given a cross-sectional arae of 1 μm^2, a maximum length of 1.5 mm, and an ingestion rate of 7 pl/s (Mittler, 1970; Chapter 3; both from artificial diets), a fluid velocity of 7 mm/s is obtained. Therefore sap can reach the pharyngeal cavety within 0.3 s. The observed D+E(c) duration is much longer (42 s in Fig. 1b, phase iii). If extracellular ingestion occurs during the D+E(c) pattern, the aphid appears to

'sample' for a much longer period than is needed. On the other hand, it seems that the extracellular D+E differs from the intracellular pattern in a number of waveform aspects which are not yet fully understood. Further research is needed to investigate the relation to ingestion of both patterns.

The time needed for a fluid to reach the pharynx is also appropriate for gustation during the short potential drops during pattern C. In this study they were not examined. In both EPGs they occur frequently (Fig. 1a and 1b). They may represent membrane penetrations in the mesophyll but because of their short duration and the assumed ability of the plasmalemma to restore a damaged area within a short time (0.3 min; Walker, 1955), the methods used in this study may not accurately test this interpretation.

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CHAPTER 6

Wire effects on aphids during electrical recording of stylet penetration

ABSTRACT

Aphids must be attached to a thin flexible goldwire (20-25 µm) with conductive silver paint when stylet penetration is recorded electrically. The paint solvents may be toxic and the electrical current passing through the aphid when it is connected to the recording circuit may cause electrical effects. The attachment itself may also disturb normal behaviour; this can be called a 'tether effect'. Longevity experiments with free and wired aphids demonstrated the absence of toxic effects but marked behavioural changes in wired aphids were found. Wiring reduced the quantitative differences between aphid reactions to host and non-host plants significantly. However, qualitative differences in penetration behaviour, such as the occurrence of sieve element penetration, remained distinct. Since electrical effects are presumably negligible (Chapter 3), the effects are supposed to be mainly due to tethering. A special experimental procedure was developed to allow comparison of penetraion patterns in free and wired aphids. This type of control experiments should be executed when interpreting results from electrical recording, especially in studies on acceptability of crop plant varieties.

INTRODUCTION

Electrical recording of stylet penetration seems an attractive method to study food plant acceptability in aphids (McLean & Kinsey, 1968; Nault & Styer, 1973). Only recently some progress seems to be made, when this method was used in combination with other techniques (Campbell, *et al.*, 1982; Montllor, *et al.*, 1983; Mentink *et al.*, 1984). An advantage of the electrical recording method is that it occurs automatically and continuously, that it enables recording of events which cannot be observed visually or recorded with other techniques, and that it generally provides a direct or indirect output on paper: the electrical penetration graph (EPG). A disadvantage is that stylet penetration cannot be recorded from free aphids, but that the insects have to be attached to thin flexible wires and connected to an electrical circuit (McLean & Kinsey, 1964, 1965; previous chapters). The attachment is usually made with conductive silver paint containing volatile organic solvents which may cause toxic or anaesthetic effects. The wire also reduces the exploratory behaviour to a limited leaf area and may provide mechanical troubles. It may also interfere with certain behavioural parameters, which have been used by other workers to study food plant acceptability, such as 'Seitenwechsel' (Wensler, 1962; Klingauf, 1970, 1972) and the normal 'interaction between walking and probing' (Ibbotson & Kennedy, 1959). These alterations may be designated as 'tether effects'. When the stylets of a connected aphid penetrate the plant the circuit is completed and an electrical current passes through the stylet canals and the aphid body. This may not only affect muscles, nerves, and sense organs, but it may also interfere with the transport of charged particles and ions in food and salivary canals. These are called electrical effects.

In Chapter 2 we reported that some individuals connected to the system survived for about 10 days and produced about 20 larvae each. These results indicate that toxic and other physiological effects are probably not significant. In Chapter 3 it was shown that diet uptake by wired aphids (*Myzus persicae* (Sulzer)) was comparable to uptake found in free aphids (Mittler, 1970). Additionally, on the basis of calculations on electrical current and fluid velocity in the food canal of the stylets, it was concluded that it is unlikely

that fluid uptake is modified considerably. On the other hand, an indication of a tether effect is given by the occurrence of repetitive stylet penetration on non-host plants in 24 h tests (McLean & Kinsey, 1968), which is in conflict with the observation that free aphids leave a non-host plant within a few hours (Tjallingii, 1976).

In the present study we will attempt to define which parameters wiring can be expected to affect, what the consequences will be, and which remedial measures can be taken. For this purpose we compared free and wired aphids of two species, on host and non-host plants in experiments lasting from 30 minutes to 15 days.

MATERIALS AND METHODS

Our experiments included all 8 combinations of three factors: (i) aphid species, (ii) plant type; host or non-host, and (iii) locomotion constraint; free or wired aphids (c.f., Table 1). Two aphid species were used, the cabbage aphid, *Brevicoryne brassicae* (L.), and the pea aphid *Acyrthosiphon pisum* (Harris). All test animals were apterous, virginoparous adults, 2-5 days after moulting. They were born on the same day from mothers which were isolated from the stock culture. The stock cultures were kept at 15-17°C whereas the test aphids were reared at 20-22°C, both on potted plants at 16 h light per day.

Aphid host plants were Brussels sprouts (*Brassica oleracea* (L.), cv. Stiekema) and broad beans (*Vicia faba* (L.), cv. Driemaal wit) respectively. The host of one aphid species served as the non-host to the other and vice versa.

Freely moving aphids were compared to aphids wired as for EPG recording (see below). All 8 combinations were tested in three different experiments: (1) an experiment lasting 15 days to determine effects on longevity and fecundity, (2) an experiment lasting 5 h to test for differences in settling behaviour, and (3) an experiment in which penetration behaviour was observed during the initial 30 min of plant access.

Longevity and fecundity data were obtained by scoring daily the number of surviving adults and new-born nymphs on potted plants. Nymphs were removed after being counted. To prevent escaping from the plants free aphids were kept individually in clip-on cages.

'Settling' was defined as an aphid being in a feeding posture (Wensler, 1962) at the same spot on the leaf during two successive observations 15 min apart. The number of settled aphids as percent of the total is the settling ratio. Aphids which left a plant were scored as not settled.

Penetration behaviour was directly observed for a period of 30 min using a light 10x stereo microscope (Olympus, VT II), which has operated free hand. Aphids were collected from plants and kept for about 15-90 min in a Petri dish. Then a free test aphid was put on a 1 cm paper disc which was mounted on the upper side of the leaf of a potted plant. The plant was placed on a small table (15 cm diam.) which could be rotated by hand, thus enabling observations when the aphid moved behind a leaf or stem. Observation time started when the aphid crossed the rim of the paper disc. A contact of the proboscis (labium) with the leaf (Tjallingii, 1976), which persisted for more than 10 s was scored as a (stylet) penetration. Tapping of the leaf with the labium was not scored. The term probing, usually meaning short periods of exploratory stylet penetration, or sometimes also for stylet penetration in general, will not be used in this paper to prevent confusion. In our observations on penetration behaviour we have distinguished the following parameters: the duration of the first non-penetration period, the duration of the first penetration period, the number of penetrations and the (total) penetration time. The latter is the summed time of the successive penetration periods. When using these terms in the following, we refer to mean values of 'n' test aphids. As a derived parameter also the mean penetration period is used, which is the penetration time divided by the number of penetrations (and not the sum of individual mean penetration periods divided by 'n').

To wire aphids a thin gold wire (25 µm) 2-3 cm long was attached to the aphid abdomen with a droplet of silver paint. The other end of the wire was likewise fixed to a small connector. In the longevity and fecundity experiment the connectors of 2 to 4 wired aphids were plugged into holes in a plastic cap which was clipped against the leaf in the same way as were the clip-on cages for free aphids. Scoring occured similarly as in free aphids.

The figures on settling (5 h) and on penetration behaviour (30 min) of wired aphids were obtained from experiments in which EPGs

were recorded from the four different aphid-plant combinations. These experiments are referred to as the '6 h EPGs'. During electrical recording the aphid electrode was connected to a (50x) DC amplifier, and the other electrode was connected to an excised leaf of the test plant (Chapter 2 and 3). When the wired aphids were placed on the leaf, electrical recording was started. The signals were recorded on an FM tape recorder (Racal, 7DS; Philips, Analog 7) and a chart recorder (0-75 Hz; Watanabe WTR 771). The graphs allowed checking whether an aphid started a new penetration or not at each 15 min interval, thus providing the same information as in the settling behaviour experiments with free aphids. Also, the same parameters as in the 30 min observations on penetration behaviour could be derived from these graphs.

Some more detailed information on penetration activities of wired aphids was obtained from the 6 h EPGs. The penetration period was measured throughout the EPGs, which provided penetration times. Also the wave form patterns were determined after 0.5 h, 1 h, and at 5 more consecutive 1 h intervals from the beginning. At each interval the number of times that a certain pattern was scored provided a pattern frequency. Five different wave form patterns were distinguished. The first pattern is the EPG base line, which indicates that no penetration occurs. The second is pattern C, including A and B, which can be considered related to the stylet pathway (Chapter 2). The next three patterns are combinations with pattern D: D+E(c), D+E(pd) and D+F. Pattern D is a quiet pattern with low amplitude, which is seen during uptake of artificial diets (Chapter 2). In graphs, pattern D+E is shown at two electrical potential levels. It may occur at the same level as pattern C, referred to as D+E(c), or at the lowered level of a potential drop: D+E(pd). The potential drop level indicates an intracellular position of the stylet tips and the pattern C level indicates an extracellular position (Chapter 4). A continued D+E(pd) pattern of longer than about 8 min was found to reflect sieve element penetration (Chapter 5). When pattern D is combined with the F pattern (D+F), the relation to food intake is uncertain. This pattern could not be related to the intake of radioactive diet (Chapter 2), since it only occurs in plant penetrations. Unpublished data suggest for pattern F a closer relation to mechanical penetration activities

than to ingestion. Therefore a distinction was made between F and the other patterns in combination with pattern D. D+F occurs in the figures directly following pattern C.

A special experiment was executed to investigate the occurrence of EPG patterns in free and wired aphids. In this experiment, referred to as the '16 h experiment', the EPG pattern was determined at 0.5, 1, 2, 4, 8 and 16 h after placing free and wired *Brevicoryne* on excised *Brassica* leaves. A special procedure was developed to record EPGs from free aphids. Prior to the experiment a droplet of silver paint (without a wire) was applied to the aphid dorsum and allowed to dry. These aphids were given access to a plant which was connected to the electrical recording system. When an observation was started, a thin (50 µm) copper wire with dried silver paint at the end was brought into contact with the dorsal spot, using a micromanipulator. Signals were then recorded for about 30 s. In order to exclude the scoring of a pattern caused by disturbance of the aphid, only the first few seconds of the recorded signals were used to determine the wave form patterns. To avoid an influence of the test procedure on the recorded pattern of the next observation period, the free aphids were used only once. For each observation period, patterns were determined from different individuals. Normally wired aphids were used for all observations from 0.5 to 16 h because recording did not disturb the aphids. The wires of 7 aphids were connected to a 7 position choice switch on a holder, mounted on the amplifier input. This set-up allowed recording for about 20 s from each individual aphid in succession. The aphids were placed on top of the leaf, so that if they happened to lose hold they could contact the leaf again. Because the recorded signals were discontinuous the potential level of the D+E pattern could not be determined in this experiment. Consequently, only four patterns were obtained from the normally wired aphids as well as from the free aphids.

To summarize, five comparative experiments were executed. Three of them provide figures on the complete set of 8 combinations: (1) the longevity and fecundity experiment, (2) the settling experiment and (3) the 30 min observations. One experiment, the 6 h EPGs, gives data which are restricted to wired aphids (4 combinations). The last experiment, the 16 h experiment, only provides data on free and wired *Brevicoryne* on *Brassica* (2 combinations).

RESULTS AND DISCUSSION

Before discussing the quantitative results of the various experiments, we will first consider some qualitative aspects. During the daily care of the stock cultures of *Brevicoryne* and *Acyrthosiphon*, and also during the manipulations and pretreatments of the experiments, some characteristic differences in the reactions of the two aphid species were observed. In order to collect test aphids, *Brevicoryne* needed to be brushed gently and repeatedly before stylets were withdrawn and the insects could be removed. In *Acyrthosiphon* a single touch or even a gentle blow was sufficient to collect a whole population when a Petri dish was held under the plant. In the latter species, the collected aphids often showed for some time a 'reflex immobilization' (Wiggleworth, 1972). When this reflex lasted for more than 5 min after the aphid was put on a plant for the 30 min observations, the insect was discarded.

In the data of wired aphids, derived from 6 h EPGs, zero values of non-penetrating individuals were rejected in order to get a similar selection of data as in free aphids. Though in *Brevicoryne* reflex immobilization is less apparent, also in this species some free and wired individuals did not penetrate during the first 30 min, and their results were discarded.

Stereomicroscopic observations showed that *Acyrthosiphon* had striking difficulties walking on the waxy surface of *Brassica* leaves. The aphids carefully followed the major veins or the rim of a leaf, which provided a certain grip. Wired aphids had less problems because they were more or less supported by the wire. These phenomena may have influenced our results, but we have no direct indications in what sense.

Another point is the comparability of results from the different experiments. In the 6 h EPGs we scored pattern frequencies at discrete intervals (Fig. 4a), and added them to frequencies of the total experiment for each pattern (Fig. 4b). The data from the 16 h experiment are similarly presented in Fig. 5a and b. A direct comparison of the 6 h and 16 h data with the data from the 30 min observations however, is not possible, because in the latter experiment the penetration time was scored and not the occurrence of penetration at a certain moment. From the 6 h EPGs, penetration time

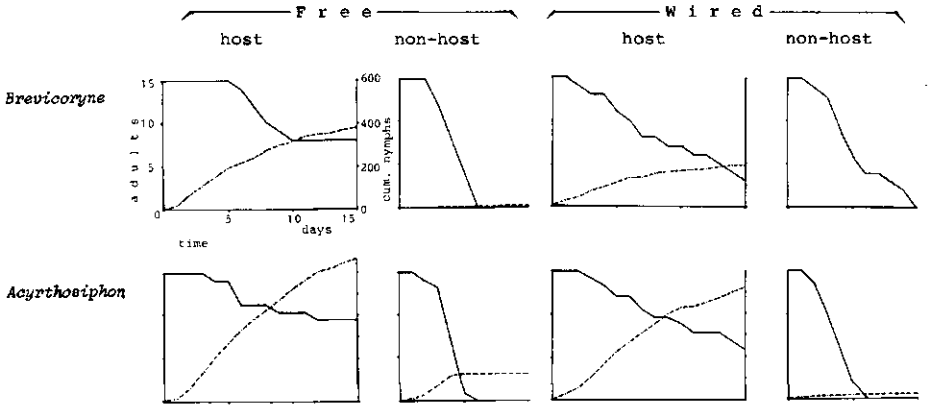


Fig. 1. Longevity and fecundity. The daily scored number of surviving adults, y-axis left, and new-born nymphs, cum. y-axis right. Two species of free and wired aphids tested on host and non-host plants (n = 15).

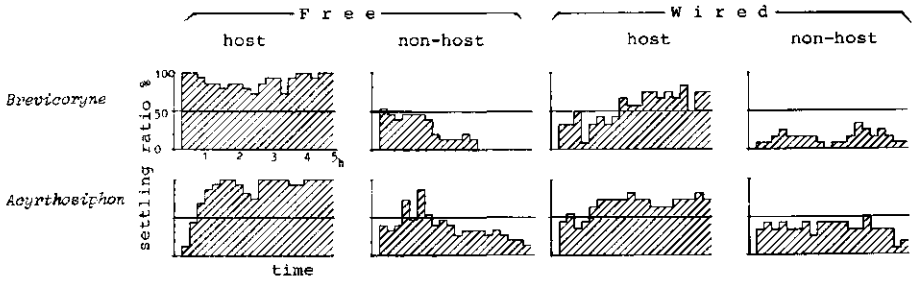


Fig. 2. Setting ratios in free and wired aphids of two species on host and non-host plants during 5 h after access. Figures of free aphids from visual observations (n = 15), of wired aphids from graphs (n = 12).

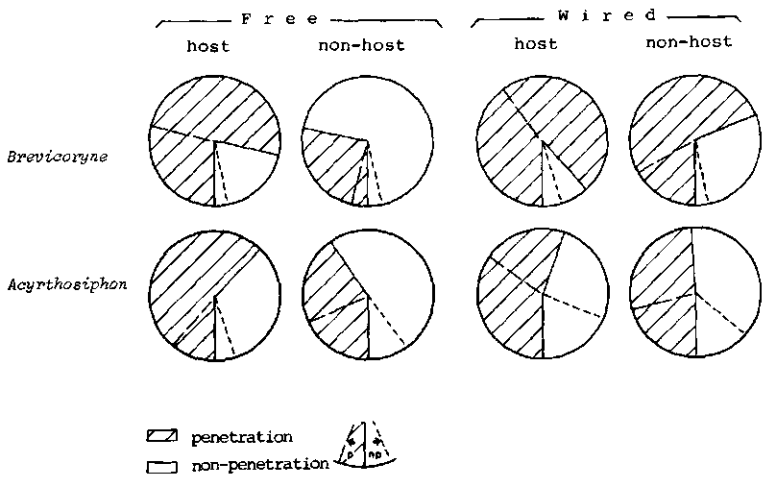
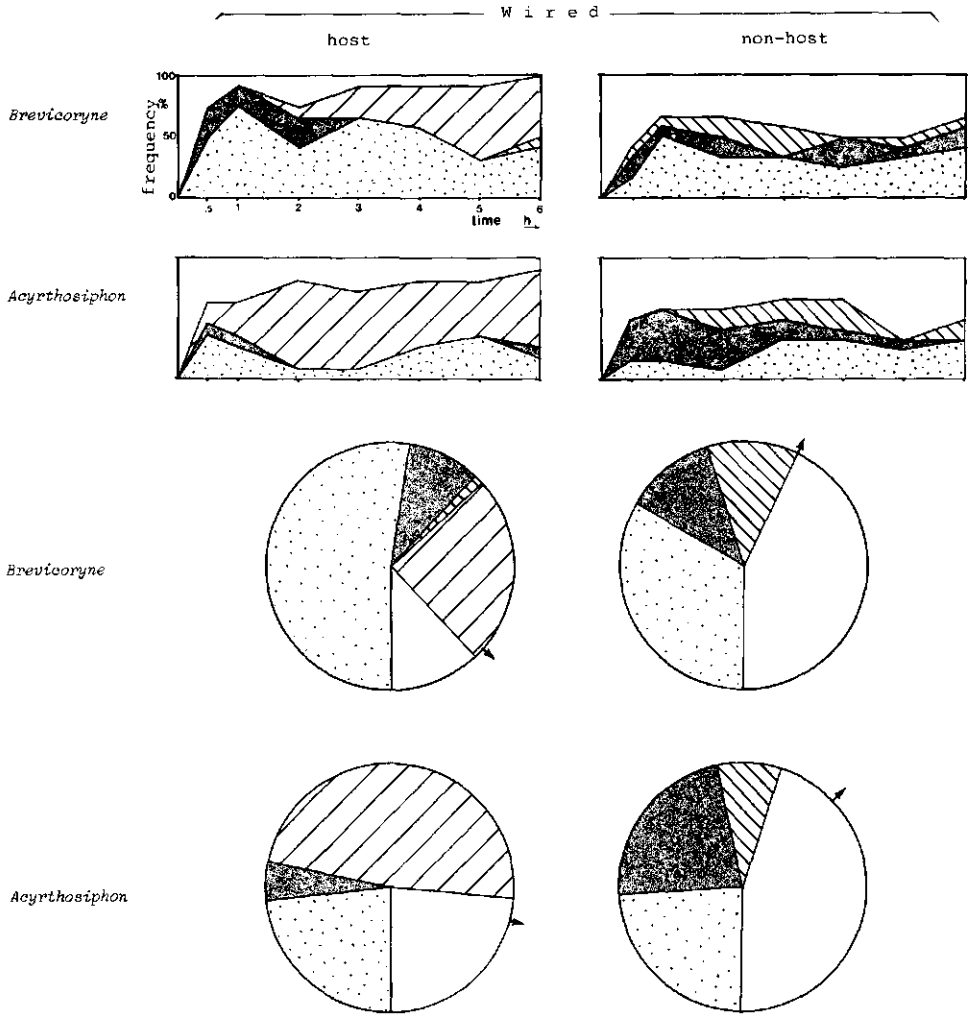


Fig. 3. Penetration time as part of the 30 min observation time. Free and wired aphids of two species on host and non-host plants. p, clockwise angle, duration of first penetration period; np, counter clockwise angle, duration of first non-penetration period. (Number of penetrations and mean penetration period given in Table 1 (n = 10-15).

		free		wired	
		host	non-host	host	non-host
Brevicoryne	p.t.	23.6	8.5	26.5	20.6
	n.p.	4.5	8.1	3.1	4.7
	$\bar{p}$	5.2	1.0	8.6	4.4
Acyrthosiphon	p.t.	18.6	12.2	16.6	14.7
	n.p.	6.3	5.3	2.4	2.4
	$\bar{p}$	3.0	2.3	7.0	6.2

Table 1. Penetration parameters from 30 min observations of free and wired aphids on host and non-host plants. p.t., total penetration time; n.p., number of penetration periods; p, mean penetration period (seconds, n = 10-15).



Legend ➡

Fig. 4. EPGs during 6 h from two aphid species on host and non-host plants. Base line and 4 penetration patterns scored at 7 consecutive intervals (n = 12).
a. Frequency distribution in the course of time.
b. Summed frequencies from a in proportion to total frequency. Arrows, clockwise angle from 6 o'clock to arrow, proportion of penetration time to observation time (= 360°) obtained from the same graphs.

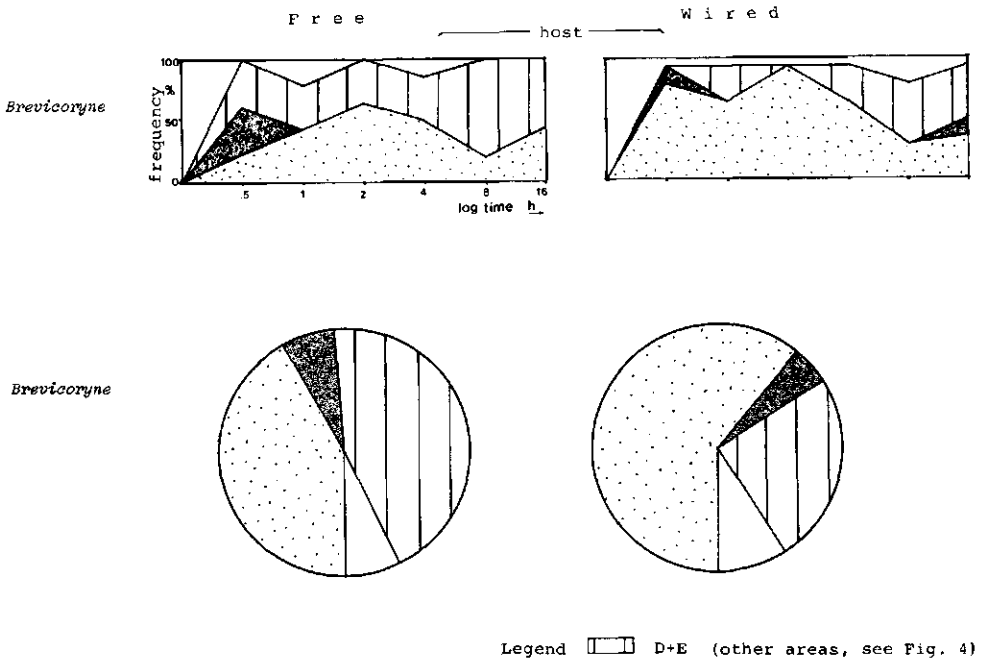
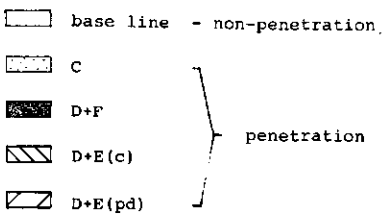


Fig. 5. EPG pattern frequencies as in Fig. 4. from free and wired *Brevicoryne* on *Brassica* (n = 12).
a. Frequencies at different intervals after attachment.
b. Summed frequencies in proportion to total frequency.



per aphid was also obtained additionally to the scored frequencies. This enabled comparison of the relative frequency of penetration patterns (non-penetration excluded) to the penetration time in proportion to the total EPG time (6 h = 360°, Fig. 4b, arrow). Since the penetration time was only slightly different from the frequency of penetration patterns, it was concluded that comparison of a pattern time with a pattern frequency, or vice versa, was allowed.

The penetration time of *Acyrthosiphon* on *Vicia* found in our experiment (77%, Fig. 4b) agrees with results of McLean and Kinsey (1968) on the same aphid-plant combination (85%).

Aphids effects. The developmental rate of the two aphid species on their host plants differed somewhat. In *Brevicoryne* it was about 14 days from birth to adult, whereas in *Acyrthosiphon* it was about 10 days at 20-22°C. Longevity and fecundity were higher in *Acyrthosiphon* than in *Brevicoryne* (Fig. 1). In settling experiments it appeared that some aphids left the test plants in the course of time, especially when non-hosts were used. *Brevicoryne* left *Vicia* plants sooner than *Acyrthosiphon* left *Brassica*. In Fig. 2 (free/non-host combination) this is reflected by an earlier decrease of the settling ratios in *Brevicoryne* than in *Acyrthosiphon*. In the 30 min observations the differences between the aphid species were neither distinct nor consistent, as demonstrated by the penetration time, the first non-penetration period and the first penetration period (Fig. 3, vertical comparison), and also by the number of penetrations and the mean penetration period (Table 1). From the 6 h EPGs (Fig. 4b) it may be concluded that the penetration time (arrows) is about the same for both species but the pattern frequency distribution is different, particularly on host plants. *Acyrthosiphon* showed nearly twice as much pattern D+E(pd) than *Brevicoryne*, whereas in *Brevicoryne* there was more pattern C. Since continued D+E(pd) reflects sieve element penetration (Chapter 5), this suggests that *Acyrthosiphon* penetrates its host plant more efficiently than *Brevicoryne*. These experiments provide no information whether this phenomenon is due to characteristic differences between the two aphid species or to different properties of their host plants.

Plant effects. In contrast to the aphid effects, the effects of host and non-host plants on our parameters were rather distinct. Both insects showed on non-hosts a reduced longevity and fecundity (Fig. 1). Also their settling ratios were lower (Fig. 2), and penetration time was shorter in the 30 min observations (Fig. 3) as well as in the 6 h EPGs (Fig 4b). Fig. 3 also shows that the first non-penetration period hardly differed between host and non-host plants, but that the first penetration period was distinctly longer on hosts. Klingauf (1970) and Tjallingii (1976) found comparable effects of host and non-host plants on these parameters. The 6 h EPGs show that D+E(pd) was found only in host plants whereas D+E(c) was almost completely restricted to non-host plants (Fig. 4b). Mentink *et al.* (1984) found however, that D+E(pd) occurred also when *Brevicoryne* penetrated the non-host *Tropaeolum majus* (L.) and when *Nasonovia ribisnigri* (Mosley) penetrated resistant lettuce cultivars. However, in their experiment the duration of the D+E(pd) patterns was much smaller in these plants than on host plants or susceptible cultivars. In both aphid species the occurrence of pattern D+F (Fig. 4a) was limited to the early penetrations in the case of host plants, but on non-host plants this pattern was distributed more evenly. The total D+F pattern frequency (Fig. 4b) differed more between host and non-host for *Acyrthosiphon* than for *Brevicoryne*. We did not examine these differences into more detail, because that would go beyond the scope of this study. However, it may be concluded that the D+E patterns are of great importance in further studies on host/non-host differences.

Wire effects. No serious effect of wiring on longevity of aphids was observed (Fig. 1), which may indicate that there are no direct toxic effects of the solvents of the silverpaint on the aphids. Fecundity on hosts was much lower in wired aphids than in free. Furthermore wiring appeared to reduce the settling ratio on host plants obviously, but only slightly on non-host plants (Fig. 2). As a consequence,the difference between hosts and non-hosts decreased by wiring, in respect to the settling ratio, particularly in *Acyrthosiphon.* The settling ratios on non-hosts is sustained in wired aphids, whereas in free aphids these ratios decrease after 2-3 h. As discussed above (aphid effects) the decrease is caused

by departures from the test plants. In wired aphids leaving is prevented as a matter of fact.

Settling ratios seem to disagree to a certain extent with the results of the 30 min observations. The contradiction between the settling ratios and the 30 min parameters is presumably mainly due to the fact that the settling criterion is too rough to be a good estimator for the penetration time in particular. Especially in the beginning of a settling experiment, when the penetration periods are rather short (Table 1), the number of 'settled aphids' depends very much on accidental events. Moreover, since we discarded immobile aphids in the 30 min observations, and not in the settling experiments, settling data are somewhat biassed. For these reasons settling data are difficult to compare with the results from 30 min observations.

In the 30 min observations wiring left the penetration time more or less unchanged on hosts, but increased it markedly on non-hosts in *Brevicoryne* (Fig. 3, Table 1). Though in *Acyrthosiphon* this parameter was less altered, in both species wiring reduced the differences between host and non-host plants for penetration time, and also for the number of penetrations (Table 1). In *Brevicoryne* on its host the penetration time of the 30 min observations (Fig. 3), and the frequency of penetration patterns of the 16 h experiment (Fig. 5b), are approximately equal for free as well as for wired aphids. However, Fig. 5b also shows that the pattern frequency distribution changes distinctly by wiring. Wired aphids showed a lower frequency of pattern D+E and a higher frequency of pattern C than free aphids, but pattern D+F was unaltered. All pattern frequencies corresponded fairly well to those found in the 6 h EPGs of *Brevicoryne* on its host plant (Fig. 4b). Also the change of the pattern frequency distribution in the course of time (Fig. 4a and 5a) was very similar in the two experiments for this aphid-plant combination, in spite of the different observation intervals and the different durations of the experiments (6 vs. 16 h). It can be seen from Fig. 5a that the decreased D+E frequency in wired aphids recovers in course of time, a tendency which is supported by the frequencies in Fig. 4a. This suggests that the wire effect is greater in the beginning of the experiment than later on.

There are no data available of wire effects on pattern frequencies in the non-host situation. We may assume on the basis of the foregoing that the total frequency of penetration patterns is presumably increased by wiring, similarly to the increase of penetration time in the 30 min observations. The results of the other experiments suggest that wiring changes the behaviour on non-hosts in a direction of host plant behaviour, but it is not clear whether this would mean in our circumstances that in wired aphids the D+E frequency would be higher or the C frequency lower than in free aphids. As discussed above (plant effects), the differences between hosts and non-hosts found in the 6 h EPGs are more extreme than has been demonstrated between other hosts and non-hosts (Mentink *et al.*, 1984). It might be possible that this is partly due to the rather rough analysis of the EPG patterns by determining pattern frequencies. Small periods of a certain pattern may have escaped our observations.

All wave form patterns found in free aphids were also observed in wired aphids, which indicates that there are neither new patterns induced nor specific patterns eliminated as a result of wiring.

CONCLUSIONS

Attachment of a wire to an aphid hardly shortened the longevity in the two species studied, but fecundity was significantly decreased. Although the wired aphids in the longevity and fecundity experiment were not connected to an electrical circuit, we assume that these results are also representative for aphids linked to the complete electrical recording system. The results from the 16 h experiment suggest that the reduced fecundity in wired aphids may be caused by a decreased feeding, since the frequency of the sieve element penetration pattern D+E(pd), is reduced.

Wiring caused a reduction of the differences which are normally found between aphid behaviour on host and non-host plants with respect to longevity, settling ratio, penetration time, and number of penetrations. Our short term experiments indicate that when the latter two parameters are used, the non-host properties of a plant will be underestimated. Presumably this is mainly due to the

inability of wired aphids to leave the unfavourable foodplant condition. This effect may be designated as the 'tether effect'. Therefore, it is concluded that the number of penetrations and the total penetration time, as parameters derived from electrically recorded graphs, are less suitable for determining host plant acceptability than has been suggested by Holbrook (1980).

Although wiring did not change total frequency of penetration patterns of *Brevicoryne* on *Brassica*, the distribution of EPG pattern frequencies did change. Patterns which have been related to stylet pathway activities increased, whereas those which have been related to sieve element penetration decreased. In spite of these quantitative changes of penetration patterns due to wiring, distinct qualitative differences between penetration patterns on host and non-host plants were observed in our experiments with wired aphids. The sieve element penetration pattern D+E(pd) was only found on host plants and the pattern D+E(c) occurred almost exclusively on non-host plants. The question arises whether the wire effects on penetration patterns would not overrule the difference between susceptible and resistant cultivars. Host and non-host are extremes in the foodplant range of an aphid, which are of less interest to applied entomologists. Mentink *et al.* (1984) found that *Nasonovia ribisnigri* (Mosley) showed the presence of the D+E(pd) pattern on susceptible as well as on resistant cultivars of lettuce. Quantitative differences between aphid behavioural responses to more closely related plants are presumably more sensitive to wiring efects than the qualitative differences found between hosts and non-hosts.

This study does not intend to provide a very accurate description of the effects of wiring on longevity, fecundity and penetration behaviour of the two different aphid species, on host and non-host plants. If that were the case, each of these topics would require a more elaborate study. Our data show some incompleteness, the parameters used are to a certain extent rough, and the numbers of test animals may be regarded as fairly small. This gives our study a preliminary character. In spite of this we think that the results obtained may serve as a warning against blind extrapolation to the natural situation, of results collected with electrical recording methods. Control experiments on free aphids are absolutely

needed before reliable conclusions on aphid-plant relationships can be made.

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SUMMARY

The composition of stylet penetration behaviour in aphids and its possible role in food-plant selection is the subject of these studies. Evidence is presented that the labium is devoid of external chemoreceptors (Chapter 1). In addition to other morphological data (Wensler & Filshie, 1969; Wensler, 1974) this suggests that internal chemoreceptors are involved in assessing plant quality. Internal gustation can only occur during stylet penetration. Another indication of a central role of stylet penetration in food plant selection is provided by behavioural observations (Klingauf, 1970; Tjallingii, 1976; Chapter 6) which show that differences in the reactions to host and non-host plants are inconspicuous before stylet penetration, but very distinct from the time of first penetration.

For further investigation of stylet penetration a DC modified method of electrically recording this behaviour was developed. In the recorded signal, the electrical penetration graph (EPG), six different wave form patterns, A to F, have been distinguished. Some of these patterns have been correlated experimentally with penetration activities (Chapter 2). Pattern A, B, and C were correlated principally with saliva secretion and pattern D with ingestion, but some ingestion occurs during A, B, and C. Pattern E and F occur almost exclusively in combination with pattern D. During pattern D+E saliva pump muscle activity was correlated with the pulses of the E pattern. Pattern D+F could not be correlated with ingestion. These relations are somewhat coarse and mainly based on statistical correlations, not on causitive relations. A more refined analysis of the EPG relations requires knowledge of the physical backgrounds of the electrically recorded signals. In Chapter 3 it is shown that the signal is caused by resistance fluctuations in the stylet region (resistance component) and by electro motive force (emf) sources in the aphid as well as in the plant (emf component). The contribution of each component to the signal depends on the input specifications of the amplifier used, especially its input resistance. The DC method is compared with the AC method used by McLean and Kinsey 1964, 1965).

In Chapter 4 and 5 the 'potential drop'(pd), a phenomenon due to an emf source, is described and experimentally related to the penetration of a plant cell membrane by the stylet tip. The membrane potential causes the sudden drop of the potential in the signal, which may last for short or longer periods. A short pd reflects a protoplast puncture for 5 to 20 s. During long pds, lasting about 10 min to several hours or longer, the signal pattern D+E is recorded (abbr.: D+E(pd)). When stylets were amputated during long pds, phloem sap immediately exuded from the severed stump in the leaf. Subsequent EM showed the maxillary stylet tips being penetrated beyond the plasmalemma into a sieve element cell.

It was shown that penetration behaviour is affected by wiring and connecting an aphid to the electrical circuit (Chapter 6). Since toxic and electrical effects can probably be ruled out, the effects are presumably due mainly to locomotion restraints. The effects of wiring on some penetration behaviour parameters of aphids on host plants appear to be opposite to that of aphids on non-host plants. It is concluded that electrical recording of stylet penetration should not be applied without control experiments with free aphids, especially when susceptibility or resistance of plants to aphids is studied.

SAMENVATTING

In dit proefschrift wordt evidentie aangevoerd voor de centrale rol van styletpenetraties in het voedselselectiegedrag van bladluizen. Een morfologische aanwijzing daarvoor vormt het feit dat er geen smaakzintuigen op de labiumpunt aanwezig zijn (Hoofdstuk 1). Soortgelijke gegevens voor zowel het labium als de in de plant penetrerende styletten (Wensler, 1974, 1977) leiden mede tot de conclusie dat de monddelen geen uitwendige smaakzin bezitten. Aangezien er wel een inwendig smaakorgaan in de mondholte aanwezig is (Wensler & Filshie, 1969), kan de chemische identiteit van stoffen in de plant pas na penetratie door het insekt worden vastgesteld. Gedragsonderzoek (Hoofdstuk 6) heeft bevestigd dat de periode die voorafgaat aan de eerste styletpenetratie, op een waardplant niet langer duurt dan op een niet-waardplant, zoals ook reeds eerder werd gevonden (Klingauf, 1970; Tjallingii, 1976). Daarentegen is de duur van de eerste penetratie, en ook van de totale penetratietijd, op waardplanten wel groter. Hieruit blijkt dat vooral na de eerste styletpenetratie de bladluis een verschillend gedrag gaat vertonen op waardplanten in vergelijking tot niet-waardplanten.

Voor het onderzoek aan het styletpenetratiegedrag werd een gemodificeerde methode van elektrische registratie gebruikt (Hoofdstuk 2 t/m 6). De signaalpatronen die met deze DC-methode (gelijkspanningsmethode) werden verkregen, vertonen weinig overeenkomsten met de signaalpatronen zoals die beschreven zijn met gebruikmaking van een AC-methode (wisselspanningsmethode; McLean & Kinsey, 1963, 1964, 1967). Daarom werd, op basis van onze registraties, een nieuwe indeling gemaakt van de signaalpatronen die tijdens het penetratiegedrag kunnen worden afgeleid (Hoofdstuk 2). Daarbij werden zes verschillende patronen (A t/m F) van elkaar onderscheiden. Proeven met radioisotopen tonen aan dat de patronen A, B en C in verband staan met speekselsecretie, maar tevens bleek dat tijdens deze patronen een geringe voedselopname plaats vindt. Van patroon D kon met isotopen alleen een relatie met voedselopname worden aangetoond. De patronen E en F worden vrijwel uitsluitend aangetroffen in combinatie met D, en slechts sporadisch in combinatie met C. Met behulp van andere technieken kon voor de regelmatige piekjes van patroon E worden vastgesteld dat deze mogelijk samenhangen met activiteit

van de speekselpompspieren. Patroon A, B en C hangen dus niet uitsluitend met speekselsecretie samen en patroon D (in de combinatie D+E) niet uitsluitend met voedselopname. De gevonden relaties geven statistische correlaties aan en geen causale verbanden. Patroon F, of eigenlijk de combinatie D+F, bleek later (Hoofdstuk 6) waarschijnlijk geen relatie met voedselopname te bezitten.

De elektrische signalen bevatten frequenties van 0-75 Hz. Alle onderdelen van het meetsysteem moeten daarom eenzelfde of groter bereik hebben. Diverse auteurs hebben apparatuur gebruikt met een geringer bereik of een onderbroken bereik (McLean & Weigt, 1968; Brown & Holbrook, 1976), hetgeen de geringe overeenkomst tussen onze signalen en de door deze auteurs gepubliceerde patronen ten dele kan verklaren.

De DC-methode weerspiegelt nauwkeurig de weerstandsveranderingen die in de styletkanalen tijdens de penetratie plaats vinden (Hoofdstuk 3). Dat geldt ook voor de weerstandsfluctuaties in de direkte nabijheid van de uiteinden van het styletkanaal, zowel in de plant als in de bladluis. Deze invloed van de weerstandsverandering op de geregistreerde potentiaal vormt de weerstandscomponent van het signaal. Bovendien verschaft deze methode, in tegenstelling tot de AC-methode (McLean & Weigt, 1968), een goede weergave van potentialen en potentiaalveranderingen afkomstig van bronnen in de bladluis en in de plant: de emk-component (elektromotorische kracht) van het signaal. De mate waarin beide componenten aan het signaal bijdragen kan verschillen en is sterk afhankelijk van de ingangsweerstand van de gebruikte versterker. Is deze ingangsweerstand ongeveer even groot als de elektrische weerstand van de bladluis ($10^8\Omega$), dan wordt voornamelijk de weerstandscomponent in het signaal teruggevonden. Is de ingangsweerstand veel groter (groter dan $10^{10}\Omega$) dan worden de emk-componenten belangrijker. Met de gangbare versterkers ($10^8\Omega$) worden alleen de zeer krachtige potentiaalbronnen gemeten. Een speciaal ontwikkelde versterker ($10^{11}\Omega$) diende ertoe de emk-componenten nader te onderzoeken.

Een belangrijke emk-component blijkt ten grondslag te liggen aan negatieve potentiaalsprongen met waarden tussen -100 en -180 mV, de zgn. 'potential drop' (pd, Hoofdstuk 4), die regelmatig tijdens een penetratie worden waargenomen. Korte pd's houden 5-20 seconden aan, waarna de potentiaal terugkeert naar het uitgangsniveau. Ook treden

er langere pd's op, met een duur van één tot enkele minuten, en tenslotte zeer langdurige, van 10 minuten tot enkele uren of langer. De pd's worden uitsluitend aangetroffen tijdens styletpenetraties in planten, nooit tijdens styletpenetraties in kunstmatig dieet. Als echter in een suspensie van geïsoleerde levende plantenprotoplasten wordt gepenetreerd, dan worden wel weer pd-verschijnselen waargenomen. Het blijkt dus dat het voorkomen van protoplasten, al of niet in een plant gesitueerd, een voorwaarde is voor het optreden van pd's. Bovendien komt de potentiaalwaarde van de pd (ca -150 mV) overeen met de waarde die bekend is van membraanpotentialen in plantecellen. Dit leidde tot de hypothese dat de pd een intracellulaire punctie door de bladluisstyletten representeert. Tijdens een korte pd worden karakteristieke golfpatronen waargenomen. Tijdens een lange, of zeer lange pd wordt het patroon D+E waargenomen. Dit patroon D+E kan op twee verschillende potentiaalniveaus voorkomen: het uitgangsniveau, meestal dat van patroon C en daarom aangeduid als D+E(c) en het pd-niveau en daarom aangeduid als D+E(pd). Uitgaande van bovengenoemde hypothese, zou D+E(pd) dus betekenen dat gedurende het D+E patroon de styletpunten zich binnen het plasmalemma van een cel bevinden. Aangezien de pd niet onderbroken wordt, moet worden aangenomen dat deze intracellulaire positie van de styletten gedurende de volledige duur van dit patroon gehandhaafd blijft. Het ligt voor de hand te veronderstellen dat dit met name het geval is wanneer er een periode van voedselopname uit het floeem plaats vindt. Deze veronderstelling werd experimenteel getoetst (Hoofdstuk 5) door eerst de penetratie-activiteit van een bladluis elektrisch te registreren totdat het D+E(pd) patroon vertoond werd. Op dat moment werden de styletten geamputeerd, waarna het stukje blad met daarin de achtergebleven styletstomp histologisch onderzocht werd met behulp van de elektronenmicroscoop (EM). Bij penetraties in het floeem kunnen, na styletamputatie, druppeltjes vloeistof worden waargenomen die, als gevolg van de hydrostatische druk in het floeemsap, via de styletstomp naar buiten komen (Mittler, 1957). Het bleek dat bij amputatie tijdens het D+E(pd) patroon floeemsapbloeding optrad als de aan de amputatie voorafgaande periode van D+E(pd) langer dan ca.10 minuten had geduurd. Was deze periode korter geweest, dan werd slechts in ruim de helft van de gevallen (7 van de 12) floeemsap waargenomen. Amputatie

tijdens andere patronen leverde nooit floeemsapdruppeltjes op. In twee gevallen met floeemsapbloeding bleek op EM foto's dat de punten van de maxillaire styletten zich binnen het plasmalemma van een zeefvatcel in het floeem bevonden. Dit onderzoek bevestigt dus de veronderstelling dat het D+E(pd) patroon, zij het onder bepaalde condities, intracellulaire penetraties in het floeem weerspiegelt. Het staat vooralsnog niet vast of er dan ook continu floeemsap wordt opgenomen.

De hier beschreven resultaten zijn voor het merendeel verkregen met behulp van een elektrische registratiemethode, waarvoor het noodzakelijk is een dun draadje op het achterlijf van de bladluis te bevestigen met zilververf. De gevolgen hiervan en van de overige ingrepen op de betrouwbaarheid van onze conclusies, worden in Hoofdstuk 6 besproken. Uit speciaal daarvoor opgezette experimenten blijkt dat er geen verminderde levensduur of directe sterfte optreedt als gevolg van eventuele toxische effecten van de zilververf. In Hoofdstuk 3 bleek reeds dat de elektrische effecten van aansluiting aan het meetcircuit waarschijnlijk geen belangrijke invloed hebben, zolang de gebruikte gelijkspanning tot max. 500 mV beperkt blijft. Andere ingrepen blijken echter niet geheel verwaarloosbaar te zijn. De bevestiging van de proefdieren aan een draad vermindert de kwantitatieve verschillen in penetratiegedrag tussen waard- en niet-waardplanten aanzienlijk, alhoewel er duidelijke kwalitatieve verschillen blijven bestaan. Zo zijn intracellulaire floeempenetraties (patroon D+E(pd)) alleen aangetroffen op waardplanten. Er is een experimentele procedure ontwikkeld om gedurende korte perioden elektrische signalen van dieren zonder draad te verkrijgen. Hiermee is het mogelijk om met bepaalde tijdsintervallen het penetratiepatroon van een aantal vrije dieren vast te stellen en op basis daarvan patroonfrequenties te bepalen. Deze kunnen dan vergeleken worden met soortgelijke gegevens van dieren die continu aan een draad zijn bevestigd. Naar aanleiding van de veranderingen in de patroonfrequenties door het aanbrengen van een draad wordt geconcludeerd dat voor de interpretatie van gegevens, verkregen met de elektrische registratiemethode, het van groot belang is controle-experimenten uit te voeren met vrije dieren, bijvoorbeeld als het gaat om onderzoek naar de acceptabiliteit of de resistentie van cultuurgewassen. De verschillen ten aanzien van het penetratiegedrag

tussen meer en minder resistente planten zullen in de regel minder groot zijn, dan die tussen de door ons vergeleken waard- en niet-waardplanten. De door ons gevonden bijna absolute verschillen in het al dan niet voorkomen van bepaalde signaalpatronen kunnen daarom bij een vergelijking tussen cultivars van één soort nauwelijks worden verwacht. De elektrische registratiemethode zal de verschillen tussen resistente en niet-resistente planten waarschijnlijk verdoezelen. Ook bij onderzoek naar de rol van styletpenetratie bij het voedselplant-keuzegedrag van bladluizen moet met draadeffecten rekening gehouden worden.

Bij metingen aan vrije bladluizen werden geen nieuwe en onbekende signaalpatronen waargenomen, noch ontbraken er bepaalde patronen. Het lijkt dus zeer onwaarschijnlijk dat als gevolg van de gebruikte methode bepaalde onderdelen van het penetratiegedrag worden onderdrukt, of dat andere activiteiten, die in de natuurlijke situatie niet voorkomen, worden geïnduceerd. Het feit dat dit soort onacceptabele effecten ontbreken, rechtvaardigt het gebruik van deze registratiemethode bij verder onderzoek aan het voor bladluizen zo belangrijke styletpenetratiegedrag.

ABBREVIATIONS

A	pattern A of the recorded EPG
B	pattern B of the recorded EPG
B-waves	slow wave forms characteristic to pattern B
C	pattern C of the recorded EPG
C_a	capacitance value of the aphid in the primary circuit
C_i	capacitance of the amplifier input
c-level	level of the signal potential according to pattern C, used as an indication for extracellular presence of stylet tips
D	pattern D of the recorded EPG. Not occurring as a separate pattern, but only in combination with others. If other patterns cannot be distinguished clearly, then used separately.
D+E	pattern D and E superimposed
D+E(c)	as D+E, specified on c-level
D+E(pd)	as D+E, specified on pd-level
D+F	pattern D and F superimposed
E	preparation potential. Summed stationary potential from sources in the preparation, including the electrode contacts.
E	pattern E of the recorded EPG. E always superimposed on pattern D or C.
E-pulses	characteristic short pulses of pattern E
F	pattern F of the recorded EPG. Exclusively superimposed on pattern D.
pd	potential drop. Sudden change of the signal potential. The duration of the pd can be short, long, continued, not-continued, etc. In Chapter 2 indicated as 'voltage drop'.
R_a	resistance value of the aphid in the primary electrical circuit.
R_f	resistance of the feeding substrate in the primary electrical circuit.
R_i	resistance of the amplifier input
R_s	series resistor used in a test circuit to measure C_i.

V circuit potential. Summed potential of all sources in the primary circuit.

V_i signal potential. Electrical potential across R_i that forms the recorded signal when amplified.

V_s supplied potential. Adjustable potential or voltage that is supplied to the feeding substrate.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 30 december 1943 te Venlo geboren. In 1962 behaalde hij het diploma HBS-B aan het Wagenings Lyceum. Na zijn vervangende dienstplicht te Vledder (Dr.) begon hij in 1965 met zijn studie Biologie aan de Rijksuniversiteit te Groningen. In 1969 behaalde hij het kandidaatsexamen (k) en in 1972 het doctoraal examen (cum laude) met als afstudeervakken Dieroecologie, Ethologie en Toegepaste Entomologie.

Sinds juli 1972 is hij verbonden aan de vakgroep Dierfysiologie van de Landbouwhogeschool, waar hij het onderzoek verrichtte dat leidde tot dit proefschrift, en waar hij tevens onderwijs verzorgt voor met name de studierichting Biologie.