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Charlotte Henriette Hänisch ten Cate

ABSCISSION OF FLOWER BUD PEDICELS IN BEGONIA

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
PROF.DR.IR. H.A. LENIGER,
HOOGLERAAR IN DE LEVENSMIDDELENTECHNOLOGIE,
IN HET OPENBAAR TE VERDEDIGEN OP
WOENSDAG 8 MEI 1974 DES NAMIDDAGS TE VIER UUR
IN DE AULA VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

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STELLINGEN

I

Bloemknopval in Begonia wordt veroorzaakt door een verlaagde auxine-uitvoer uit deze knoppen.

dit proefschrift, IV

II

Het causaal verband tussen cellulaseactiviteit en abscissie is ondanks de veelvuldig gevonden correlatie niet zeker.

III

De conclusie van Jackson en Osborne, dat abscisinezuur de abscissie slechts bevordert door het vervroegen van de toename van de ethyleenproduktie, is onjuist.

Jackson, M.B. en D.J.Osborne, J.Exper.Bot.23,849-862,1972

IV

De parthenocarpe vruchtontwikkeling zou te bevorderen zijn door het verwijderen van meeldraden en kroonbladeren, daar deze waarschijnlijk stoffen produceren welke de celdeling van het vruchtbeginsel remmen.

V

De"beperkte heterogeniteit"van de zogenaamde zure chromatineeiwitten sluit niet uit, dat deze eiwitten een regulerende functie bij de genexpressie hebben.

VI

De conclusie van Rast et.al.,dat de betacyaninesynthese gemedieerd wordt door cyclisch AMP,is onjuist.

Rast D.,R.Skrivanova en R.Bachofen, Phytochem.12,2669-2672,1973

VII

Er zijn redenen om aan te nemen dat de verdeling van droge stof over de verschillende organen van de tulp niet in de eerste plaats energetisch wordt geregeld, maar afhangt van de stimulering van de strekking van de bloem en de bolinductie gedurende de voorafgaande koudebehandeling.

Nard M.Le, J.Cochat, Ann Amélior.Pl.18, 181-215, 1968

VIII

Het verdient geen aanbeveling het hypanthiaal ovarium gelijk te stellen met het gynaeciaal hypanthium.

Leins P., Ber.Deutsch. Bot.Ges. 85, 294-297, 1972

IX

Opheffing van de diversiteit van grondbezitters houdt het gevaar in dat het Nederlandse landschap net zo eentonig wordt als de nieuwbouwwijken.

Proefschrift van Ch.H.Hänisch ten Cate

Dit proefschrift met stellingen van
CHARLOTTE HENRIETTE HÄNISCH TEN CATE
biologisch doctorandus, geboren te
Hilversum op 8 april 1942,
is goedgekeurd door de promotor
Prof.Dr. J.Bruinsma, hoogleraar in de
plantenfysiologie aan de Landbouwhogeschool.

De Rector Magnificus van de Landbouwhogeschool,

Wageningen, 19 maart 1974 H.A. Leniger

INHOUD

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Samenvatting XXII

Voorwoord.

Bij het tot stand komen van dit proefschrift ben ik de vele mensen dankbaar zonder wier hulp dit nooit gelukt zou zijn.
Mijn promotor Prof. Dr. J.Bruinsma heeft mij aan een promotie onderzoek gezet. Zonder zijn bemoedigende woorden, en waardevolle suggesties zou het onderzoek nog niet beëindigd zijn.
Alle medewerkers van het Bot. Lab. hebben aan dit proefschrift meegewerkt.
In het bijzonder moeten Dr. C.M.Karsen en Dr. E.Knegt genoemd worden, die mij steeds met raad en daad hebben bijgestaan. Het vele werk verricht door en de vriendschappelijke samenwerking met Mevr.I.Schmidt-Stolk, Mevr.C.Tjallingii-Mol en Mej.J.van Houdt werd zeer gewaardeerd. Ook vele studenten hebben tijdens hun doctoraal studie hun bijdrage aan dit proefschrift geleverd. Hiervoor hartelijk dank Hanneke, His, Yvonne, Jan, Jan, Ton, Klaas en Freek.
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Het typwerk verricht door Mevr.J.Tak-Vis en Mevr.J.v. Welzen-v.Ast en het stencillen door de heer E.Rothuis werd zeer geapprecieerd.
Ook ben ik de Landbouwhogeschool zeer erkentelijk, dat zij mij dit onderzoek hebben laten verrichten.

INLEIDING

Abscissie is het proces waarbij bepaalde organen of delen van een plant (o.a. takken, bladeren, bloeiwijzen, knoppen, bloemen, bloemdelen, vruchten of zaden) afvallen. Dit kan door middel van mechanische krachten, celwandoplossing of door beide tot stand komen (ADDICOTT en LYNCH 1955). De abscissie door mechanische krachten wordt veroorzaakt door spanningen, welke het gevolg zijn van verschillende graden van uitdroging van naast elkaar liggende weefsels, zoals bijv. bij het los laten van zadenuit peulen. De abscissie ,die het gevolg is van celwandoplossing, vindt vaak in een specifieke zone, de abscissiezone, plaats. Het loslaten komt tot stand, doordat enzymatisch alleen de middenlamel, de middenlamel en een deel van de primaire celwand, of hele cellen oplossen (ADDICOTT en LYNCH 1955). De abscissiezone bestaat vaak uit een zone van dunwandige cellen. Bij bladeren en vruchten ontstaat zij doordat na de celstrekking in de blad- en vruchtsteel, een of twee lagen van reeds gestrekte cellen zich weer gaan delen. Bij mannelijke bloemen wordt zij tijdens de ontwikkeling van de bloemsteel reeds gevormd en bestaat zij uit een zone van dunwandige weinig gestrekte cellen.

1. UITWENDIGE FACTOREN, DIE DE ABSCISSIE IN VIVO BEÏNVLOEDEN.

Vele factoren beïnvloeden de natuurlijke abscissie. Hun werking kan vaak terug gebracht worden tot een effect op de regulatie van de vorming of op de werking van de celwandafbrekende enzymen. ADDICOTT (1968) heeft een overzicht gegeven van de verschillende uitwendige factoren.

a. Uitwendig toegevoegde plantenhormonen beïnvloeden de abscissie. Auxinen en cytokininen remmen, gibberellinen (GA), ethyleen en abscisinezuur (ABA) bevorderen de abscissie. In deze inleiding volgt een uitgebreider overzicht betreffende hun werking.

b. Normale fysiologische temperaturen zijn nodig voor abscissie, daar de celwand afbraak een enzymatisch proces is. Het temperatuuroptimum bij de abscissie van petioolexplantaten van boon ligt tussen de 25° en 30°C. Lichte vorstschade bevordert de abscissie, terwijl zware vorstschade deze remt, daar de abscissiezone dan dusdanig beschadigd is, dat er geen celwandafbrekende enzymen meer gevormd kunnen worden. Lichte vorstschade laat de abscissiezone intact, maar veroorzaakt wel beschadigingen, welke tot een verhoogde ethyleenproduktie leiden. Zo vonden YOUNG en MEREDITH (1971) bij bladeren van Citrus, dat lichte vorstschade tot een verhoogde ethyleenproduktie leidt, welke gecorreleerd is met een grotere bladval.

c. Licht kan de abscissie via de fotosynthese en de fotoperiode beïnvloeden.

   i. Fotosynthese: BIGGS en LEOPOLD (1957) toonden aan, dat een gebrek en een overmaat aan saccharose de abscissie remmen. Een gebrek aan koolhydraten remt de abscissie, omdat er te weinig energie voor het proces is. Bij een overmaat worden dikkere celwanden gevormd, die langzamer oplossen, waardoor er eveneens een abscissieremming optreedt.

   2. Fotoperiode: het lichtregime is van invloed op de abscissie. In de herfst vallen in gematigde streken, ten gevolge van de korte dag (KD) en van lagere temperaturen, de bladeren van de bomen. Bomen die onder lantaarnpalen staan houden hun bladeren langer vast,omdat zij in lange dag (LD) staan. NITSCH (1963) wees er op, dat KD-planten relatief veel groei-remmers (inclusief ABA) en weinig auxinen en gibberellinen, terwijl LD-planten relatief veel auxinen en gibberellinen en weinig remmers zouden bevatten. KD-planten hebben dus een hormoonbalans welke gunstig en LD-planten een hormoonbalans welke ongunstig voor abscissie is.

d. Water: Zowel bij watergebrek als bij overmaat vindt geen abscissie plaats. Een extreem watergebrek veroorzaakt waarschijnlijk beschadigingen aan cellen, waardoor de celwandafbrekende enzymen niet gevormd worden of hun werking niet kunnen uitoefenen. Bij waterovermaat , verdrinking, worden een aantal metabolieten niet gevormd, omdat er dan tegelijkertijd ook zuurstofgebrek is. In de tropen wordt de abscissie door droogte geïnitieerd, al vindt de uiteindelijke abscissie soms pas in de volgende natte periode plaats. MIZRAHI et al. (1971) toonden bij tabaksplanten aan, dat de bladeren van planten, die gedurende 24 uur bij hoge vochtigheid hadden gestaan, een veel lager ABA-gehalte bevatten dan de bladeren van planten, die gedurende dezelfde tijd bij lage vochtigheid hadden gestaan. De inductie van de abscissie door droogte zou het gevolg kunnen zijn van een stijging in het ABA-gehalte in de bladeren.

e. Ademhaling is nodig voor de abscissie. O_2 gebrek en remmers van de ademhaling (KCN, arsenaat, azide, fluoride enz.) remmen dit proces. Ook hoge CO_2-concentraties remmen de abscissie daar CO_2 een competetieve remmer van ethyleen is (BURG and BURG 1967).

f. Mineralen zijn door hun invloed op verschillende metabolische wegen vaak nodig voor de abscissie. Ca^{2+} wordt in pectine ingebouwd en stabiliseert membranen, een gebrek veroorzaakt minder stevige celwanden. Zn^{2+} is nodig voor de synthese van tryptofaan, een precursor van indolazijnzuur (IAA); een gebrek leidt dus tot lagere IAA-concentraties en daardoor tot een bevordering van de abscissie. Een overmaat aan metaalionen, vooral als ze op de bladeren gespoten worden, veroorzaakt beschadigingen, welke tot ethyleenproduktie en daardoor tot bevordering van de abscissie leiden (BEN-YEHOSHUA en BIGGS 1970).

2. LEEFTIJD EN ABSCISSIE.

In vivo vallen bladeren af als zij verouderd zijn, vruchten

als zij rijp zijn en bloemen als zij uitgebloeid zijn. De veroudering blijkt uit een afname in drooggewicht, chlorophyl-, RNA- en eiwitgehalte en uit een toename in ademhaling en ethyleenproduktie (DE LA FUENTE en LEOPOLD 1968). Gedurende de veroudering daalt het auxinegehalte en stijgen plotseling het ABA-gehalte en de ethyleenproduktie (BÖTTGER 1970b; BURG 1968 en GOLDSCHMIDT et al. 1973). Tevens bleek dat explantaten van abscissiezones van oude bladeren sneller absciseren dan die van jongere (BIGGS en LEOPOLD 1957; LEINWEBER en HALL 1959; and CHATTERJEE en LEOPOLD 1965).

3. PLANTENHORMONEN EN ABSCISSIE.

Uit het voorafgaande blijkt dat plantenhormonen een belangrijke rol bij de regulatie van abscissie spelen. Hun invloeden op de abscissie zijn uitvoerig onderzocht, voornamelijk m.b.v. blad- en vruchtsteelexplantaten van Phaseolus, Coleus, Gossypium en Citrus.

a. Cytokininen remmen in het algemeen de veroudering en de abscissie (ADEPIPE et al. 1971; BACK en RICHMOND 1971; MAYAK en HALEVY 1970), doordat zij de afbraak van RNA, eiwitten en chlorophyl remmen TAVARES 1970) of doordat zij de fotosynthese op peil houden (FLETSCHER et al. 1970). Uit later onderzoek blijkt hun werking vooral via de opbouw en afbraak van eiwitten te gaan. MARTIN en THIMAN (1972) toonden bij haverbladeren aan, dat kinetine vooral de vorming van proteasen remt. In Lemna beïnvloedt benzyladenine (BA) zowel de vorming als de afbraak van eiwitten, afhankelijk van de voedingsbodem. Onder gunstige omstandigheden bevordert BA de eiwitvorming en onder ongunstige omstandigheden remt het de eiwitafbraak (TREWAVAS 1972). Kinetine remt dus in het algemeen de abscissie, doordat het de veroudering tegengaat (OSBORNE en MOSS 1963).

b. Gibberellinen stellen de veroudering van een aantal organen uit. In de meeste gevallen bevorderen zij echter de abscissie (BORNMAN 1968; BÖTTGER 1970a; CHATTERJEE en LEOPOLD 1964; MUIR et al. 1970). Gibberellinezuur GA_3 bevordert vooral de abscissie

van jonge petioolexplantaten van Coleus, terwijl het geen effect op de abscissie van explantaten van oude bladeren heeft. BÖTTGER (1970a) kwam hierdoor tot de conclusie, dat GA niet de natuurlijke afval van Coleus bladeren reguleert. De bevorderende werking van GA wordt waarschijnlijk niet veroorzaakt door een verhoogde ethyleenproduktie (LEWIS et al. 1968). Misschien wordt de abscissiebevorderende werking van GA veroorzaakt door zijn auxinesparend vermogen, waardoor het auxine niet op de plaats van werking kan komen om de abscissie tegen te gaan (MUIR en VALDOVINOS 1970).

c. Auxine speelt een belangrijke rol in de regulatie van de abscissie. Gedurende het verouderen van Coleus bladeren neemt de hoeveelheid uit bladeren diffundeerbare auxine en de basipetale transportcapaciteit door de petiolen af (MYERS 1940; BÖTTGER 1970b; VEEN en JACOBS 1969). Wordt de bladschijf verwijderd, dan valt het petiool enige uren later af. Deze afval wordt geremd wanneer auxine op het snijvlak van het petiool wordt opgebracht. De remmende werking van het blad op de petioolabscissie wordt verklaard met de auxineuitvoer naar het petiool (JACOBS 1968). De werking van auxine is nader geanalyseerd m.b.v. explantaten. Auxinen vertragen steeds de abscissie, wanneer zij distaal in de beginperiode van de kunstmatige veroudering worden toegediend. Worden zij echter proximaal in lage concentraties of distaal na een bepaalde kunstmatige veroudering toegediend, dan bevorderen zij de abscissie. RUBINSTEIN en LEOPOLD (1963) verdelen het abscissieproces van bladsteelexplantaten in een auxinegevoelige periode (stadium I) gevolgd door een auxineongevoelige periode (stadium II). Deze twee stadia vallen samen met respectievelijk een ongevoelige en een gevoelige periode voor ethyleen, waarin dit hormoon, indien dan alleen toegediend, de abscissie niet respectievelijk wel bevordert (DE LA FUENTE en LEOPOLD 1968). Auxinen verlengen de ethyleenongevoelige periode. De remmende werking van IAA neemt op den duur af, omdat het in plantenweefsel gemetaboliseerd wordt, 2,4 -dichloorphenol (2,4D) dat niet gemetaboliseerd wordt, verliest zijn remmende werking.

niet (CRAKER et al. 1970). DE abscissie bevorderende werking van auxine wordt waarschijnlijk veroorzaakt door de door auxine geïnduceerde verhoogde ethyleenproduktie, waarvoor de explantaten dan gevoelig zijn, terwijl het auxine te laat of op de verkeerde plaats is opgebracht om de abscissie te kunnen temmen (ABELES en RUBINSTEIN 1964; HALLAWAY en OSBORNE 1968). Betreffende de manier waarop auxinen hun werking uitoefenen bestaan enkele theorieën. 1e. De auxine-gradiënt theorie; een auxine - gradiënt in de petiolen zou de abscissie tegen gaan. LOUIE en ADDICOTT (1970) toonden bij Phaseolus-explantaten aan,dat het distaal toegediend de abscissie remt en proximaal toegediend de abscissie bevordert, terwijl het effect van zowel distaal als proximaal toegediend auxine afhankelijk is van de opgebrachte concentraties. 2e. De auxine-transport theorie; mogelijk oefent auxine zijn remmende werking alleen uit wanneer het basipetaal getransporteerd wordt (OSBORNE en MULLINS 1969). Dit wordt ondersteund door het feit, dat kunstmatige auxinen steeds ook polair getransporteerd worden, terwijl derivaten, die geen auxine werking hebben, niet polair getransporteerd worden (HERTEL et al. 1969). Ook blijken remmers van het auxine-transport de abscissie te bevorderen (MORGAN en DURHAM 1972). 3e. De auxine-concentratie theorie; mogelijk is alleen de auxine-concentratie van belang om de abscissie te remmen (GAUR en LEOPOLD 1950). Soms remt ook proximaal toegediende auxine de abscissie (JACOBS 1968). Twee in vivo waarnemingen ondersteunen deze theorie. In tabak bevordert het verwijderen van jonge bladeren de abscissie van bloemen, terwijl de plaats van auxinetoediening niet van belang is om deze abscissie te remmen (YAGER 1959).Bovendien isin rozen was de bloemabscissie gecorreleerd met lage auxineconcentraties in de stengels (MOE 1970).

Het werkingsmechanisme van auxine is nog onbekend, SACHER (1957) neemt aan, dat IAA de veroudering tegengaat door het in stand houden van de membraanintegriteit. De waarneming van

van POOVIAH en RASMUSSEN (1973), dat Ca^{2+}-ionen de duur van stadium I verlengen, terwijl zij een stabiliserende werking op membranen uitoefenen (POOVIAH en LEOPOLD 1973), ondersteunt deze theorie. VICKERY en BRUINSMA (1973) toonden echter aan, dat membranen niet eerder hun integriteit verliezen dan vlak voor het afsterven van de cellen.

d. Ethyleen is een belangrijk hormoon in de regulatie van de abscissie. Het bevordert de abscissie *in vivo* van bladeren, bloemen en vruchten (BURG 1968; BUKOVAC 1971). Vaak gaat de natuurlijke en kunstmatig geïnduceerde abscissie samen met verouderingsverschijnselen en een verhoogde ethyleenproduktie van het afvallende orgaan (JACKSON en OSBORNE 1970). De effecten van vele kunstmatige abscissie-bevorderaars worden verklaard door hun stimulerende werking op de ethyleenproduktie. De belangrijke rol die ethyleen in de abscissie speelt, blijkt uit het feit, dat petioolexplantaten van *Phaseolus* niet meer afvallen, wanneer ethyleen continu uit de atmosfeer verwijderd wordt (JACKSON en OSBORNE 1970).

Ethyleen bevordert alleen de abscissie aan explantaten, wanneer het gedurende stadium II van het abscissieproces aanwezig is. In petioolexplantaten van *Phaseolus* verkort het ook de duur van stadium I, wanneer het continu aanwezig is (ABELES *et al.* 1971; JACKSON en OSBORNE 1972). Deze verkorting wordt waarschijnlijk veroorzaakt door een remmende werking op het IAA transport (BURG en BURG 1967; MORGAN *et al.* 1968). Gedurende stadium II worden de celwanden van de abscissielaag afgebroken, daar de breekkracht - de kracht die nodig is om petiolen af te laten vallen - afneemt. Gecorreleerd met deze afname nemen de activiteiten van celwandafbrekende enzymen, vooral pectinasen en cellulasen, toe (MORRÉ 1968; ABELES 1968). In overeenstemming hiermee veroorzaakt ethyleen een eerdere stijging in cellulase-aktiviteit, welke gecorreleerd is met een afname in breekkracht (ABELES *et al.* 1971; OSBORNE 1968). OSBORNE (1971) meent dat ethyleen de synthese van de celwand-afbrekende enzymen, voornamelijk cellulasen, op gang zet.

Reeds 1 uur na toediening in stadium II versnelt ethyleen de daling van de breekkracht. Wordt het hormoon vervolgens verwijderd, dan resulteert dit in een snelle terugkeer naar de oorspronkelijke snelheid van de daling van de breekkracht (DE LA FUENTE en LEOPOLD 1969). DE LA FUENTE en LEOPOLD veronderstellen dan ook, dat de door ethyleen geïnduceerde celwandafbrekende enzymen een hoge 'turn over' hebben. ABELES et al. (1971) hebben dit onderzoek verder uitgebreid door behalve de breekkracht tevens de cellulase-aktiviteit hierin te betrekken. Dit leidt tot de conclusie, dat ethyleen niet alleen de cellulase-aktiviteit verhoogt, maar tevens de uitscheiding hiervan uit de cellen bevordert. Zodra ethyleen verwijderd wordt stopt de secretie van cellulase terwijl de cellulase-aktiviteit in de cellen na de verwijdering nog iets stijgt.

e. Abscisinezuur; de rol van dit hormoon in de regulatie van de abscissie is nog omstreden. In Coleus-bladeren en katoenvruchten is het ABA-gehalte positief gecorreleerd met de abscissie (BÖTTGER 1970b; DAVIS en ADDICOTT 1972; SMITH 1969). In vivo moet ABA vaak herhaaldelijk toegediend worden, alvorens het de abscissie kan bevorderen. Ook stimuleert het de abscissie van oudere organen effectiever dan die van jongere organen (ZUCOMI et al. 1969). Vaak verhoogt een ABA-toediening de ethyleen-produktie, echter ook de tegenovergestelde correlatie is aangetoond. In Citrus-vruchten verhoogt ABA de ethyleenproduktie (COOPER en HENRY 1971). In olijven heeft ABA echter geen effect op de ethyleenproduktie (HARTMAN et al. 1968). MAYAK en HALEVY (1972) toonden aan, dat ethyleen gedurende de veroudering van rozebloemen een stijging in het ABA-gehalte veroorzaakt, hetgeen de senescentie tot gevolg heeft. In Citrus-vruchten neemt gedurende de natuurlijke en de door ethyleen geïnduceerde senescentie het ABA-gehalte in de schil sterk toe. (GOLSCHMIDT et al. 1973)

Aan explantaten toegediend, bevordert ABA bijna steeds de abscissie, maar distaal toegediend heeft het meer effect dan proximaal (SMITH et al. 1968). Volgens JACKSON en OSBORNE (1972) versnelt ABA de veroudering met de daarmee samengaande ethyleenproduktie en daardoor de abscissie. Deze theorie wordt ondersteund door de

waarneming van CHANG en JACOBS (1972), dat ABA het IAA transport remt en zo de duur van stadium I verkort. ABELES et al (1971) toonden echter in Phaseolus-explantaten aan, dat ABA nog steeds de cellulase-aktiviteit verhoogt en de abscissie versnelt in de aanwezigheid van ethyleen, in een optimale concentratie. Hierdoor komen zij tot de conclusie, dat ABA evenals ethyleen de aktiviteiten van celwandafbrekende enzymen verhoogt. Ook uit de waarnemingen van COOPER en HORANIC (1973) en van RASMUSSEN (1973) blijkt dat ABA een direkt effect op de celwandafbraak heeft. Onder verlaagde luchtdruk is ABA nog steeds in staat in Citrus de cellulase-aktiviteit te verhogen en de abscissie te induceren, terwijl cycloheximide, dat de abscissie via een verhoogde ethyleen produktie versnelt, niet meer de abscissie kan induceren.

4. DE CELWANDAFBRAAK EN ABSCISSIE.

De abscissiezone bestaat uit kleine dunwandige cellen. Door de oplossing van de middenlamel, en vaak ook van de primaire celwand, vindt de scheiding plaats. Electronenmicroscopisch is in pedicellen van Lycopersicon en Nicotiana aangetoond, dat het oplossen van de middenlamel gevolgd wordt door oplossing van een deel van de primaire celwand (VALDOVINOS en JENSEN 1968; VALDOVINOS et al. 1972). In Phaseolus nam MORRÈ (1968) oplossen van de middenlamel met behulp van het electronenmicroscoop waar, ook in Phaseolus -petiolen toonde WEBSTER (1970) met behulp van het lichtmicroscoop en histochemische kleuringen oplossing van de middenlamel en een deel van de primaire celwand aan, terwijl bij de door ethyleen geïnduceerde abscissie specifiek de primaire celwand oplost (WEBSTER 1973). Histochemisch neemt de kleurbare hoeveelheid pectinen in de abscissiezone af (BORNMAN 1967; MORRÈ 1968; VALDOVINOS en JENSEN 1968; WEBSTER 1968). MORRÈ stelde dan ook, dat het oplossen van pectinen voldoende is om de scheiding te veroorzaken. Vele onderzoekingen betreffende de correlatie tussen pectinasen abscissies gaven echter negatieve resultaten. Enkele uitzonderingen hierop zijn waargenomen o.a. door MORRÈ (1968), die

in Phaseolus-explantaten een stijging van pectine-aktiviteit voorafgaande aan de abscissie aantoonde met behulp van de gewichtsafname van pericarpcoupes van komkommer. Voorts vonden OSBORNE (1958) en YAGER (1960) een daling van pectine-methylesterase (PME)-aktiviteit,gecorreleerd met de abscissie, in respectievelijk petiolen van Phaseolus en pedicellen van tabaksbloemen. MOLINE et al. (1973) onderzochten in 7 soorten de correlatie tussen abscissie en PME-aktiviteit.Alleen in Coleus werd een correlatie gevonden, terwijl in alle andere soorten, waaronder ook Phaseolus, er geen correlatie was. Ook RATNER et al. (1969), POLLARD en BIGGS (1970), ABELES et al. (1971) en RASMUSSEN (1973) onderzochten de correlatie tussen abscissie en pectinasen, o.a. polygalacturonase, PME en andere enzymen,maar zij konden geen verband vinden. Het is merkwaardig, dat uit anatomisch onderzoek bijna altijd blijkt, dat gedurende de abscissie de middenlamel oplost, terwijl er zo weinig correlatie tussen pectinasen en abscissie zijn gevonden. Het is mogelijk dat steeds de verkeerde pectinasen zijn onderzocht of dat de plantenpectinasen zeer substraatspecifiek zijn, waardoor de appel- of citruspectinen, waarmee de aktiviteiten bepaald worden, niet door bijv. Phaseolus-pectinase worden afgebroken.
De correlatie tussen cellulasen en abscissie is steeds positief. ABELES (1969), HORTON en OSBORNE (1967), RATNER et al. (1970), POLLARD en BIGGS (1970) en RASMUSSEN (1973) vonden allen dat een stijging in de aktiviteit van cellulase aan de abscissie voorafgaat. Of cellulase het sleutelenzym voor de abscissie is, is nog niet onomstotelijk aangetoond, daar de cellulase-aktiviteit steeds met behulp van de viscositeitsdaling van carboxyl methylcellulose (CMC) is bepaald. MANDELS en REESE (1965) veronderstellen dat onoplosbare cellulosen door een C_1-cellulase veranderd worden in oplosbare cellulosen, welke op hun beurt door C_x-cellulase worden afgebroken. De CM-cellulasen, die gedurende de abscissie worden bepaald, behoren tot de groep van C_x-cellulasen. In schimmelcultures hebben KARR en ALBERSHEIM (1970) de C_1- en C_x-cellulasen gescheiden en aangetoond. In

hogere planten zijn echter nog nooit C_1-cellulasen aangetoond (PHARR en DICKINSON (1973).Ook is nog niet bewezen, dat de CM-cellulasen, die zich tijdens de abscissie ontwikkelen, onoplosbare cellulosen in de celwanden kunnen afbreken. Echter de (CM)cellulaseaktiviteit is in vele gevallen duidelijk met de abscissie gecorreleerd. De hormonale regulatie van dit enzym is uitgebreid onderzocht. Ethyleen en ABA veroorzaken een eerdere stijging in de aktiviteit, gecorreleerd met abscissie, terwijl IAA beide uitstelt (ABELES, 1969; ABELES et al. 1971a). Uit onderzoek met behulp van de eiwitsyntheseremmers cycloheximide en actinomycine D blijkt stijging in de cellulaseaktiviteit waarschijnlijk het gevolg van de novo enzymsynthese te zijn (ABELES, (1968). Met behulp van D_2O toonden VARNER en LEWIS (1970) aan dat cellulase gedurende de abscissie in petioolexplantaten van Phaseolus de novo wordt gevormd cellulasesynthese met behulp van gelelectroforese in explantaten van Citrus. POLLARD en BIGGS (1970) bevestigden de novo cellulasesynthese m.b.v.gelelectroforese in explantaten van Citrus.

5. HET FYSIOLOGISCH VERLOOP VAN DE ABSCISSIE.

In het abscissieproces van explantaten zijn de volgende fasen te onderscheiden:

a. De differentiatie van de abscissiezone kan, zoals reeds vermeld is, het gevolg zijn van de deling van enkele cellagen in de abscissiezone die, vlak voor de scheiding of reeds gedurende de ontwikkeling van het af te vallen orgaan zijn gevormd. In bladsteelexplantaten van Phaseolus vindt, 6 uur na afsnijding, de deling plaats. Auxinen kunnen deze uitstellen (WEBSTER 1968).

   De hierop volgende fase tot aan de scheiding is in 2 stadia te verdelen, met verschillende gevoeligheid voor auxine of voor ethyleen.

b. Stadium I; anatomisch zijn er gedurende deze periode geen veranderingen zichtbaar. Het auxinetransport neemt geleidelijk af. Auxinen moeten gedurende deze fase toegediend worden, om de abscissie te kunnen remmen, waarbij het geen

verschil maakt of zij aan het begin of tegen het einde worden toegediend. Ook ABA moet tijdens stadium I toegediend worden. Het heeft een optimale tijd van toediening, enkele uren na het begin, waarschijnlijk omdat het pas later zijn werking kan uitoefenen en in plantenweefsels gemetaboliseerd wordt (JACKSON en OSBORNE 1972). Ethyleen verkort de duur van stadium I hetgeen verklaard kan worden door zijn remmende werking op het auxinetransport. Waarschijnlijk wordt de duur van stadium I alleen door auxine bepaald. Komt het IAA-niveau in de abscissiezone, of daalt het IAA-transport door de abscissiezone, onder een bepaalde drempel, dan gaan de explantaten over naar het volgende stadium. ABELES et al.(1971) menen dat IAA alleen de gevoeligheid van het weefsel voor ethyleen reguleert, terwijl JACKSON et al. (1973) aannemen dat IAA niet alleen de gevoeligheid voor maar ook de synthese van ethyleen door het weefsel reguleert.

c. Stadium II; het weefsel distaal van de abscissiezone toont duidelijke verouderingsverschijnselen, hetgeen blijkt uit een daling van drooggewicht en van de hoeveelheid chlorophyl, RNA en eiwit, welke stoffen zich in de zone proximaal van de abscissie ophopen (DE LA FUENTE en LEOPOLD (1968). Dit proximale weefsel is metabolisch zeer aktief (WEBSTER 1968). De ethyleen-produktie van het distale weefsel gaat bij het begin van dit stadium sterk omhoog. Vervolgens worden de celwanden afgebroken, zoals blijkt uit de daling van de breekkracht en de stijging van de cellulaseaktiviteit. Daar verwijdering van ethyleen de abscissie voorkomt, en remmers van de eiwitsynthese de door ethyleen gestimuleerde abscissie remmen, induceert ethyleen de celwandafbrekende enzymen, en wel voornamelijk cellulasen. Maar ook ABA induceert celwandafbrekende enzymen, onafhankelijk van ethyleen, daar ABA zowel de abscissie in afwezigheid van ethyleen bevordert alsook bij optimale ethyleenconcentratie de cellulaseaktiviteit nog verhoogt en de abscissie versnelt.

JACKSON en OSBORNE (1972) verdelen de periode van stadium I en II in het abscissie proces van Phaseolus in 3 stadia: stadium I wordt door IAA gereguleerd; stadium II door een IAA/ethyleen balans, en stadium III door ethyleen. Voor deze indeling in 3 stadia zijn echter verder nog geen aanwijzingen gevonden.

d. De scheiding; de celwanden zijn af gebroken, zo dat alleen de vaatbundels nog voor een verbinding zorgen. Deze verbinding wordt door mechanische krachten verbroken.

c. Heling; na de scheiding wordt op het wandvlak een kurklaag gevormd, die het gevolg kan zijn van de aktiviteit van een kurkcambium of van de verkurking van de reeds aanwezige celwanden. Door de gevormde beschermingslaag drogen noch de overgebleven plant noch het afgevallen orgaan uit.

6. DOEL EN OPZET VAN HET ONDERZOEK.

Uit het voorgaande overzicht blijkt, dat de abscissie het gevolg is van de verandering van het orgaan. Deze veroudering gaat gepaard met hormonale veranderingen. De abscissie is voornamelijk onderzocht met behulp van explantaten van oudere blad- en vruchtstelen. Dit onderzoek betreft de hormonale regulatie van de abscissie van jonge organen, welke zich nog in hun strekkingsfase bevinden. Bij vele Begonia-cultivars, welke overvloedig bloeien, komt vaak ook een grote bloemknopval voor, waardoor deze planten minder bruikbaar zijn voor de sierteelt. Om dit probleem te bestuderen zijn door de Afdeling Tuinbouwplantenteelt 3 klonen van de kruising (Begonia cinnabarina Hook, x B.micranthera Griessl) x B davisii Veitch, alsmede een hybride van de kruising B. fuchsioides Hook. x B.Foliosa HB.etk.verstrekt. De B.davisii hybriden vormen alleen mannelijke bloemen, en zij hebben hetzelfde uiterlijk, maar zij laten in verschillende mate hun bloemknoppen vallen. De B. fuchsioides hybride vormt zowel mannelijke als vrouwelijke bloemen, echter de mannelijke bloemknoppen vallen af voordat zij zich volledig ontwikkeld hebben, terwijl de vrouwelijke bloemknoppen volledig tot bloei komen.

In deel I worden de effecten van plantenhormonen op de pedicel abscissie van de B.davisii hybride in vivo en in vitro vergeleken. Eveneens wordt de invloed van de vegetatieve plant en van de bloemknop op de pedicelabscissie bepaald. Het verschil in tendens tot knopval tussen de 3 klonen blijkt voornamelijk in de pedicellen te zijn gelocaliseerd. Daarom is in deel III de anatomische bouw van de abscissiezones van de 3 klonen vergeleken.

In deel II zijn de effecten van plantenhormonen op het abscissieproces van pedicelexplantaten nader geanalyseerd. IAA, ABA en ethyleen beïnvloeden onafhankelijk van elkaar de abscissie. IAA verlengt de beginperiode van het abscissieproces, terwijl ethyleen en ABA onafhankelijk van elkaar de afbraak van celwanden versnellen.

Deel IV behandelt de correlatie tussen tendens tot bloemknopval en hormoongehaltes in knoppen en pedicellen. In de hybride van B. fuchsioides blijken de vrouwelijke knoppen ± 100 maal zo veel IAA te bevatten als de mannelijke hetgeen samengaat met verminderde abscissie van vrouwelijke knoppen. Bij de B.davisii-hybriden bevatten de pedicellen continu een abscissiebevorderende ethyleenconcentratie. De abscissie van de knoppen wordt voorkomen door een constante IAA-uitvoer uit de knoppen naar de onderliggende pedicellen. Indien de auxine-uitvoer vermindert door minder produktie of door een blokkade van deze uitvoer, vallen de knoppen af.

In deel V is de enzymatische afbraak van celwanden nader bestudeerd. Eiwitsynthese blijkt nodig om de abscissie te doen plaatsvinden. Het oplossen van pectinen veroorzaakt de scheiding. De poging om de enzymen, verantwoordelijk voor het oplossen van de pectinen, aan te tonen, zijn echter niet gelukt. Na het oplossen van pectinen volgt het oplossen van cellulosen m.b.v. cellulase.

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Abscission of flower bud pedicels in *Begonia* I. Effects of plant growth regulating substances on the abscission with intact plants and with explants.

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Summary.

Pedicel abscission of flower buds is retarded by the presence of the bud and by IAA, the buds probably preventing abscission by auxin production. Ethylene removes the retarding effect of the buds and also accelerates the abscission of pedicel explants without buds. ABA has a small accelerating effect, gibberellic acid and kinetin are inactive. The presence of vegetative plant parts enhances the abscission rate, mainly by the supply of nutrients. The difference in flower bud abscission between three *Begonia* clones depends on a difference in the pedicels which is probably not hormonal in nature.

1. Introduction.

The abscission process has generally been regarded as a senescence phenomenon. Apart from a few exceptions (VALDOVINOS and JENSEN 1972, YAGER 1959), it has mainly been studied with such adult organs as petioles and fruit stalks (BIGGS 1971, LEOPOLD 1971). By contrast, abscission of flower buds of ornamental *Begonia* cultivars occurs at young pedicels which are still elongating. In order to compare the abscission in this juvenile tissue with the literature data on adult ones, the effects were studied of different growth regulating substances applied to flower bud pedicels which were either attached to the plant or excised, and which carried a flower bud or had their buds cut off.

Materials and methods.

The abscission was studied using three clones, selected from the crossing (Begonia cinnabarina Hook x B. micranthera Griessl) x B. davisii Veitch. These clones look very similar but differ in their tendency to bud drop. The flowers, arranged in a dichasium, are male, old plants rarely carrying some female flowers. The abscission occurs just above the branchings (Fig. 1). The clones were grown in a greenhouse at 18°C minimum temperature and 18 hrs. light per day. From October until March extra illumination was supplied from high pressure mercury lamps (Philips HPR-400W) and incandescent lamps.

With intact plants, growth regulators were applied either by injecting 2 µl solutions into the pedicels or by placing 4 µl drops on the bracts (Fig. 1). The latter application was repeated twice a week during four weeks and the abscission determined every other day, using three plants per treatment.

Explants were cut from the last but one branchings with distal ends of 0.5-2 cm and proximal ends of 1.5 cm (Fig. 1). The explants were placed through holes in plexiglass holders in Petri dishes, 20 cm diameter, 50 explants per dish, with their proximal ends generally in 250 ml of tap water, sometimes in solutions for proximal applications Distal application of growth regulators to explants carrying buds ('bud explants') occurred by injection of 2 µl solution underneath the buds, to explants without buds ('pedicel explants') by placing 2 µl solution on the cut surface. Ethylene was applied either as Ethephon or as a gas in experiments using desiccators sealed with a rubber cap, through which known amounts of water saturated with ethylene were injected; the ethylene concentration was measured with a Varian 1800 gas chromatograph.

The incubation of the explants was at 18°C in the dark, unless indicated otherwise. The abscission was determined at different points of time after gently touching the pedicels with a pair of tweezers. The interpolated time at which 50% of the pedicels had abscised (T_{50}) was used as a measure for the rate of abscission. The effect of growth regulators was indicated by expressing their T_{50}-values in per cents of those of the water-treated controls, values above and below 100% representing retardation and acceleration of abscission, respectively.

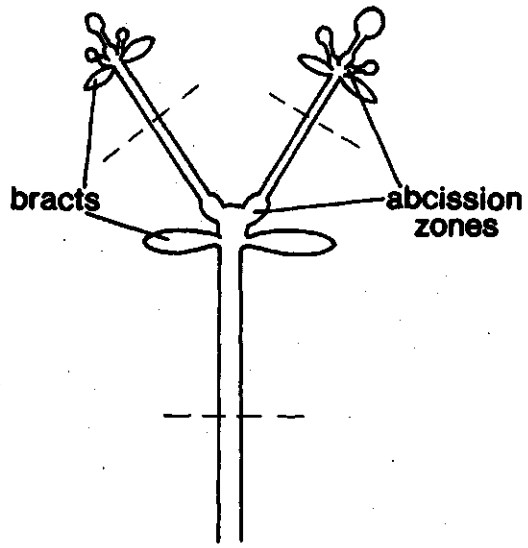

Fig. 1. Diagram of part of an inflorescence. The broken lines indicate the measure of the explants.

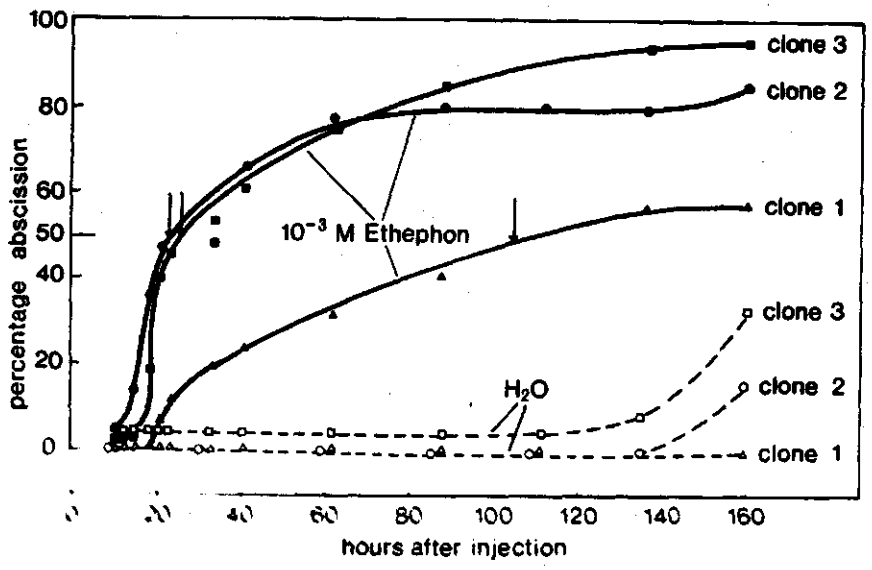

Fig. 2. Effect of a single injection of 2 µl 10^{-3}M Ethephon solution underneath the buds on the abscission of pedicels of intact plants. Arrows indicate T_{50}-values.

Results.

1. Effects of growth regulators on flower(bud) abscission.

The effects of indoleacetic acid (IAA), abscisic acid (ABA), and the ethylene-releasing substance Ethephon, when applied to the bracts of pedicels with plants in the greenhouse, are shown in TABLE 1. The three clones produced about the same amounts of flowers, but with the clones 2 and 3 a higher percentage abscised in the bud stage already. IAA prevented the abscission of flowers, particularly with the clones 2 and 3, often only the petals abscised. ABA had little effect, but Ethephon considerably increased the total drop of flowers, particularly by enhancing abscission in the bud stage.

Injection of IAA into the pedicels strongly retarded abscission, ABA was ineffective, and Ethephon highly accelerated the abscission of buds: Fig. 2.

2. Effect of the flower buds on pedicel abscission.

Experiments with explants with and without buds ('bud explants' and 'pedicel explants', respectively) were performed to analyse the influence of the flower buds on the rate of abscission of the pedicel (TABLE 2). The flower buds strongly retarded pedicel abscission: whereas the T_{50}-values of pedicel explants were always under 15 hrs., those of the bud explants invariably surpassed 80 hrs. This was mainly due to the presence of the flower buds, soon after the abscission of the flower buds the pedicel also abscised. The pedicels of both the bud explants and the pedicel explants of the clones 2 and 3 abscised significantly earlier than those of clone 1.

The effects of IAA, ABA, and ethylene on the abscission of pedicels of bud and pedicel explants, are presented in Fig. 3 in relative T_{50}-values, those of the controls being 100%. The abscission-retarding effect of IAA is always considerable, although varying from one experiment to the other. ABA has a small, ethylene a larger accelerating effect, particularly with the bud explants because it induces the buds to abscise rapidly (cf. Fig. 2).

TABLE 1.

Effects of 4 µl regulator solution, applied twice a week to the bracts, on abscission of buds and flowers of intact plants, three plants per treatment.

	total drop of buds and flowers in 4 weeks			percentage abscised as buds		
clone	1	2	3	1	2	3
H_2O	440	397	424	6	22	21
5.10^{-3}M IAA	443	238	276	9	21	30
5.10^{-4}M ABA	459	340	443	8	35	22
10^{-3}M Ethephon	529	597	663	24	67	60
LSD (P=0.05)		55			15	

TABLE 2.

Abscission of buds and pedicels of explants. Numbers followed by different indices differ significantly at P=0.05.

	Hours until 50% abscission (T_{50})		
clone	1	2	3
Bud explants			
buds	106 a	80 b	82 b
pedicels	116 a	85 b	86 b
Pedicel explants			
pedicels	14.4 c	13.6 d	13.2 d

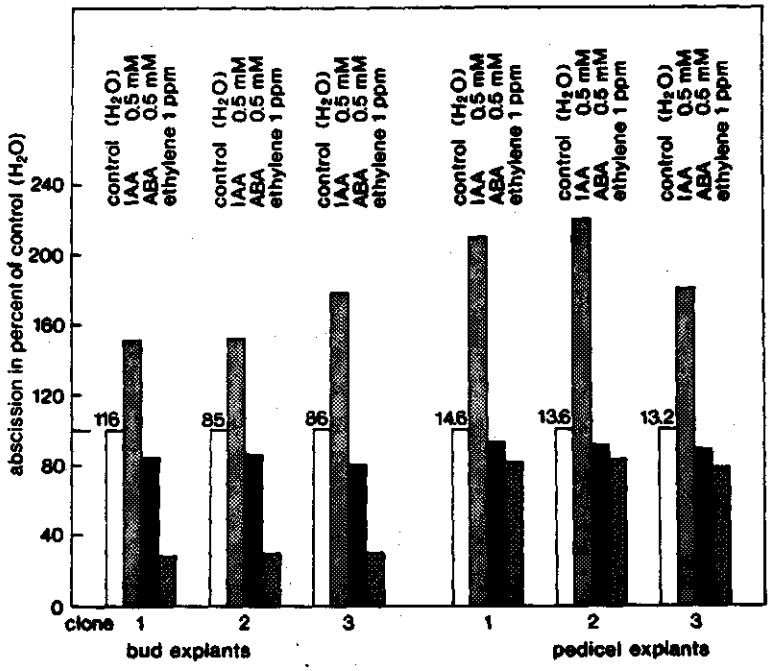

Fig. 3. Relative effects of 2 µl 5.10^{-4}M IAA, 2 µl 5.10^{-4}M ABA, and 1 ppm ethylene, on pedicel abscission of pedicel and bud explants. The T_{50}-values of the controls, in hours, are indicated above the bars.

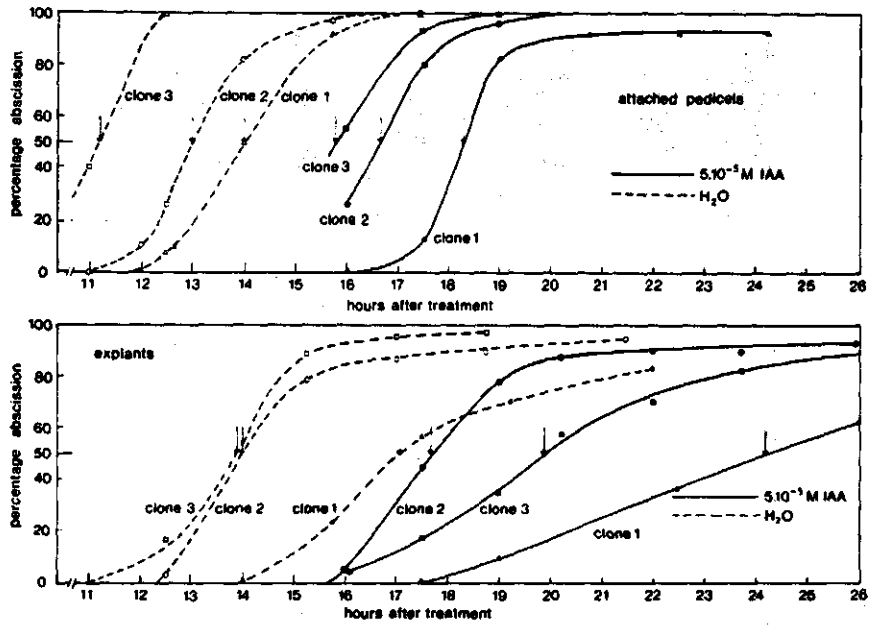

Fig. 4. Effect of 2 µl 5.10^{-5}M IAA at the cut surface of disbudded pedicels, either attached to the plants or excised. Arrows indicate T_{50}-values.

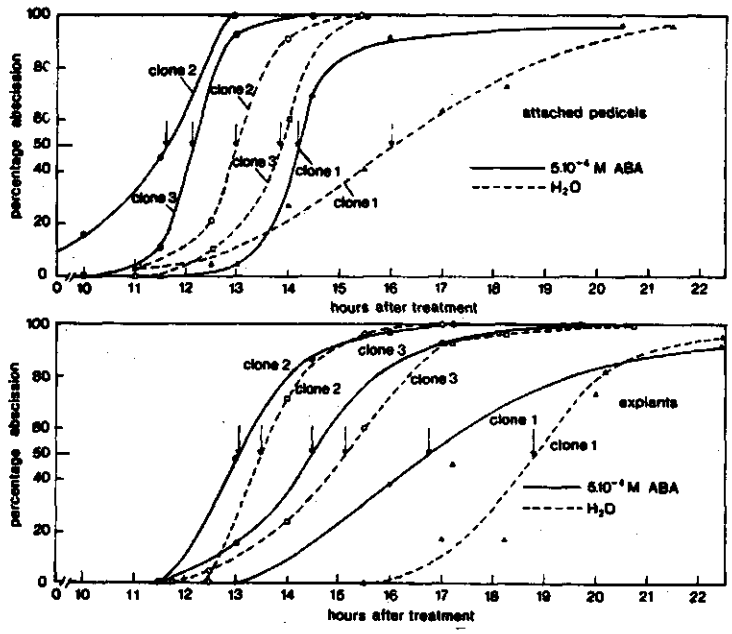

Fig. 5. Effect of 2 µl 5.10^{-4}M ABA at the cut surface of disbudded pedicels, either attached to the plants or excised. Arrows indicate T_{50}-values.

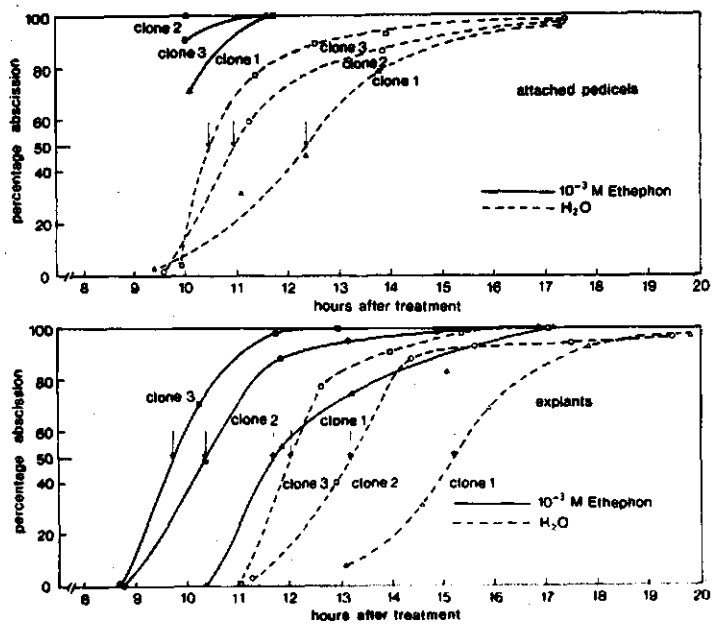

Fig. 6. Effect of 2 µl 10^{-6}M Ethephon at the cut surface of disbudded pedicels, either attached to the plants or excised. Arrows indicate T_{50}-values.

3. Effect of the plant on pedicel abscission.

Figures 4, 5 and 6 show the rates of abscission of pedicels with their buds cut off, either being still attached to the plant or as pedicel explants. The experiments were performed in the greenhouse, the Petri dishes with the explants being under the same conditions as the plants. To the distal ends of the pedicels 2 µl water or regulator solution were applied. Again, IAA retarded and ABA and Ethephon promoted abscission, and their relative effects are the same for the three clones, irrespective whether the pedicels are attached or excised (TABLE 3).

In all experiments the abscission of attached pedicels proceed significantly more rapidly than that of excised pedicels, indicating an abscission-stimulating factor being provided by the plant (Figs. 4, 5 and 6).

Experiments with pedicel explants, placed with their proximal ends in glucose solutions or in water, indicate that the supply of assimilates accelerates the energy-requiring abscission process (TABLE 4). Comparison with attached pedicels shows that this nutrient supply accounts for the larger part of the effect of the presence of the plant.

4. Effects of different growth regulators on pedicel abscission.

The preceding experiments show that the presence of the flower buds and of the plant do not affect the sensitivity of the pedicel to growth regulators, except that ethylene is far more active in the presence of buds. This allows to determine the effects of different growth regulators on pedicel abscission using pedicel explants.

Fig. 7 shows the concentration ranges within which IAA, ABA, and ethylene affect the abscission process, the three clones being equally sensitive to these substances. Gibberellins and cytokinins fail to influence abscission as is shown in Fig. 8 for gibberellin GA_3 and for kinetin. It made no significant difference whether the growth regulators were applied in a drop to the distal end or in the solution in which the proximal ends were immersed.

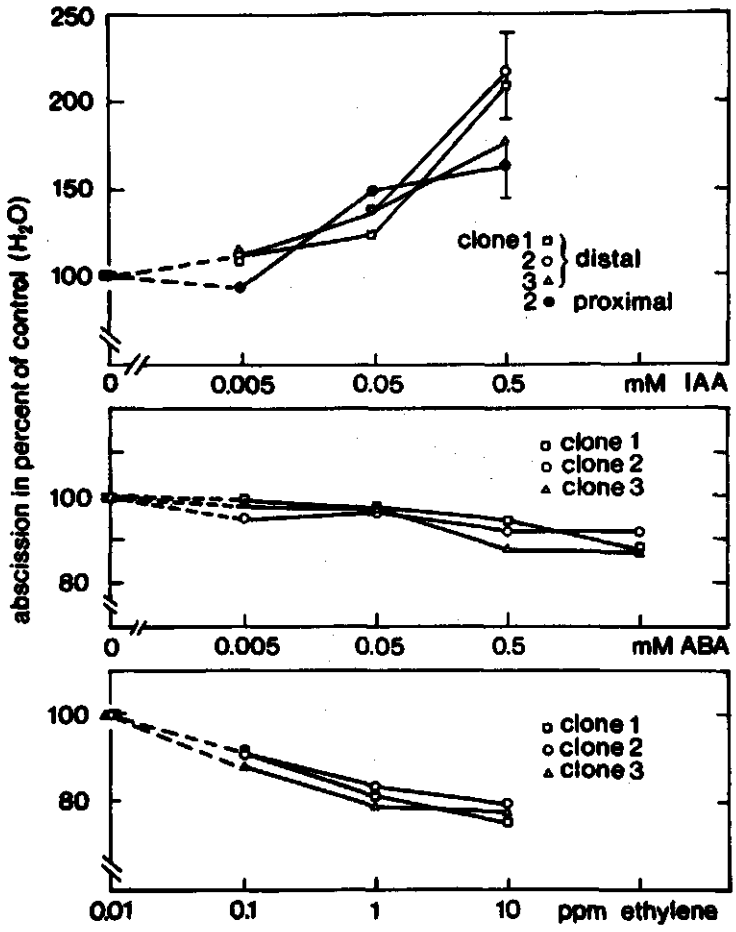

Fig. 7. Relative effects of IAA, ABA, and ethylene on the abscission of pedicel explants. IAA and ABA were distally applied in 2 µl drops and, if indicated, proximally in the solution in the Petri dish.

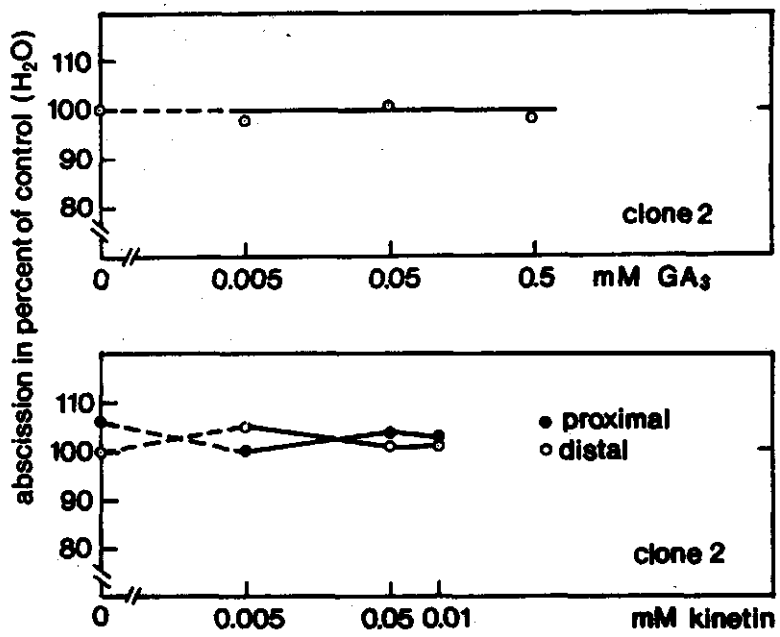

Fig. 8. Absence of effect of gibberellic acid and of kinetin on the abscission of pedicel explants.

TABLE 3.

Effects of IAA, ABA, and Ethephon on the abscission of pedicels without flower buds.

		T_{50}-values, in % of control (H_2O)		
clone		1	2	3
pedicels attached	IAA	136	131	143
	ABA	86	91	90
	Ethephon	--	--	--
pedicels excised	IAA	131	135	133
	ABA	89	93	93
	Ethephon	77	79	80

TABLE 4.

Abscission of pedicels without buds, either attached to the plant or excised, standing in water or in a 1 mM glucose solution.

	T_{50}-values, in per cents of control		
clone	1	2	3
attached pedicels	91 ± 1	90 ± 1	92 ± 2
excised pedicels			
in 1 mM glucose	93 ± 4	93 ± 4	94 ± 3
in water	100	100	100

Discussion.

The effects of growth regulating substances on the abscission of young, still elongating pedicels of flower buds of Begonia, differ in some aspects from those found in studies with adult petioles and fruit stalks (BIGGS 1971, LEOPOLD 1971). Contrary to some observations with petioles (BÖTTGER 1970, CHATTERJEE and LEOPOLD 1964), gibberellin and cytokinin do not affect the abscission of pedicels. The only small effect of ABA can be ascribed to the juvenility of the pedicels (SMITH et al. 1968, ZUCCOMI et al. 1969).

As with adult organs, IAA invariably delays abscission. The strong retarding effect of the presence of buds or flowers on the abscission of the pedicel points to a prevention of abscission by auxin produced by these organs. It is generally agreed that the auxin production of an organ is the main abscission-preventing factor (JACOBS 1968). With flowers of Nicotiana tabacum, YAGER (1959) found that removal of ovaries enhanced abscission; indoleacetic acid retarded the abscission, also with intact flowers. In the present study, only male flowers were used, their auxin production is discussed by HÄNISCH TEN CATE and BRUINSMA (in preparation). That application of IAA to the distal end of disbudded pedicels could not delay abscission to the same extent as the flower bud did, is due to the difference between a single application and continuous production of the auxin.

Ethylene strongly accelerates abscission, particularly when flowers or buds are present. This growth regulator largely removes the retarding effect of flower and bud. Ethylene is known to decrease the synthesis and transport of IAA and to increase IAA destruction (BURG 1968). The sensitivity to ethylene of intact systems has sometimes been demonstrated with leaves, too (MORGAN 1969). However, ethylene does not act by removing the effect of the flower buds only, because also the abscission rate of pedicel explants without buds is enhanced by this hormone. Similar effects of ethylene were found with petiole explants (ABELES et al. 1971).

The accelerating effect of the presence of the vegetative plant is largely due to the supply of assimilates. Because glucose is able to replace the plant, there is no indication that next to this nutritive factor also a hormonal factor is involved, e.g. a specific 'senescence factor' (OSBORNE et al. 1972).

The difference in tendency to bud drop of the three clones is reflected in the difference in abscission rate of the pedicels, either attached or excised, irrespective whether flower buds are present or not. The difference can, therefore, be located in the pedicel itself. It is probably not hormonal in nature because the relative sensitivity of the three clones to the various growth-regulating substances was remarkably similar. The anatomical features of the abscising pedicels are described elsewhere (HÄNISCH TEN CATE et al. 1973).

Acknowledgements.

The three Begonia cultivars were kindly supplied by the Director of the Department of Horticulture of the Agricultural University at Wageningen, Prof.Dr. J. Doorenbos. The co-operation of Mrs. C. Tjallingii-Moll, Miss J. van Houdt, and Mr. K. Boeve is gratefully acknowledged.

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Abscission of flower bud pedicels in *Begonia* II. Interaction and time sequence of plant growth regulating substances on the abscission with explants.

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Abscission of flower bud pedicels in *Begonia*.

II Interaction and time sequence of plant growth regulating substances on the abscission with explants.

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Summary.

The influences of indoleacetic acid (IAA), abscisic acid (ABA), and ethylene on the abscission of *Begonia* pedicels were studied by estimating the interval of sensitivity to them and by analyzing their interactions.
Except for the alternation of sensitivity to IAA and to ethylene, the hormones affect the abscission independently from one another.

Introduction.

In the first paper of this series (HÄNISCH TEN CATE and BRUINSMA 1973) it was shown that indoleacetic acid (IAA) inhibits the pedicel abscission of intact plants and of explants of *Begonia* cultivars, whereas abscisic acid (ABA) and ethylene promoted the process. To obtain a more detailed analysis of these hormonal effects, the present study deals with the interactions between IAA, ABA and ethylene, and with an estimation of the periods during the course of the abscission process, during which the process is sensitive to these hormones.

Material nnd methods.

Three *Begonia* clones were selected from the crossing (*Begonia cinabarina* Hock x *B micranthera* Griessl) x *B davisii* Veitch.
The growing, preparation, and hormonal treatment of the pedicel explants are described by HÄNISCH TEN CATE and BRUINSMA (1973).
Because the sensitivity of the three clones to the hormones is the same (HÄNISCH TEN CATE and BRUINSMA 1973), the three clones have been used in turn.

When hormones were given to the explants several hours after excision, the distal 1 mm of tissue was cutt off to enable application to a fresh surface.

The rate of abscission (T_{50}) is expressed by the interpolated time at which 50% of the pedicels had abscised (HÄNISCH TEN CATE and BRUINSMA, 1973). The effects of growth regulators were indicated by expressing their T_{50}-values in per cents of those of the controls, values above and below 100% representing retardation and acceleration of abscission, respectively. Per treatment 50 explants were used in at least duplicate experiments.

Results.

In a first series of experiments two growth regulators were applied simultaneously to the distal ends of the pedicel explants, directly after excision. From the combinations of IAA with either ABA or ethylene (Figs. 1 and 2), it can be concluded that the retarding effect of IAA is reduced by both substances. The effects of ABA and ethylene are larger in the presence than without IAA. However, because in the presence of IAA the curves run about parallel instead of diverging, the hormones influence the abscission process independently of one another.

The simultaneous application of ABA and ethylene results in a mere addition of their separate effects (Fig. 3).

In a next series of experiments it was estimated during which period of time the different hormones are effective. To obtain the abscission promotive effect of ethylene, the hormone must be present in the gas atmosphere from the sixth hour after excision on, a treatment during the first three or six hours only being ineffective (Table 1). Only when ethylene is applied after nine hours, its promotive effect is considerably reduced (Fig. 4). Therefore the explants become sensitive to ethylene between six and nine hours after excision, the presence of the hormone before six hours after excision does not influence its action in the sensitive period.

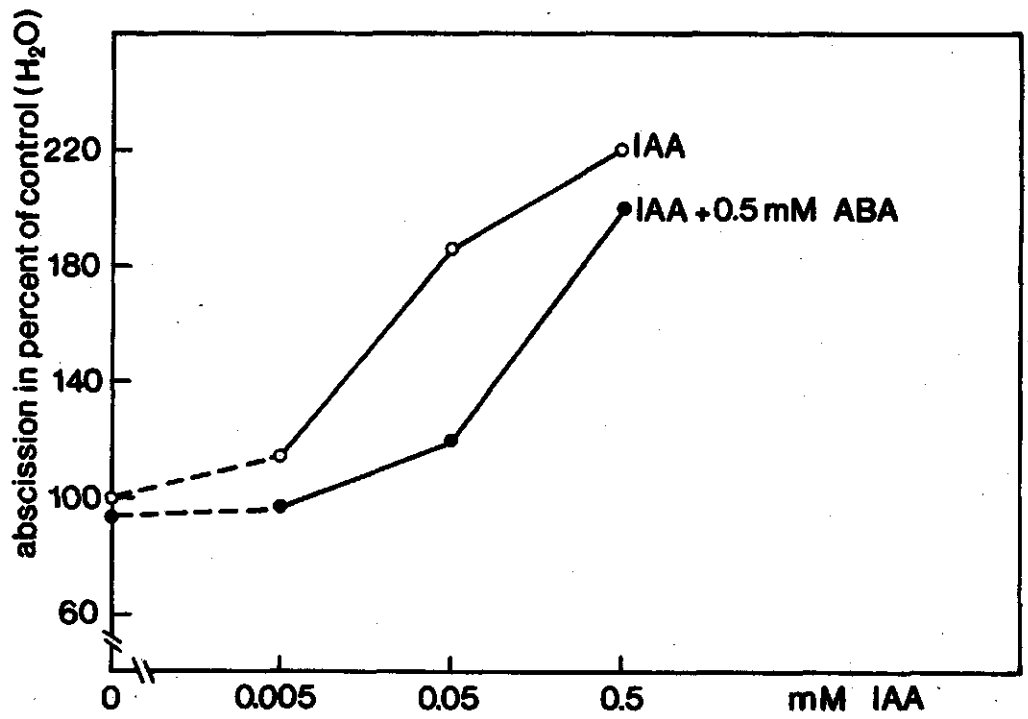

Fig. 1. The interaction of IAA and ABA, distally applied in 2 µl solutions, directly after excision, in the abscission of pedicel explants of clone 2.

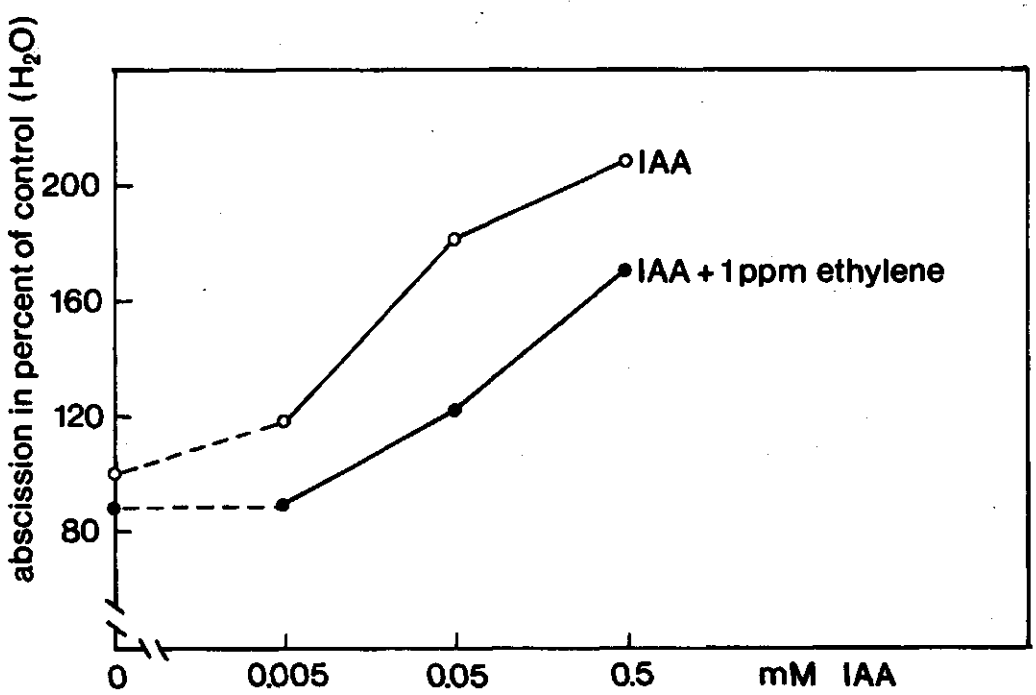

Fig. 2. The interaction of IAA, distally applied in 2 µl solutions, and of 1 ppm ethylene in the gasphase, directly after excision, in the abscission of pedicel explants of clone 2.

Table 1.

Effect of interval between excision and ethylene treatment, alone and in combination with 2 µl ABA or IAA applied distally directly after excision. The T_{50}-values are expressed in per cents of the control values that are given between ( ). (clone 2).

1 ppm ethylene from	--------	ABA 5.10^{-4}M	IAA 5.10^{-4}M
0-3 hrs	100 ± 3	100 ± 2	100 ± 3
0-6 "	99 ± 2	102 ± 2	101 ± 3
0-9 "	81 ± 6	89 ± 4	104 ± 4
continuously	76 ± 5	85 ± 3	90 ± 8
no ethylene	100 (13.9 hrs)	100 (12.5 hrs)	100 (16.9 hrs)

Table 2.

Effect of interval between excision and the distal application of 2 µl 5.10^{-4}M IAA, alone and in combination with 2 µl 5.10^{-4}M ABA at 0 or 3 hrs, or ethylene directly after excision applied. The T_{50}-values are expressed in per cents of the control values that are given between ( ). (clone 1).

Interval of IAA application	--	ethylene 1 ppm	ABA at 0 hrs.	ABA at 3 hrs.
0 hrs	134 ± 27	124 ± 9	116 ± 4	_
3 "	131 ± 23	123 ± 6	119 ± 5	119 ± 5
6 "	130 ± 23	127 ± 3	116 ± 2	116 ± 7
9 "	97 ± 3	_	106 ± 1	112 ± 3
no IAA	100(15,3 hrs)	100(12.1 hrs)	100(13.1 hrs)	100(12.8 hrs)

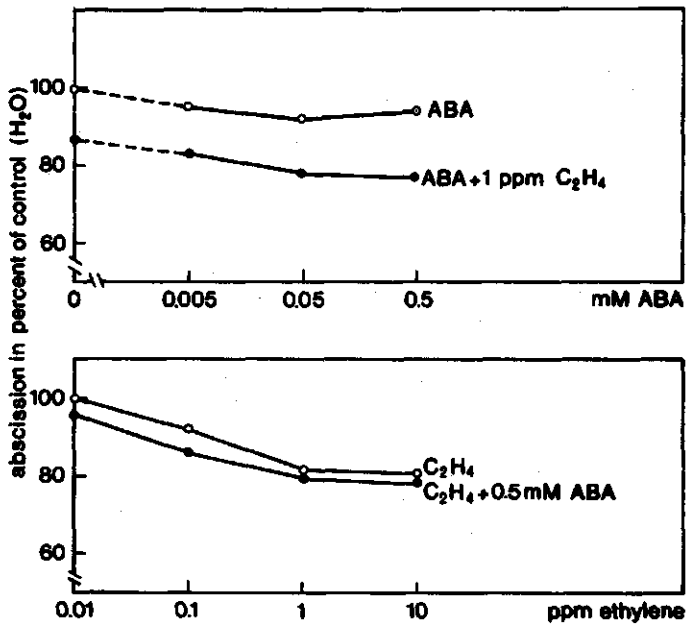

Fig. 3. The interaction of ABA, distally applied in 2 µl solutions, and of ethylene in the gasphase, directly after excision, in the abscission of explants of clone 2.

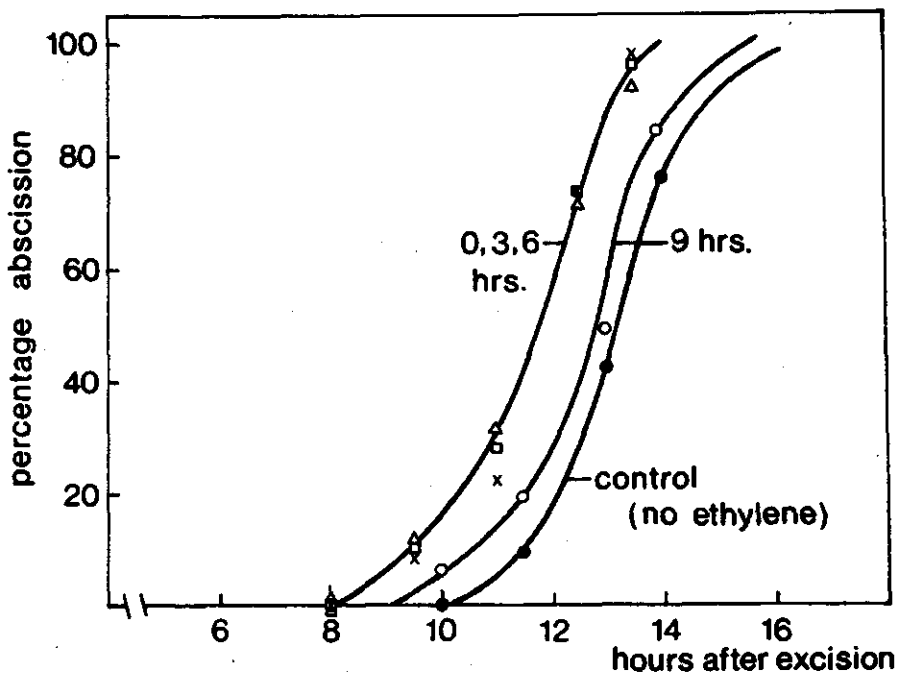

Fig. 4. The effect of application of 1 ppm ethylene, 0, 3, 6 and 9 hours after excision, on the course of abscission of pedicel explants of clone 2.

IAA, when applied directly after excision, postpones the sensitivity to ethylene until after nine hours, whereas ABA has no effect (Table 1).

The abscission retarding effect of IAA is obtained when the hormone is applied before nine hours after excision (Table 2). In contrast with ethylene (Table 1), IAA is effective during the whole first period. The length of the period of sensitivity to IAA is increased by ABA, both when the incubation with ABA starts immediately after excision and after three hours. Ethylene does not shorten this period, it is unlikely that it would prolong it, but this could not be established because the explants start to abscise already after a nine hours treatment with ethylene (Table 2).

The explants are also immediately sensitive to ABA, but this substance is most effective when applied three hours after excision (Table 3). IAA and ethylene tend to prolong the period of sensitivity to ABA without shifting the optimum time of application.

Discussion.

In the course of abscission of pedicel explants of Begonia cultivars, an IAA-sensitive period can be distinguished from an ethylene-sensitive one, the former preceding the latter. This pattern is in accordance with the stages I and II which DELA FUENTE and LEOPOLD (1968) describe for petiole abscission, but not with the three stages course presented by JACKSON and OSBORNE (1972). IAA prolongs the duration of the ethylene insensitive period (stage I) as effectively when applied at the beginning or at the end of this phase, while ABA and ethylene have no reducing effect on the duration of this period (Table 2). The way in which IAA prolongs stage I is unclear in view of the different hypotheses that have been proposed: its transport (OSBORNE and MULLINS, 1969), its gradient over the abscission zone (LOUIE and ADDICOTT, 1970), or its concentration (JACOBS, 1968). The earlier described experiment with proximal application of IAA (HÄNISCH TEN CATE and BRUINSMA 1973) is in favour of the last hypothesis.

The abscission accelerating substance, ethylene, fails to affect the first stage but shortens stage II. In this respect Begonia pedicels differ from bean petioles where ethylene shortens stage I too (ABELES et al. 1971; JACKSON and OSBORNE 1972).

Table 3.

Effect of interval between excision and the distal application of 2 µl 5.10^{-4}M ABA, alone and in combination with 2 µl IAA or ethylene, given directly after excision. The T_{50}-values are expressed in per cents of the control values that are given between ( ). (clone 3).

Interval of ABA application	--	ethylene 1 ppm	IAA 5.10^{-4}M
0 hrs	94 ± 2	93 ± 1	95 ± 2
3 "	88 ± 4	87 ± 7	91 ± 2
6 "	99 ± 2	92 ± 4	93 ± 2
9 "	99 ± 2	–	97 ± 2
no ABA	100 (14.5 hrs)	100 (13.1 hrs)	100 (16.0 hrs)

The main effect of ethylene in abscission,however, is generally thought to be the activation and secretion of cellwall degrading enzymes, especially cellulase, during stage II (ABELES et al., 1971; HOLM and ABELES, 1967; LEWIS and VARNER, 1970; POLLARD and BIGGS, 1970).

The role of the abscission promoting hormone, ABA, is still controversial. In Begonia pedicels, ABA accelerates the abscission when applied during stage I, with an optimum time of application at about three hours after excision. This optimum time may be due to metabolism of ABA in the explants, while it has its effect only later in the course of abscission (JACKSON and OSBORNE, 1972). JACKSON and OSBORNE suggest that ABA and IAA regulate the abscission by acceleration or retardation of the production of ethylene which subsequently induces cellulase synthesis. In Begonia pedicels however, ABA certainly does not shorten stage I, and,moreover,has an additive effect next to that of ethylene (Fig. 3). This agrees with the view of CRAKER and ABELES (1969) that ABA increases cellulase activity independently of ethylene.

It can be concluded that the abscission course of Begonia pedicels consists of two stages: a stage I which is prolonged by IAA and not shortened by ethylene or ABA; and a stage II which is accelerated both by ABA and by ethylene. Within these stages the hormones exert their effects independently from one another.

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Abscission of flower bud pedicels in *Begonia* III. Anatomical pattern of abscission.

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Abscission of flower bud pedicels in *Begonia*.
III Anatomical pattern of abscission.

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Summary.

The abscission in *Begonia* pedicels was studied anatomically. The abscission zone, consisting of small non-elongated cells, is present already in very young pedicels. The separation proceeds from the outside inward. The pattern is identical in three different clones and is not affected by growth regulators delaying or accelerating the abscission.

Introduction.

The difference in the degree of flower bud abscission between three clones of *Begonia* is located in the pedicel and is probably not of a hormonal nature (HÄNISCH TEN CATE and BRUINSMA 1973). The present paper studies the anatomy of the abscission pattern in the three clones, including the effects of the abscission accelerating and delaying hormones, abscisic acid (ABA) and indoleacetic acid (IAA), on the course of abscission.

Material and methods.

Three *Begonia* clones were selected from the crossing (*Begonia cinabarina* Hook x *B. micranthera* Griessl) x *B. davisii* Veitch. The growing, preparation, and hormonal treatment of pedicel explants were described by HÄNISCH TEN CATE and BRUINSMA (1973). Explants were fixed in formalin: glacial acetic acid: ethanol (50%) = 90:5:5, during at least 24 hours (JENSEN 1962), dehydrated through the standard tertiary butylalcohol series (JOHANSEN 1940), and embedded in Paraplast (melting point 56°-57°C, Sherwood Medical Industries Inc., Missouri, USA). The sections (10 μ) were stained with fast green and safranin according to JOHANSEN (1940).

Results.

Particularly with older pedicels, the abscission zones are visible from the outside as lightly coloured thickenings near to the base. Longitudinal sections of very young pedicels show already an abscission zone of four to ten layers of small flat cells (Plate 1, Fig. 1), which form is maintained throughout the lifetime of the pedicel (Plate 1, Fig. 2). Cell divisions prior to abscission were never observed.

The separation starts from the periphery in the cortex by a loosening of cell walls, the cell contents remaining intact (Plate 2, Fig. 3). The first visible sign often is a gap between the epidermis and cortex (Plate 2, Fig. 4). Subsequently, the separation of cell walls proceeds towards the centre of the pedicels (Plate 3, Fig. 5). The pith within the five vascular bundles is the last part to show separation of cells. After the completion of the separation a protective layer of suberin is formed (Plate 3, Fig. 6).

When the abscission is accelerated by ABA, or retarded by IAA, the anatomical course is not changed. The three clones invariably show the same anatomical pattern.

Discussion.

The abscission zone of pedicels of male Begonia flowers is already present at a very young developmental stage when the pedicel is still elongating. Contrary to the formation of petiole abscission zones, which are often formed by cell divisions just before the separation or earlier during the lifetime of the petiole (WEBSTER 1968, BORNMAN et al. 1967), in Begonia pedicels cell divisions never occur. A preformed separation zone was also observed in pedicels of flowers of Nicotiana (KENDALL 1918).
The comparison of the anatomical abscission pattern with the three clones gives no clue as to their difference in bud abscission, so that this difference is probably neither hormonal (HÄNISCH TEN CATE and BRUINSMA 1973) nor anatomical in nature.

Plate II.

Fig. 3. Separation of cortical cells in the abscission zone.

Fig. 4. The first sign of the starting of abscission by a loosening of the epidermis.

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Abscission of flower bud pedicels in *Begonia* IV. Regulation by endogenous hormones; translocation and metabolism of IAA-1-^{14}C in pedicel segments.

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Abscission of flower bud pedicels in Begonia
IV. Regulation by endogenous hormones; translocation and metabolism of IAA-1-^{14}C in pedicel segments

By

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Abstract

In order to analyse the hormonal regulation of flower bud shedding in Begonia, the concentrations in buds and pedicels of the substances involved, indoleacetic acid (IAA), abscisic acid (ABA), and ethylene, were followed. Moreover, the translocation and metabolism of ^{14}C-labelled IAA in pedicel segments was studied.

In a monoecious Begonia fuchsioides hybrid, the abscising male flower buds contain only about 1% of the IAA present in the non-abscising female flowers. In a male-flowering Begonia davisii hybrid, the seasonal variation in bud shedding coincides with changes in the IAA content of the buds. In this hybrid, the release of IAA from the flower bud to the pedicel is also hampered which increases the tendency to pedicel abscission.

The internal atmosphere of the abscission zone of these pedicels permanently contains the abscission-

promoting concentration of 0.2 to 0.3 ppm ethylene. IAA prevents the tissue to respond to this concentration with abscission unless its supply from the flower bud becomes insufficient. The buds also contain ABA but this does not considerably affect the abscission response.

Pretreatment with ethylene or ABA fails to influence the transport of IAA-1-^{14}C in pedicel segments. The rate of this transport is 4 to 6 mm.hr^{-1}, the capacity depends on the diameter of the segments. In young segments, particularly, IAA is actively decarboxylated and also metabolised in other ways.

Introduction

In a previous paper the question was raised whether the different tendency to flower bud abscission in three clones of Begonia could be ascribed to a hormonal difference (Hänisch ten Cate and Bruinsma 1973a). The different tendency to flower bud abscission was found to be located in the pedicels, which were equally sensitive to various growth regulating substances. An initial sensitivity to indoleacetic acid (IAA) was replaced by sensitivity to ethylene; abscisic acid (ABA) acted additively to ethylene (Hänisch ten Cate and Bruinsma 1973b).

The present study deals with the internal levels of hormones in flower buds, the export of IAA from these buds, and its transport and metabolism in the pedicels. Also

the ABA content of flower buds and the ethylene levels of the pedicels during the course of abscission are determined and the effects of these substances on the translocation of IAA are investigated.

Material and methods

The growing of three Begonia clones, with only male flowers, from the cross (Begonia cinnabarina Hook. x B. micranthera Griessl) x B. davisii Veitch, was described by Hänisch ten Cate and Bruinsma (1973a). A monoecious hybrid of Begonia fuchsioides Hook, and B. foliosa HB. et K., was grown in the greenhouse under the same conditions; the male flowers, in the centre of the dichasium, generally abscise before anthesis, whereas the two lateral female flowers reach full bloom.

The IAA content in buds and flowers was determined according to Knegt and Bruinsma (1973). The ABA content was determined by a gas chromatographic method, with correction for losses during extraction (Lenton et al. 1971).

The IAA release was studied by floating about 180 freshly harvested buds or 50 flowers, without pedicels, on 250 ml 0.01 M K_2HPO_4 in Petri dishes, 20 cm diameter, during 24 hours at 18°C in the dark, and determining the IAA content of the diffusates.

The IAA transport through pedicel sections of 4.2 mm was measured according to Veen and Jacobs (1969). IAA-1-^{14}C (over 95% pure, specific activity 52 mCi/mM, from Radiochemical Centre, Amersham, U.K.), was dissolved to a concentration of 0.4 mM in 1.5% (V/W) agar disks of 4 mm diameter, 2 mm thick.
The radioactivity in these donor and similar receiver blocks was counted after extraction of the disks into 2 ml 50% ethanol, using a Nuclear Chicago Mark I liquid scincillation spectrometer. The scintillation fluid contained 667 ml toluene, 333 ml Triton X-100, 4 g PPO, and 0.2 g POPOP per liter. The counts were corrected for background.

To identify the radioactive substances in the agar blocks, 5 blocks were extracted with 4 ml methanol during 24 hours in the cold. The extract was reduced at low pressure, spotted on a thin layer Silica G (Merck, 0,5 mm thick), and chromatographed with isopropanol: ammonia (25%): H_2O=10:1:1. Radioactive spots were located by exposure to a Kodak medical X-ray no screen film, covered with a 6.25 µ Melinex polyester sheet. After 10 days the films were developed. For identification of the radioactive substances in the tissues, 10 sections were frozen in liquid nitrogen and extracted with 4 ml methanol during 24 hours in the cold. The methanol extract was treated as described above. The remaining radioactivity in the tissue was determined by drying and burning the extracted material,

and counting the released ^{14}C after collecting the CO_2 in phenylethylamine.

The effect of ABA on the IAA transport was determined by placing 10 pedicel segments in 0.01 M phosphate buffer, pH=6.0, with or without 0.5 mM ABA. After 20 min. pretreatment the IAA transport in the pedicels was measured as described above. To determine the ethylene effect, dichasia were placed in beakers with tap water in dessicators containing 1 ppm ethylene or air (ethylene concentration less than 0.01 ppm), at 25°C in the dark. After 11 hours pretreatment, 4.2 mm segments were cut from the pedicels and the IAA transport was measured in an atmosphere containing 1 ppm or less than 0.01 ppm ethylene.

The ethylene concentration in the intercellular space of pedicel explants during the course of abscission was determined after extracting samples of 100 explants under low pressure according to Beyer and Morgan (1970).

Results

(A) Content and release of IAA of flower buds

In the Begonia fuchsioides hybrid the central male flowers abscise before anthesis, whereas all the lateral female flowers reach full bloom. This difference in abscission tendency is correlated with a difference in IAA content, the female flowers containing about one hundred times as much IAA as the male ones (Table 1). The buds

Table 1. Bud abscission and IAA content of female and male flowers in the Begonia fuchsioides x B. foliosa hybrid.

	female buds	male buds
per cent abscission	0	99
ng IAA per bud	10.7 ± 0.7	0.12 ± 0.02

Table 2. Seasonal variation in abscission and IAA content of flower buds of two clones of a Begonia davisii hybrid.

	clone 1		clone 3	
	July	January	July	January
per cent absc.	2	10	20	40
ng IAA per bud	1.2±0.1	0.55±0.05	4.6±0.2	0.54±0.07
ng IAA per fl.	5.3±1.6	6.5±0.7	11.1±0.2	11.0±0.4

Table 3. Course of the IAA content, in ng per flower (bud), during the flower development of Begonia davisii hybrid. July, 1972.

	clone 1	clone 2	clone 3
small buds	0.08±0.01	0.47±0.04	1.25±0.05
half-grown buds	0.65±0.01	0.67±0.04	4.64±0.08
large buds	1.18±0.12	1.67±0.07	4.60±0.18
half-open flowers	0.87±0.06	1.84±0.14	3.20±0.40
open flowers	5.26±1.60	5.66±0.08	11.1±0.20

Table 4. Abscisic acid content of buds and flowers of the Begonia davisii hybrid. November, 1973.

	ng ABA per bud or flower		
	clone 1	clone 2	clone 3
experiment 1			
large buds	4.3	-	10.5
open flowers	5.7	-	4.9
experiment 2	-	-	
abscising buds	-	9.4±2.6	9.7±0.2
attached buds		10.7±0.4	6.3±2.1

were collected in a nearly full-grown stage.

In the three Begonia davisii clones the tendency to flower bud abscission changes in the course of the year. Clone 1 varies from 0% in summer to 10% in the winter, clones 2 and 3 vary from 20 to 50% flower bud abscission depending upon the season. These changes are also correlated with changes in the IAA content of the buds, once opened the flowers attain at about the same level (Table 2).

However, the difference in tendency to flower bud abscission between the three clones cannot be ascribed to different IAA contents (Table 3). During the development of the flowers, the IAA content gradually augments with a large increase after a transient fall at about opening. Contrary to the expectations, the flower buds of clone 1, which are most resistant to abscission, contain the least IAA; the IAA content of flower buds of clone 3 is by far the largest, although these buds drop as easily as those of clone 2 (Hänisch ten Cate and Bruinsma 1973a).

The content of IAA may not be an appropriate measure for the release of IAA from the bud to the pedicel. On the contrary, blocking this release might increase the IAA content of the bud. That, indeed, the diffusion of IAA from the flowers and buds tends to decrease at increasing IAA-contents, is indicated by experiments as represented in Figure 1.

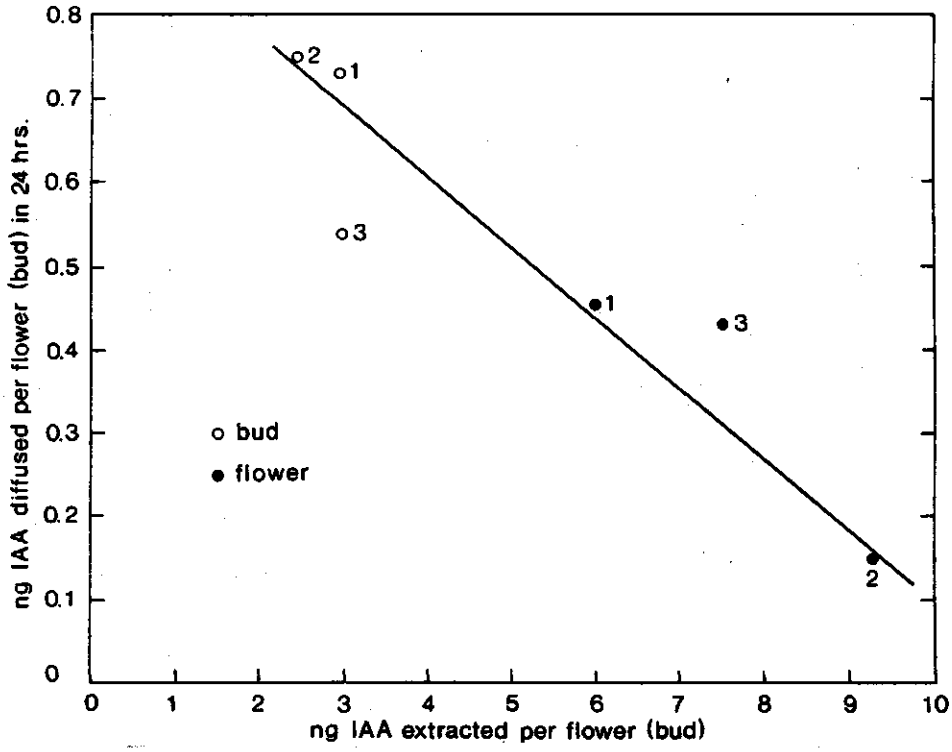

Fig. 1. Content and release of IAA of buds and flowers. Large buds and open flowers are either extracted or allowed to diffuse during 24 hrs. Numbers indicate the three clones. March, 1973.

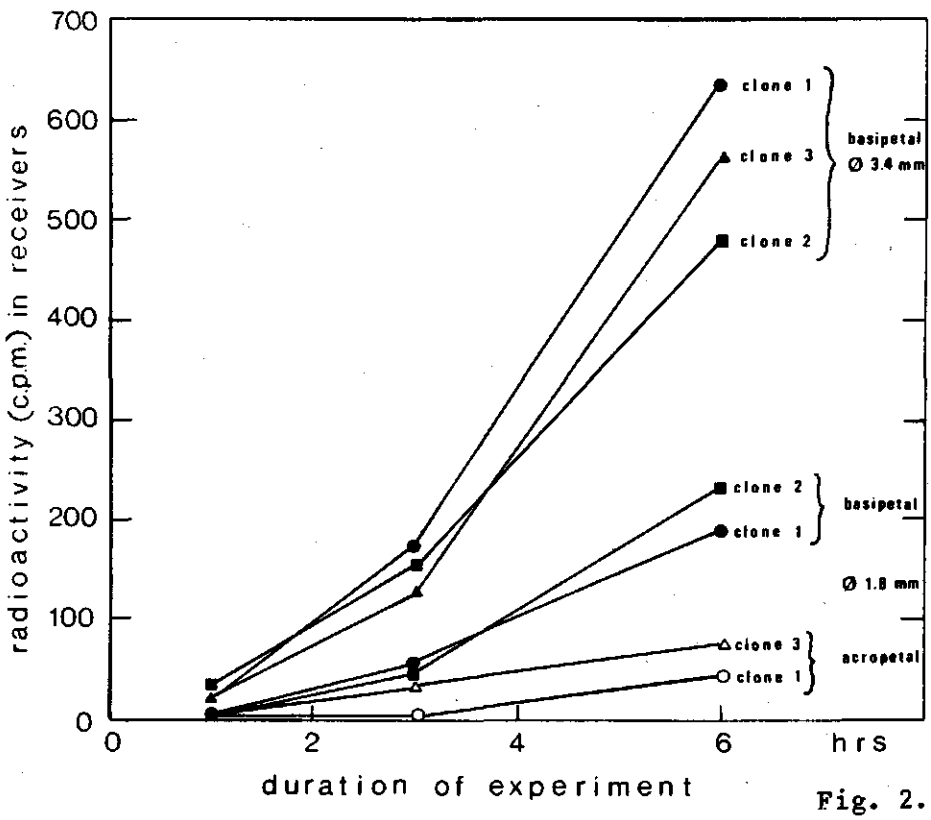

Fig. 2. Basipetal and acropetal transport of IAA-1-^{14}C in 4.2 mm dichasium segments of different diameters.

(B) Translocation and metabolism of IAA-1-^{14}C in pedicels

The fragile pedicels turned out to be poor media for the translocation of ^{14}C-labelled IAA from donor to receiver agar blocks (Fig. 2). The acropetal transport of IAA was very small, the basipetal one showed no significant differences between the three clones. The rate of the IAA transport was usually 4 to 6 mm per hour. The capacity of the transport increased proportional to the diameter of the segments, so that lower branchings of the dichasium were found to be more suitable for these studies than the otherwise used pedicels. However, also in these lower branchings no significant differences in the rate or capacity of the IAA-transport occurred.

The total radioactivity, finally recovered from donor and receiver blocks and from the tissue, was always smaller than the amount initially applied in the donor block, indicating that decarboxylation of IAA occurred. With young pedicel segments, 80 to 90 per cent of the IAA leaving the donor block was thus found to be decarboxylated, with segments from older parts of the inflorescences a quarter to a third of the radioactivity, lost from the donor blocks, could be recovered.

The radioactivity, recovered from the receiver blocks, was confined to IAA, but the tissue was shown to contain metabolites, too, particularly with segments from young parts of the dichasium. As shown in Fig. 3, in segments of

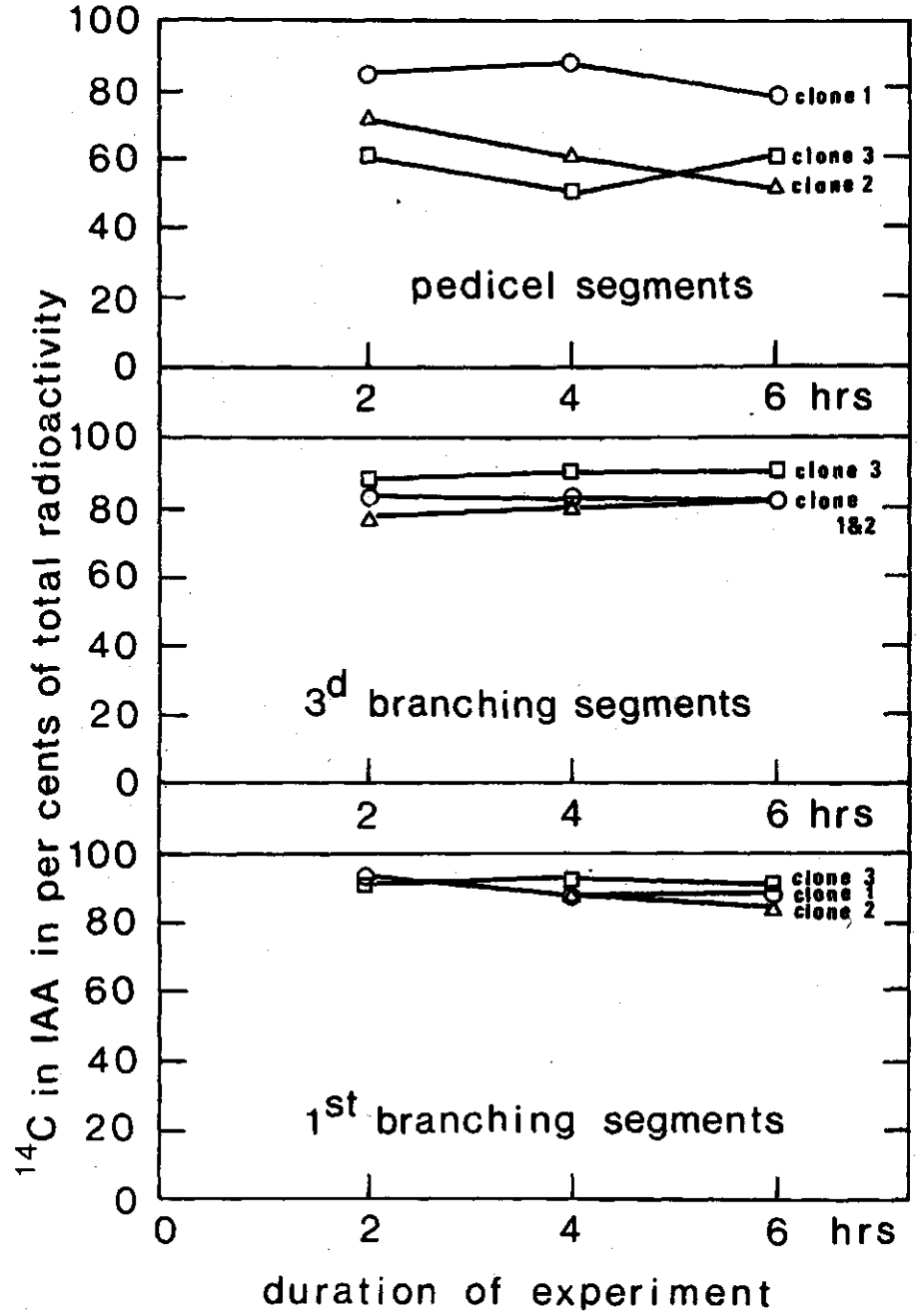

Fig. 3. Recovery of IAA-1-^{14}C in per cents of the total radioactivity on thin layer chromatograms of tissue extracts, in the course of basipetal IAA transport in 4.2 mm segments from different parts of the inflorescence.

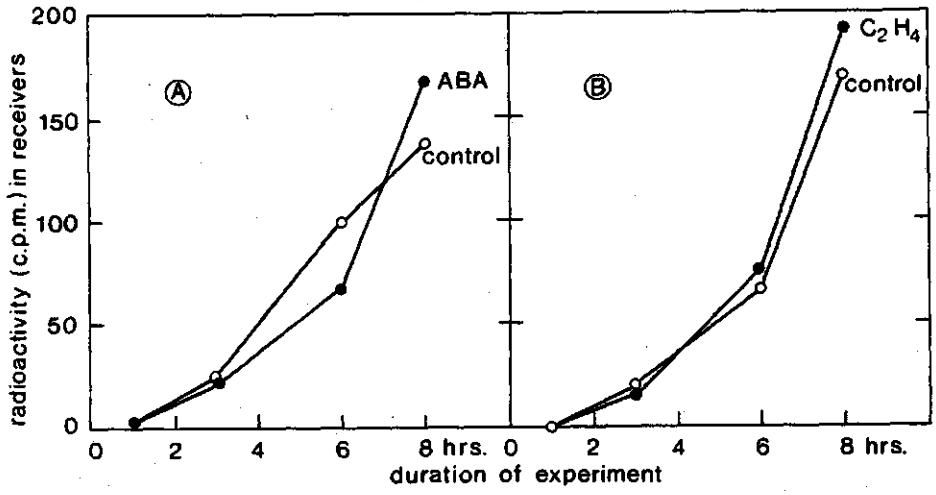

Fig. 4. Basipetal transport of IAA-1-^{14}C in 4.2 mm pedicel segments of clone 1. A: Effect of 20 min. pretreatment of segments in 0.5 mM ABA solution. B: Effect of 11 hrs. pretreatment of dichasia in air containing 1 µl ethylene per l.

the older parts, over 80 per cent of the radioactivity was still in IAA. In the pedicel segments, however, IAA was metabolised to a larger extent, particularly in the clones 2 and 3.

(C) Concentrations and effects of abscisic acid and ethylene

Whereas auxin inhibits pedicel abscission, abscisic acid and, particularly, ethylene were shown to accelerate this process (Hänisch ten Cate and Bruinsma 1973a, b). Flower buds of the Begonia davisii hybrid contain, indeed, appreciable amounts of abscisic acid when they are about to open (Table 4). However, the differences in ABA-content were too small, if any, to account for the differences in abscission between either the clones or the individual buds within the clones.

In petiole abscission, the ethylene concentration in the internal atmosphere of the abscission zone is often found to increase at the onset of the abscission process (Jackson et al.1972,1973).During the whole course of pedicel abscission, however, the internal ethylene concentration was found to remain remarkably constant at about 0.2 to 0.3 µl/l, neither increasing before, nor decreasing after completion of the process (Table 5).

Pretreatment of pedicel segments with an abscission-promoting concentration of abscisic acid, or of inflorescences with ethylene, failed to alter the rate or capacity of the IAA-transport in pedicel segments (Fig. 4).

Table 5. Internal ethylene concentrations, in µl/l, during the course of abscission. Determined at samples of 100 pedicel explants, prepared after Hänisch ten Cate and Bruinsma (1973a).

hrs. after excision	clone 1	clone 2	clone 3
0	0.25	0.25	0.28
4	0.23	0.23	0.20
8	0.19	0.25	0.31
12	0.33	0.19	0.25
16	0.21	0.18	0.20
20	0.32	0.20	0.20
24	0.23	0.18	0.20
32	0.25	0.16	-
50% abscission at:	18.3 hrs	16.9 hrs	14.5 hrs

Discussion

Although Begonia flower buds contain considerable amounts of abscisic acid, no clear relationship was found between the tendency of the pedicels to abscise and the ABA content of the buds. This is in accordance with results from earlier experiments with pedicel segments, showing that these explants are little responsive to exogenous ABA (Hänisch ten Cate and Bruinsma 1973a and b). In these earlier experiments, abscission turned out to be promoted by 0.1 ppm of ethylene already, but indoleacetic acid prevents the tissue to become sensitive to ethylene. The rather constant ethylene concentration in the internal gas phase of the abscission zone, 0.2 to 0.3 ppm throughout the course of abscission, points to IAA as the main regulator of pedicel abscission, determining whether or not the tissue responds to the abscission-promoting ethylene concentration already present. Although abscission in petioles is generally accompanied by a transient rise in the ethylene concentration of the intercellular space (Jackson and Osborne 1970; Jackson et al. 1972, 1973), this correlation is not always found (Lewis et al. 1968; Feldman et al. 1971; Blanpied 1972). In Begonia pedicels IAA does not regulate the production of ethylene, as in bean petioles (Jackson et al. 1973), but rather controls the sensitivity of the tissue to ethylene. According to Abeles et al. (1971), IAA acts as a juvenility factor which has to disappear in order to allow abscission in a senescing tissue.

It is the auxin production of the flower bud in the first place which prevents the pedicel to abscise prematurely. This is demonstrated by the difference in bud drop between male and female flowers of the monoecious Begonia fuchsioides hybrid (Table 1) and by the seasonal variation in bud abscission of the male Begonia davisii hybrid (Table 2). Chatterjee and Chatterjee (1971) found seasonal fluctuations in abscission of cotton flowers to be correlated with their IAA content; according to Moe (1971) seasonal variations in the IAA content of rose shoots may depend on the light intensity.

If the release of IAA from the bud to the pedicel is blocked, as is evidently the case with the Begonia davisii hybrid (Fig. 1), then the relation between auxin content of the bud and prevention of pedicel abscission is rather expected to be negative. It is uncertain, as to how far the different tendencies to bud drop of the 3 clones of the Begonia davisii hybrid can be ascribed to this restriction of auxin release by the bud; also the intrinsic differences in abscission rate of excised pedicels, as described by Hänisch ten Cate and Bruinsma (1973a), and possibly a different ABA-production of the bud may also contribute to the differences in their disposition to bud drop. The results of the present study confirm the use of auxin sprays in practice to prevent premature shedding of flower buds of Begonia cultivars.

this view but rather confirm the earlier finding (Hänisch ten Cate and Bruinsma 1973b) that neither ABA nor ethylene interfere with the action of IAA in pedicel abscission.

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Abscission of flower bud pedicels in *Begonia* V. Protein synthesis and activities of cell wall decomposing enzymes.

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Abscission of flower bud pedicels in Begonia
V.Protein synthesis and activities of cell wall decomposing enzymes

By

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Abstract

The role of protein synthesis during the course of abscission of pedicel explants in a Begonia davisii hybrid is established by determining the effects of inhibitors of protein synthesis on this course. Decomposition of cell walls, necessary for the cell separation, was followed by measuring the solubility of pectins and the activities of pectinases and cellulases in the abscission zones of pedicel explants during the course of abscission.
Actinomycin D, chloramphenicol, and dinitrophenol inhibit abscission to a small degree only, whereas cycloheximide has a much larger inhibiting effect. Cycloheximide retards abscission when applied until 10.5 hours after excision, indicating that cell wall-dissolving enzymes are synthesized only after an ageing period of over 10 hours.

Separation of cells takes place by dissolution of pectins. Activities of the tested pectinases,however,

did not correlate with the abscission process. Pectin methylesterase was present in the intercellular space and in the cells of the abscission zone, whereas polygalacturonase, pectic acid transeliminase and pectin transeliminase were totally absent in the abscission zone. Endo- and exocellulase activities in the abscission zone increased only about 10 hours after the onset of abscission, indicating that the decomposition of primary walls is a secondary phenomenon of already separated cells only.

Introduction

This paper deals with protein synthesis and enzymatic dissolution of cell walls during the course of abscission of pedicel explants in a Begonia davisii hybrid. Anatomical studies suggested abscission in these pedicels to be caused by separation of intact cells in the abscission zone, that is by dissolution of the middle lamella (Hänisch ten Cate et al. 1973). In the literature, however, enzymatic studies about the correlation between pectinase activity and abscission mostly gave negative results, whereas a correlationship between cellulase activity and the abscission process has frequently been demonstrated (Ratner et al. 1969; Biggs 1971). In the present paper, the activities of endo- and exoenzymes decomposing pectins and cellulose were followed during the course of the abscission process,together with the effects of

antibiotic inhibitors of protein synthesis on this process.

Material and methods

From three Begonia clones, selected from the crossing (Begonia cinnabarina Hook.x B. micranthera Griessl) x B. davisii Veitch, pedicel explants were prepared and treated as described by Hänisch ten Cate and Bruinsma (1973a). Aqueous solutions of actinomycin D (AMD), chloramphenicol (CAP), 2,4-dinitrophenol (DNP), and cycloheximide (CHI), or water, were applied to the distal cut surface, injected into the pedicels near the abscission zone, or applied to the bracts. The abscission is expressed by the interpolated time at which 50% of the pedicels had abscised (T_{50}). The effects of the treatments are indicated by expressing their T_{50}- values in per cents of those of the water-treated controls, values above and below 100% representing retardation and acceleration of abscission, respectively. Bacterial contamination was never observed.

Cellulase activity was measured in extracts of abscission zones of explants according to an adapted method after Horton and Osborne (1967). From 25 explants 2 mm sections of abscission zones were cut. For the determination of cellulase activity in the free space (exocellulase), the sections were incubated during 24 hours at $0^{o}C$ in 3 ml 0.5 M phosphate buffer, pH 7.0, containing 0.2% NaF and 0.25% polyvinyl pyrrolidone (PVP). When also the endocellular cellulase activity (endocellulase)

was determined, after removal of the exocellulase diffusate the sections were washed three times with 3 ml of the phosphate buffer, ground with 3 ml of the phosphate buffer in a mortar, centrifuged during 20 minutes at 10,000 g , and the supernatant was collected for the determination. For the cellulase assay, 0.5 ml enzyme solution was added to 2 ml 0.9% Na-carboxymethylcellulose (CMC, British Drug House LTD, Poole, England), and the decrease in viscosity of the mixture was measured at five points of time. The percentage of decrease in viscosity after 20 hrs incubation, calculated per abscission zone, was used as a measure of cellulase activity.

Pectinase activities were assayed according to methods described by Voragen (1972), the pectin substrates were gifts of the Department of Food Science of the Agricultural University. The exo- and endocellular enzyme extracts for polygalacturonase (PG), pectin-methylesterase (PME), pectic acid transeliminase (PATE), and pectin transeliminase (PTE), were obtained as described for the exo- and endocellulases. For PG, PATE, and PTE activities, a 0.1 M citrate/phosphate buffer, pH 6.0, containing 0.25% PVP, was used, 150 abscission zones per 3 ml buffer. The PATE and the PTE activities were assayed by determining the increase of absorption at 232 nm during 1 hr of a mixture of 1 ml diffusate or extract and 1 ml 0.2% pectic acid or Brown Ribbon pectin,

respectivily, at 23°C. The PG activity was determined by the decrease in viscosity of a mixture of 1 ml diffusate or extract and 1 ml 2% pectic acid at 25°C. For the PME activity the buffer was replaced by H_2O, the same procedure as described above being used. The PME activity was assayed by adding 1 ml diffusate or extract to 1 ml .1% Green Ribbon pectin, the pH was adjusted to pH 7.0 with 0.1 N NaOH. During 3 hrs the solution was titrated with 0.01 N NaOH to maintain a pH value of 7.0 at 23°C.

Pectins were determined according to a modified method of Tibensky et al. (1963). In 6 ml H_2O 200 abscission zones were evacuated, incubated during 24 hrs at 0°C, and the soluble pectins were determined in the diffusate. The segments were subsequently ground in a mortar with 10 ml H_2O, 2 ml 1N NaOH being added, incubated by stirring during 1 hr at ambient temperature, and centrifugated at 18,000 g during 30 minutes. The hydrolyzed pectins were determined in the supernatant. The pH of the pectin solutions was adjusted to 4.0 with 2 M acetic acid, 2 ml 1% $CuSO_4$ was slowly added and after 5 minutes the sedimented pectins were centrifuged at 18,000 g during 30 minutes. The pellets were 3 times resuspended in 10 ml H_2O to remove all free Cu^{2+}-ions. The pellets were dissolved with 5 ml 1.5 M ammonia/citrate buffer, pH 9.5. To 2.5 ml of the pectin solutions 2.5 ml 0.5% cuprizon was added and after 30 minutes the absorption of the solutions at 590 nm was measured.

The soluble pectins were calculated in per cents of the total pectin content of the abscission zones.

Results

Effects of metabolic inhibitors

The role of protein synthesis in the abscission process of pedicel explants was studied by analyzing the effects of AMD, CHI, CAP, and DNP on the course of abscission. The place of application influenced the effects of these metabolic inhibitors. When AMD was applied at the distal cut of the explant, 1 cm from the abscission zone, this inhibitor of the nucleic acid synthesis slightly enhanced abscission. Injected into the abscission zone, however, AMD retarded abscission in concentrations up to 30 ppm, a higher concentration had no effect or promoted abscission (Fig. 1). Optimum concentrations of AMD, CAP and DNP retarded abscission only slightly, whereas the inhibitor of cytoplasmatic protein synthesis, CHI, postponed the process much more effectively (Fig. 2). When CHI was applied up to 10.5 hrs after excision, it retarded abscission, while application 14 hrs after excision turned out to be too late (Fig. 3).

Cell wall solubilization

Abscission of explants was accelerated by ethylene and retarded by indoleacetic acid (IAA); the occurrence

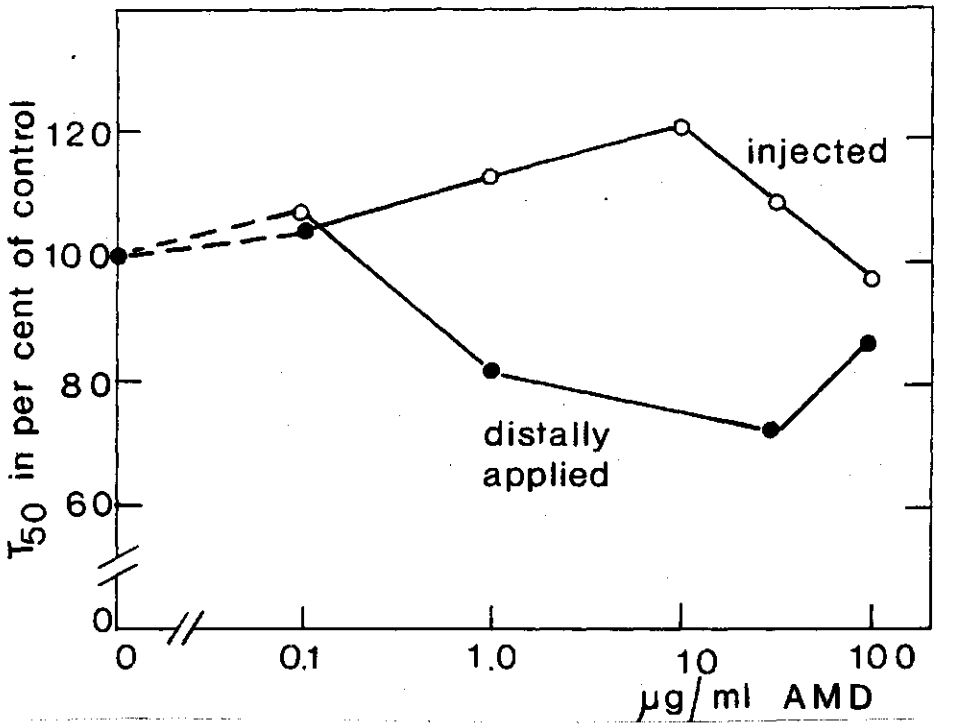

Fig. 1. Effect of 4 µl AMD solutions, injected just after excision near the abscission zone or applied to the distal cut of pedicel explants. The T_{50}-values are expressed in per cents of those of the water control.

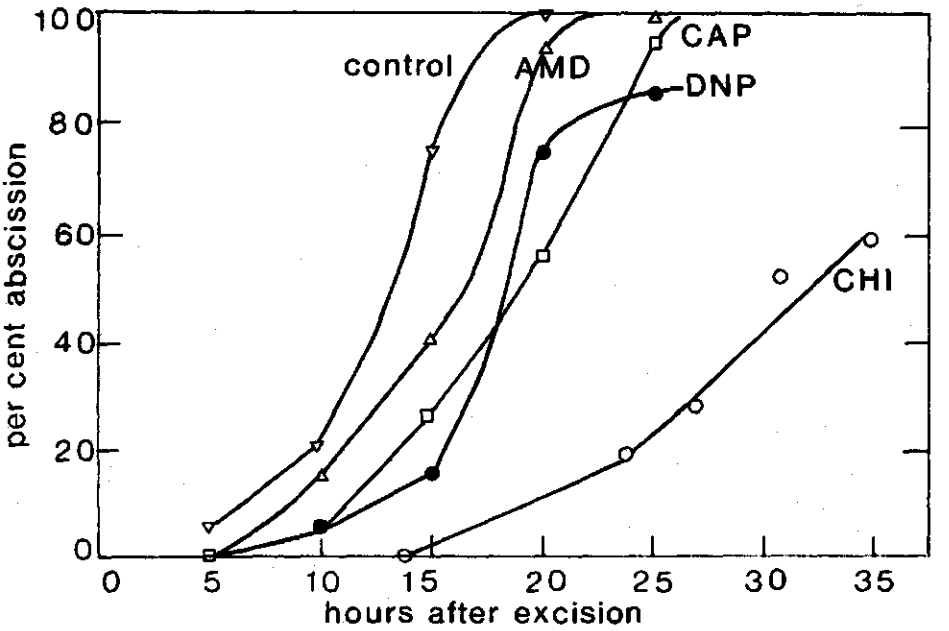

Fig. 2. Abscission of pedicel explants, injected just after excision near the abscission zone with solutions of H_2O (control), 10 µg/ml AMD, 100 µg/ml CHI, 30 µg/ml CAP and 10^{-4} M DNP.

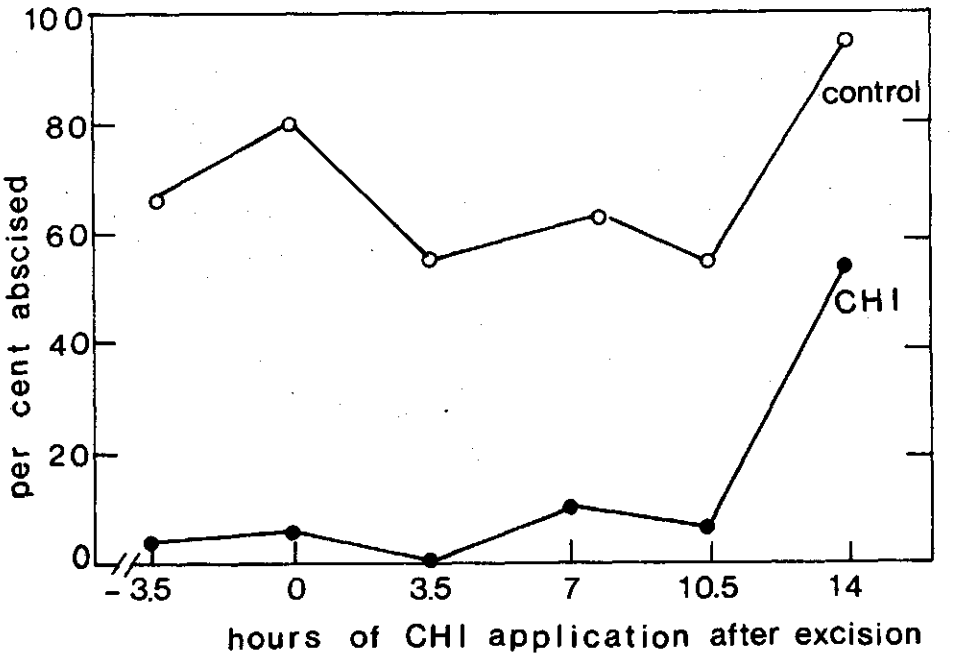

Fig. 3. Effect of the interval between excision and injection of 4 µl 100 µg/ml CHI near the abscission zone, on the percentage abscission 15 hrs. after excision.

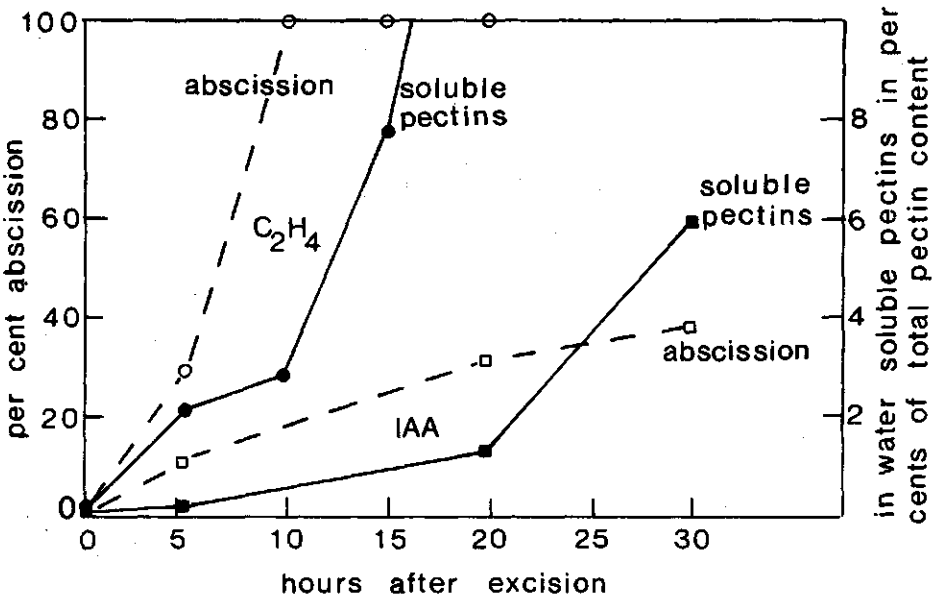

Fig. 4. Abscission and water-soluble pectins in per cents of total pectin in the abscission zones of pedicel explants, treated during the experiment with 1 µl/l ethylene in the atmosphere or with 2 µl 0.5 mM IAA on the distal cut surface.

of soluble pectins in the abscission zone was found to be correlated with an increase in abscission (Fig. 4). However, the activities of the tested pectinases in the free space or in the cell failed to increase during abscission. PME activities were present in low values in both exo- and endocellular fractions, whereas PG, PATE and PTE activities were totally absent in the diffusates and extracts of the abscission zones.

The hydrolysis of cell wall cellulase was studied by determining the exocellulase activity during the course of abscission. At the time of excision of the explants, hardly any cellulase occurred in the abscission zones, although considerable activity was detected in the central part of the explants, under the scar of the abscised central flower. The increase in exocellulase activity in the abscission zones followed only about 10 hrs after the beginning of separation (Fig. 5). Ethylene accelerated, IAA postponed ,and abscisic acid (ABA) did not influence the onset of this increase in cellulase activity. The initial absence of endocellulase suggests that cellulase was synthetized only towards completion of the abscission process, whereas CHI strongly inhibited this synthesis (Fig. 6). Apparently, cellulase is excreted soon after its production.

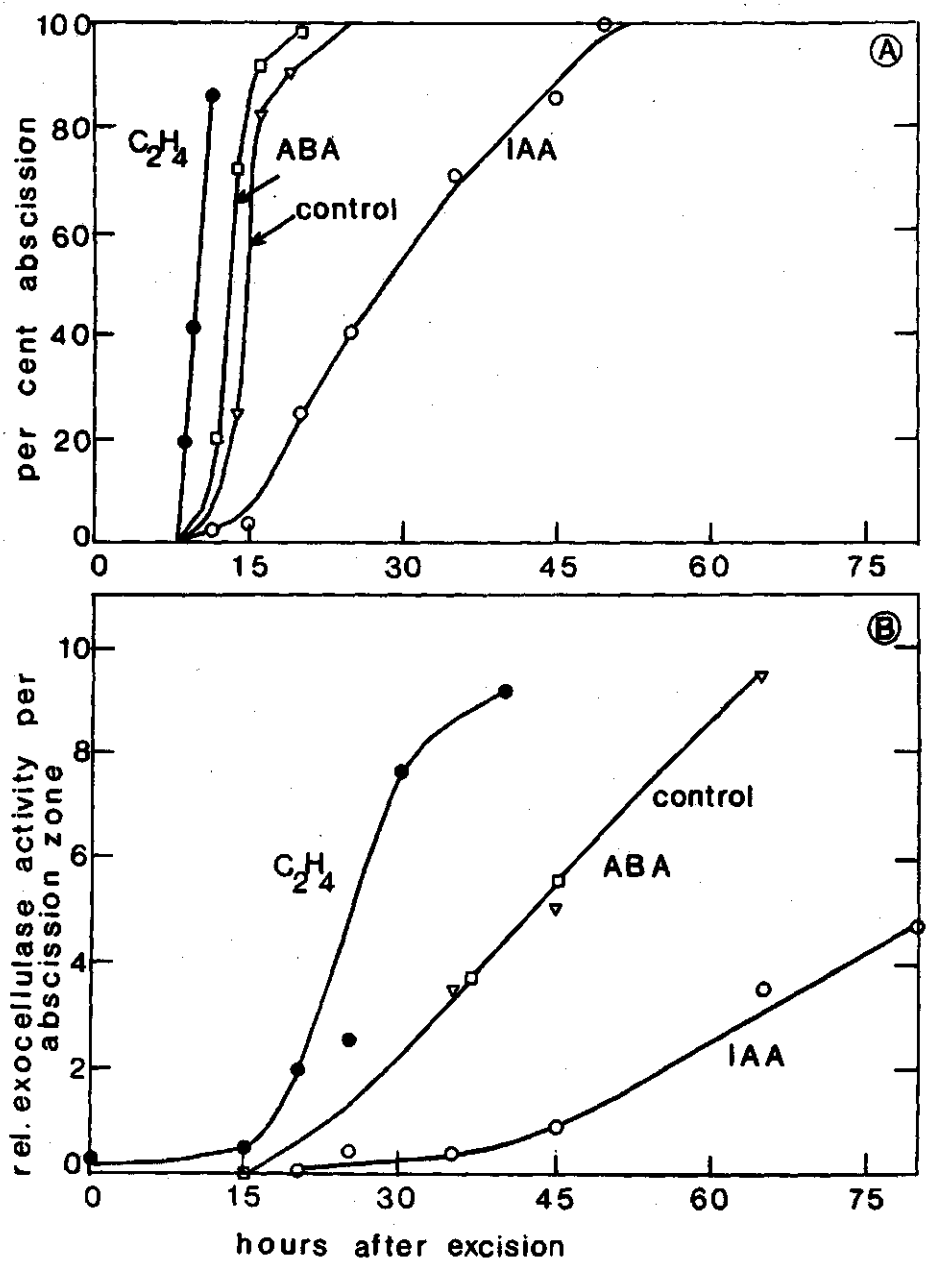

Fig. 5. A. Abscission, and B: Exocellulase activity in the abscission zones of pedicel explants, treated as in Fig. 4 with 1 µl/l ethylene, 0.05 mM IAA, 0.5 mM ABA, or H_2O (control).

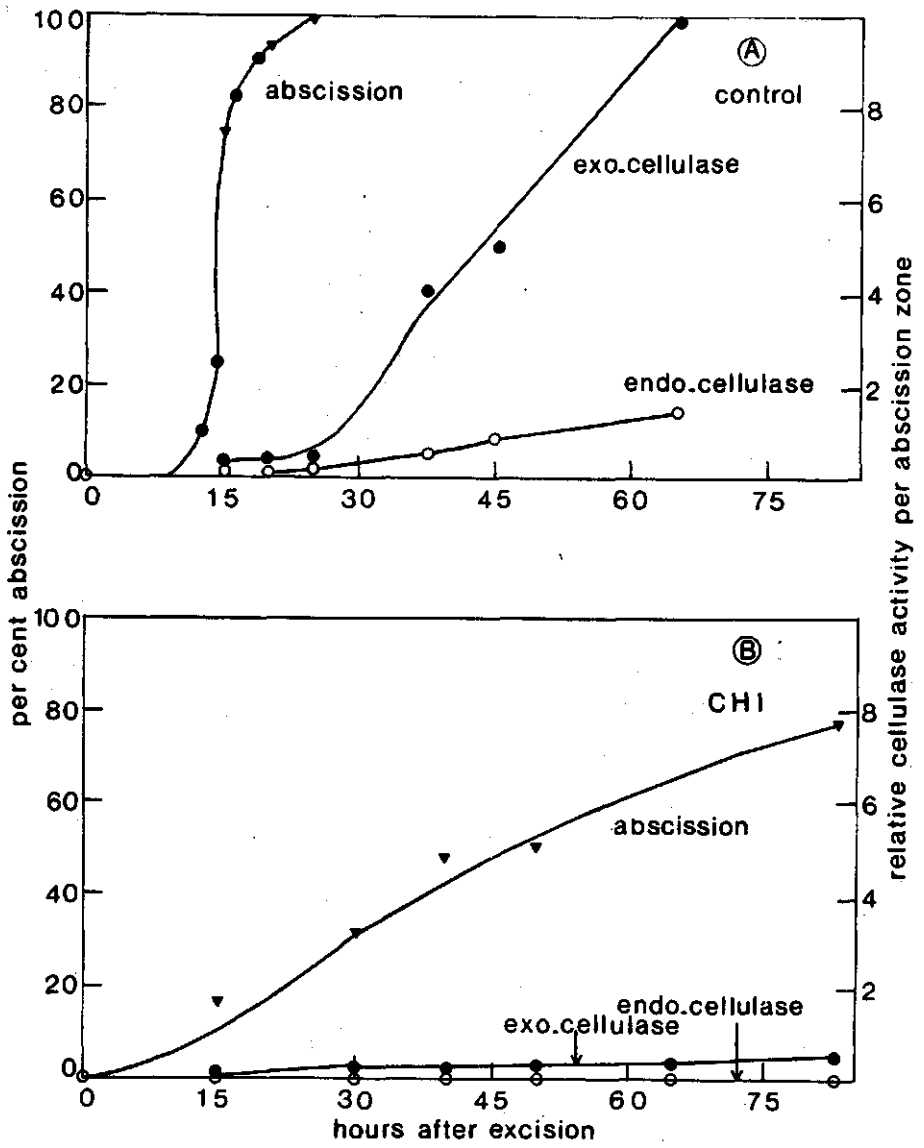

Fig. 6. Abscission and endo- and exocellular cellulase activities treated with A: H_2O, or B: 2 µl 100 µg/ml CHI, applied to the bracts just after excision of the explants.

Discussion

Of all the metabolic inhibitors used only CHI considerably retarded abscission of pedicel explants. The promotive effect of AMD, when applied to the distal cut surface, might be ascribed to increased ethylene evolution (Biggs 1971; Cooper and Horanic 1973). The retarding action of CHI, when applied to the abscission zones, indicates that protein synthesis on cytoplasmatic ribosomes is required for abscission, the genetic information being already present in the cytoplasm. This synthesis starts only after an ageing process of over 10 hrs upon excision of the explants. It was shown earlier (Hänisch ten Cate and Bruinsma 1973b) that the sensitivity to ethylene in these explants develops between 6 and 9 hrs after excision. The only small retarding effects of AMD, CAP and DNP may be ascribed to their interference with the supply of energy, required for the abscission process (Glasziou 1969; Hänisch ten Cate and Bruinsma 1973a).

Cell separation in Begonia pedicels coincides with the occurrence of soluble pectins and is only followed by dissolution of cellulase (Figs. 4,5). These results indicate that the cell wall separation is due to dissolution of the middle lamella which is composed of pectins. They agree with the results of the earlier anatomical study of the course of abscission in these pedicels, which showed separation of intact cells (Hänisch ten Cate et al. 1973).

However, the present attempt to reveal the nature of the enzymes involved in this pectin dissolution was not succesful. One explanation for this failure is that enzymes other than PG, PME, PATE and PTE are active in the dissolution of the middle lamella, another that the substrates, with which their activity was determined,apple fruit pectins, cannot be decomposed by pectinases from Begonia pedicels. Possible inhibitors of the action of these enzymes were counteracted by PVP.

Nearly all the studies about the correlation between pectinases and abscission gave negative results, too (Ratner et al. 1969; Pollard and Biggs 1970; Abeles et al. 1971; Rasmussen 1973). However, Morré (1968) showed that in Phaseolus petioles the pectinase activity, measured as the decrease in weight of cucumber pericarpdisks increased just prior to abscission. Moline et al. (1971) demonstrated a correlation between PME activity and abscission in Coleus petioles, which they failed to find in six other species, including Phaseolus.

Correlations between an increase in cellulase activity and abscission are generally positive (Horton and Osborne 1967; Abeles 1969; Ratner et al. 1969; Pollard and Biggs 1970; Rasmussen 1973). An increase in cellulase activity has often been found to precede or coincide with abscission, suggesting that abscission should be brought about by cellulase activity. However, this conclusion is

not supported by anatomical studies. In tomato and tobacco pedicels, dissolution of the middle lamella has been shown to precede the decomposition of the primary wall, to which cellulose is confined (Valdovinos and Ernest 1968; Valdovinos et al. 1970). In Phaseolus pectioles, too, dissolution of the middle lamella was observed (Morré 1968). Webster (1973) found that in the abscission zone of these petioles the primary wall desintegrated upon audition of ethylene.

The present experiments demonstrate that in Begonia pedicels cellulase activity is not the cause of cell separation, but onde of the after effects of abscission, increasing only after completion of the process.

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SAMENVATTING

De in dit proefschrift beschreven experimenten zijn in 5 artikelen samengevat.

De inleiding geeft een literatuuroverzicht betreffende de abscissie, welke vooral aan verouderende organen, zoals stelen van bladeren en vruchten, is bestudeerd. Abscissie blijkt in het algemeen met de veroudering van het afvallende orgaan te zijn gecorreleerd. In hoeverre veroudering noodzakelijk voor abscissie is, is nog niet zeker, daar soms ook jonge organen afvallen. De abscissie van jonge organen komt o.a. in een aantal Begonia variëteiten veel voor. Deze variëteiten bloeien overvloedig, maar laten ook veel bloemknoppen vallen. De stelen der bloemknoppen, pedicellen, zijn jonge organen, die zich nog in hun strekkingsfase bevinden. In de teelt en handel van Begonia's is de bloemknopabscissie dan ook een probleem.

In dit proefschrift is de hormonale regulatie van de bloemknopval bestudeerd m.b.v. 3 geselecteerde klonen van de kruising (Begonia cinnabarina Hook.x B. micranthera Griessl) x B. davisii Veitch, welke hetzelfde uiterlijk hebben, alleen mannelijke bloemen vormen, maar in verschillende mate hun bloemknoppen laten vallen; en m.b.v. een hybride van de eenhuizige kruising Begonia fuchsioides Hook. x B. foliosa HB et K. waarvan de mannelijke bloemknoppen in het algemeen afvallen terwijl de vrouwelijke bloemen zich volledig ontwikkelen.

In deel I worden de effecten van exogeen toegediende plantenhormonen bij de 3 B. davisii klonen vergeleken bij pedicellen aan de plant (in vivo) met en zonder bloemknoppen en bij geïsoleerde pedicellen (in vitro) met en zonder bloemknoppen. Auxine (IAA) remt de abscissie van de knoppen en de pedicellen in vivo en in vitro, ethyleen bevordert, terwijl abscisinezuur (ABA) alleen in vitro bevordert. Cytokininen (kinetine) en gibberellinen (gibberellinezuur) hebben geen effect. De bloemknoppen geven een stof aan de onderliggende abscissie-

zone af, waardoor deze niet afvallen. Waarschijnlijk is deze stof IAA, daar IAA de remmende werking van de knoppen voor een deel kan vervangen. Uit de plant worden abscissie-bevorderende stoffen naar de abscissiezone gevoerd, waarschijnlijk assimilaten, daar glucose de bevorderende werking van de plant kan vervangen. Uit de vergelijking van de in vivo en in vitro verrichte experimenten blijkt dat de pedicel-abscissie van explantaten de bloemknopabscissie aan de plant redelijk representeert. De verschillen in tendens tot bloemknopval tussen de 3 klonen is gecorreleerd met verschillen in abscissietijd van de pedicellen.

In deel II worden de interacties tussen IAA, ABA en ethyleen dan ook m.b.v. pedicelexplantaten nader onderzocht. IAA, ABA en ethyleen beïnvloeden de abscissie onafhankelijk van elkaar. In het abscissieproces van explantaten kan een IAA-gevoelige periode onderscheiden worden, gevolgd door een ethyleen-gevoelige periode. ABA moet gedurende de IAA-gevoelige periode toegediend worden, het heeft een optimale tijd van toediening maar oefent zijn effect waarschijnlijk gedurende de ethyleen-gevoelige periode uit.

Daar in deel I was gebleken dat de verschillende tendens in knopval van de 3 klonen correspondeert met verschillen in de abscissietijd van pedicelexplantaten, wordt in deel III de anatomie van de abscissiezone gedurende het abscissieproces in de 3 klonen bestudeerd. De scheiding vindt plaats doordat de intacte cellen, beginnend in de cortex, van elkaar loslaten. Dit suggereert, dat de scheiding het gevolg is van de oplossing van de middenlamel. Tevens blijkt dat de door IAA, ABA, of H_2O beïnvloede abscissie anatomisch niet van elkaar verschillen. Ook de 3 klonen vertonen anatomisch geen verschillen.

In deel IV wordt de hormonale regulatie van de bloemknopval geanalyseerd door middel van hormoonbepalingen in pedicellen en knoppen. De knoppen van de B. davisii-klonen bevinden zich continu in een abscissie-inducerende omstandigheid, doordat ethyleen continu in abscissie-bevorderende concentratie in de pedicellen aanwezig is. ABA is in hoge concentraties in de bloemknoppen aanwezig.

Het induceert de abscissie echter niet, omdat de knoppen van de 3 klonen geen significant verschil vertonen in hun ABA gehalte en de pedicellen weinig gevoelig voor ABA zijn.

Het IAA-gehalte in de vrouwelijke knoppen van de eenhuizige B. fuchsioides-hybride is ± 100 maal zo hoog als in de mannelijke knoppen, hetgeen gecorreleerd is met hun respectievelijke knopval. In de B. davisii-hybriden is de correlatie tussen knopval en IAA-gehalte ingewikkelder. In de winter vallen meer bloemknoppen af, gecorreleerd met een lager IAA- gehalte, dan in de zomer. Echter, de bloemknoppen van kloon 3, welke samen met kloon 2 de meeste knoppen laat vallen, bevatten het meeste IAA, terwijl de gehalten in bloemknoppen van kloon 1 en 2 niet veel verschillen. Het blijkt dat de IAA-export uit bloemknoppen en bloemen omgekeerd evenredig is met het IAA-gehalte. Hieruit volgt dat behalve het IAA-gehalte de uitvoer uit de bloemknoppen de abscissie bepaalt. Tevens geven deze resultaten een verklaring voor het feit dat ondanks de in de pedicellen aanwezige abscissiebevorderende ethyleen-concentratie de bloemknoppen niet afvallen, daar in hoofdstuk II is gebleken dat IAA het weefsel ongevoelig voor ethyleen houdt. Ook zijn nog enkele experimenten beschreven betreffende het transport van radio-actief gemerkt IAA, IAA-1-^{14}C, door pedicelsegmenten. De IAA-transport-capaciteit is afhankelijk van de diameter van het pedicel. De snelheid van het IAA-transport bedraagt 4-6 mm/uur en is dezelfde in jonge en oude pedicelsegmenten. ABA noch ethyleen remmen het IAA-transport. De aard van de verschillende tendens in knopval van de 3 klonen is nog niet geheel opgelost. Waarschijnlijk wordt deze veroorzaakt door een complex van factoren: o.a. IAA-produktie en-afgifte door de bloemknop en de omzetting ervan in de bloemsteel.

Tenslotte is in deel V de rol van de eiwitsynthese in het abscissieproces en de aard van de celwandoplossing onderzocht. Eiwitsynthese blijkt nodig voor de abscissie te zijn, daar cyclo-heximide deze zeer effectief remt. Echter, de informatie voor celwandafbrekende enzymen moet reeds in het cytoplasma aanwezig zijn, daar actinomycine D (AMD) de abscissie slechts in geringe

mate remt. De abscissieremming door AMD kan door remming van de energievoorziening veroorzaakt zijn, daar chloorамphenicol en 2, 4-dinitrophenol de abscissie ongeveer gelijkelijk remmen.

De scheiding is gecorreleerd met een stijging van oplosbare pectinen. Echter de pogingen om de enzymen hiervoor aan te tonen waren niet succesvol. Pectinemethylesterase-activiteit is zowel in de intercellulaire ruimte als in de cellen van de abscissiezone aanwezig, terwijl activiteiten van polygalacturonase, pectinezuurtranseliminase en pectinetranseliminase volledig afwezig waren. Cellulase, een enzym dat in het algemeen positief met de abscissie van petiolen en vruchtstelen is gecorreleerd, volgt de abscissie van Begonia pedicellen wanneer deze bijna voltooid is, de afbraak van de primaire celwand volgt dus na het scheiden van de cellen door oplossing van de middenlamel.

CURRUCULUM VITAE

In 1960 behaalde de auteur het diploma HBS-B aan de Gem. Hogere Burgerschool te Hilversum. In datzelfde jaar begon zij aan de studie in de biologie aan de Rijks Universiteit te Utrecht. Op 15 februari 1965 werd het kandidaatsexamen afgelegd, op 8 juli 1968 volgde het doctoraalexamen met de hoofdvakken plantenfysiologie en paleobotanie en het bijvak biofysische chemie. Van 1 december 1968 tot 1 december 1973 was zij in dienst van de Landbouwhogeschool, verbonden aan de Afdeling Plantenfysiologie als wetenschappelijk medewerker, van juli 1972 af als wetenschappelijk medewerker eerste klasse.