G. Dijst

The effect of chemical haulm destruction and haulm pulling on potato black scurf caused by *Rhizoctonia solani* AG-3

Promotor : dr ir J. Dekker, hoogleraar in de fytopathologie

Co-promotor: dr ir L.J. Turkensteen, wetenschappelijk onderzoeker IPO

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op dinsdag 7 februari 1989
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

WOORD VOORAF

Dit proefschrift bespreekt de resultaten van onderzoek dat van juni 1981 tot eind 1985 werd uitgevoerd op het Instituut voor Plantenziektenkundig Onderzoek (IPO) te Wageningen, naar aanleiding van de problemen met lakschurft in de pootaardappelteelt.

Al enige jaren voor de aanvang van dit project was geconstateerd dat er meer fundamentele kennis nodig was om tot een betere beheersing van lakschurft te kunnen komen. Het gewenste onderzoek kwam tot stand dankzij de coördinatie voor aardappelonderzoek van de Directie Landbouwkundig Onderzoek (DLO) en het IPO. Het project werd geformuleerd door dr ir L.J. Turkensteen (IPO). Samen met dr ir D.E. van der Zaag (DLO) en dr ir A. Tempel (IPO) droeg hij in belangrijke mate bij tot de start en de continuering van het project.

Het onderzoek kon beginnen dankzij een subsidie van het fonds MPW (Maatschappelijke Plaats Wageningse Afgestudeerden). Al snel werden positieve resultaten verkregen en besloot het bedrijfsleven financieel bij te springen. Na enig pro-deo werk en een jaar steun via de regeling Tijdelijke Arbeids Plaats van het Ministerie van Sociale zaken werden de laatste drie jaren mede-gefinancierd door het aardappelbedrijfsleven. Alle anonieme donateurs ben ik bijzonder veel dank verschuldigd voor hun krediet. Het MPW fonds is in haar doelstelling geslaagd om in een periode van grote werkeloosheid een afgestudeerde aan werk te helpen. Haar initiatief heeft vijf goede jaren onderzoek opgeleverd.

De spontaan aangeboden begeleiding van drs G. Jager (IB, Haren), ir A. Mulder en ing. J. Roosjen (Hilbrands Laboratorium voor Bodemziekten, Assen) was van grote waarde in de onzekere startperiode. Zij troffen de voorbereidingen voor veldproeven, die samen met het IMAG en later ook het PAGV werden uitgevoerd. In de laatste drie jaren werd verantwoording afgelegd aan een begeleidingscommissie die bestond uit dr ir P. van Halteren (PD), mw drs K. Hartmans (IBVL), dr ir A. Tempel, dr ir L.J. Turkensteen en dr ir D.E. van der Zaag (voorzitter). Ik ben hen dankbaar voor de doelgerichte wijze waarop dat gebeurde en de vrijheid die zij mij lieten bij het onderzoek.

In het voorlaatste jaar spoorde prof. dr J. Dekker mij aan de resultaten te bundelen tot een proefschrift. Ik ben hem dankbaar voor de geboden gelegenheid en voor zijn hulp bij het schrijven van deze dissertatie. De belangeloze inzet van dr ir M. Gerlagh en de vele anderen die delen van dit proefschrift inhoudelijk en taalkundig becommentarieerden heb ik bijzonder gewaardeerd. Daarbij ben ik vooral veel dank verschuldigd aan de redactie en referees van het Netherlands Journal of Plant Pathology die zo nauwgezet de verschillende hoofdstukken bekritiseerden. De directies van het IPO en het Proefstation voor de Akkerbouw en Groenteteelt in de Vollegrond (PAGV) moet ik bedanken voor de tijdsruimte die zij mij gaven om te schrijven tijdens mijn detachering op het PAGV.

Tijdens het onderzoek heb ik veel gehad aan de bijeenkomsten van de Rhizoctonia-werkgroep, onderdeel van de NRLO contactgroep 'bodempathogenen en bodemmicroörganismen'. Ook kijk ik dankbaar terug op de plezierige discussies met mw drs K. Hartmans; bij haar kon ik op elk

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STELLINGEN

1. Pas na loofdoding stimuleert de aardappelknol de vorming van lakschurft ongeremd. *Dit proefschrift*

2. Jonge aardappelknollen kunnen waarschijnlijk beter beschermd worden tegen virusinfectie en overmatige lakschurftvorming door een werkwijze, die de stolonen breekt en daarna de knollen gescheiden van de overige plantedelen in losse grond laat afharden dan door de huidige methoden van loofvernietiging.
Dit proefschrift

3. Indien bij een pootgoedteelt looftrekken onmogelijk is, kan bij gebrek aan beter, geadviseerd worden het loof chemisch te vernietigen met gelijktijdig doorsnijden van de wortels.
Dit proefschrift

4. *Rhizoctonia solani* lijkt volledig toegerust voor haar instandhouding en verspreiding. Het toch voorkomen van de perfecte vorm *Thanatephorus cucumeris*, de homothalli en groepsgewijze anastomose ondersteunen echter de gedachten van P.H. Gregory (1984, blz.14):
,,Besides their function as colonizers spores may perhaps act as an unreliable airmail service transmitting genes between established mycelia. ..... In most species the prevention of vegetative out-fusion must somehow be reconciled with the usual requirements for outbreeding."
Gregory, P.H., 1984. In: Jennings, D.H. & Rayner, A.D.M. (Eds), 1984. The ecology and physiology of the fungal mycelium. Cambridge University Press, London etc., pp. 564.

5. Het is niet uitgesloten dat de initiatie van de infectiekussentjes en van de (pseudo-)sclerotiën door *R. solani* in wezen berusten op overeenkomstige processen.

6. De resultaten van Pressey sluiten niet uit dat het polygalacturonase PG-1 van nature aanwezig is in rijpende tomaten.

Pressey, R., 1988. Reevaluation of the changes in polygalacturonases in tomatoes during ripening. Planta 174: 39-43.

7. Vanuit een fysiologisch oogpunt is het de moeite waard om na te gaan of besmetting met een niet-pathogeen organisme als VAM (vesiculaire arbusculaire mycorrhiza veroorzakende schimmel) een aspecifieke tolerantie induceert in plantgoed tegen de nadelige gevolgen van biotische en abiotische bodemfactoren in het veld.

8. Het verdient aanbeveling om ook potgrond op de markt te brengen die vrij is gemaakt van ziektekiemen; de meerprijs zou via een kwaliteits-certificaat gecompenseerd kunnen worden.

9. Voor de bestudering van planteziekten als gevolg van bodempathogenen is het zinvol om de term 'bodem-ontvankelijkheid' of 'bodem-receptiviteit' in te voeren om weer te geven in welke mate een bodem een dergelijke ziekte toelaat op grond van zijn intrinsieke eigenschappen (bv. % afslibbaar) en zijn eigenschappen die afhankelijk zijn van externe invloeden (bv. bodemstructuur of bodemmicroflora).

10. Bij de planteveredeling op weerbaarheid tegen bodempathogenen die zoals *R. solani* ook als zwakteparasiet kunnen optreden, moeten omgevingsfactoren zoals de bodemreceptiviteit voor die ziekte mede beschouwd worden als variabelen bij de verklaring van resultaten. Hetzelfde geldt voor onderzoek naar andere niet-chemische beheersmethoden tegen die pathogenen.

11. Het promoveren op artikelen stelt, gezien het nauwgezet corrigeren door de referees, zwaardere eisen aan de vindingrijkheid van de opponenten bij het verzinnen van niet-inhoudelijke vragen.

12. Het ontbreken van (werk-)colleges 'agrarische ethiek' wekt ten onrechte de indruk dat ethische problemen in deze sector ontbreken. De snel wisselende belangen van de landbouweconomie zijn vaak discutabel en tegenstrijdig aan die van de natuur en het milieu. Voorts kan zo'n leerstoel bijdragen tot het creëren van procedures welke de integriteit waarborgen van geprivatiseerde diensten.

13. Wie allang *'Gewasbescherming'* in zijn *dossier* heeft, zou zich zorgen kunnen maken over de bescherming van *'Gewasbescherming'*.

14. Een regering, die emancipatie beoogt en het gezin als hoeksteen van de samenleving wenst te behouden
    1. dient te zorgen dat kinderopvang een basisvoorziening wordt
    2. dient voorwaarden te scheppen opdat zowel in overheidsdiensten als in het particuliere bedrijfsleven een werknemer, indien die dat wenst, zijn functie tot vier jaar in verzekerde bewaring kan geven om tijdelijk een taak te vervullen die de kwaliteit van de samenleving ten goede komt, bv. ouderschap of een politiek ambt.

15. De ontwikkeling van het 'Agrobusiness Park Wageningen' is even afhankelijk van goede woonvoorzieningen als van wetenschappelijke initiatieven.

16. Het plaatsen van nieuws over Suriname op de 'binnenland' pagina van NRC-Handelsblad is wellicht een freudiaanse verdrukking.
    (zie bv. NRC-Handelsblad 29-07-88)

Proefschrift Gerda Dijst.
The effect of chemical haulm destruction and haulm pulling
on potato black scurf caused by *Rhizoctonia solani* AG-3.
Wageningen, 7 februari 1989.

moment terecht voor advies, bemiddeling en hulp voor chemische analyses. Voor advies en het beschikbaar stellen van apparatuur ben ik veel dank verschuldigd aan dr L.W. van Broekhoven (CABO), dr ir L.C. Davidse (LUW, Fytopathologie), ir L.G. Tuinstra en ing. G. Traag (RIKILT) en ir E. Woltering (Sprenger Instituut). Op het gebied van de statistiek en computergebruik heb ik veel geleerd van drs J. de Bree (GLW, DLO). Ik ben hem dankbaar voor het vele werk dat hij mij uit handen nam bij de verwerking van de veldresultaten.

Veel dank ben ik verschuldigd aan de inschikkelijke Ipo-nezen die mij lieten delen in het gebruik van hun werkruimte en materialen.

De dagelijkse gang van zaken werd mij bij tijden bijzonder veraangenaamd door hulp en gezelligheid van stagiairs. Ing. R. van der Schaaf (Ruud) en mw ing. J. Borsboom (Jolanda) hielpen met verve bij de veldproeven. Ing. G. Wever (Gerrit) heeft in een heel plezierige samenwerking en met eindeloos geduld het drooggewicht bepaald van heel veel priegelige sclerotiën. Ir J.M. van der Wolf (Jan) ontpopte zich als een uiterst precies en fanatiek periderm-cel-teller, waarmee hij een lastige klus klaarde. Ing. H. Schroën (Har) hielp bij de opzet van de eerste experimenten met vluchtige componenten. En uiteindelijk heeft ir A. van Dalen (Ton) zeer zelfstandig de experimenten met vluchtige exudaten voltooid. Ik was erg gelukkig met de inbreng van ing. J. Verheggen (Jos), die tijdelijk assisteerde als pro-deo medewerker. Zijn engelengeduld, opgewektheid en gemotiveerde inzet waren een plezier en stimulans voor mij en de toen aanwezige stageairs.

Het onderzoek vergde erg veel kasruimte onder tamelijk strenge klimatologische condities. Dankzij het miraculeuze organisatievermogen van J.H. den Dunnen, was er toch altijd weer voldoende ruimte. Zelfs in hoogtij periodes wist hij nog handen vrij te maken om te helpen bij het inzetten van planten of waarnemen in het veld. Met name de heren H. Floor, M.R. Holdinga, R.J. Jansen, G.H. Kramp en G.M. Willemsen hebben vaak geassisteerd, waarvoor ik ze bijzonder wil bedanken. Bij het opzetten van nieuwe experimenten kon ik steeds een beroep doen op de uitgebreide kennis en vindingrijkheid van B. van Rheenen. De huishoudelijke dienst o.l.v. B. van Holland werkte bergen vuil glaswerk weg en voorzag in de continue behoefte aan agarmedia. Mw. T.W. Jansen wil ik bedanken voor de vriendelijke wijze waarop ze, toen nog per kamer serverend, ons van koffie en thee voorzag.

Huisgenoten en vrienden boden me buiten werktijd een onmisbaar en fijn tegenwicht tegenover het arbeidsintensieve onderzoek. Mijn echtgenoot ir S.H.W.M. Mannaerts (Bas) wil ik bedanken voor zijn geduld, gewillig oor en zijn kritiek met betrekking tot dit werk. Met dochtertje Esther was er thuis ruim voldoende plezier en afleiding tijdens de laatste loodjes van het schrijven. Last but not least wil ik mw ir C.C. Florschütz (Christa), medewerkster van de Stichting Landbouw en Milieu, en dr G.O.M. Leone (Gionata), mycoloog in tijdelijke dienst van het IPO, bedanken voor hun bereidheid om als paranymf op te treden.

The investigations were carried out from 1981 to 1986 at the Research Institute for Plant Protection at Wageningen. The project has been financially supported by the Foundation MPW (Maatschappelijke Plaats Wageningse Afgestudeerden) and by the Board of the Netherlands Seed Potato Growers and Traders Organization (PCC).

CONTENTS

1 INTRODUCTION

In the Netherlands, haulm destruction is a mandatory measure when growing seed potatoes. The shoots are killed in order to prevent virus infection of the tubers. However, after haulm destruction, black scurf formation on the tubers often accelerates. High levels of black scurf lead to losses at grading. The rate of black scurf formation is influenced by the method of haulm killing: haulm pulling enhances black scurf formation less than chemical destruction. The question arose which factors promote black scurf after haulm killing and why the two methods available differ in effect.

Black scurf is caused by *Rhizoctonia solani* anastomosis group 3 (AG-3). This fungus forms pseudo-sclerotia on the surface of young growing potato tubers. These resting structures appear as small black crusts attached to the tuber skin and are commonly called 'sclerotia' or 'black scurf'. During the early phases of tuber growth the number of these sclerotia remains low and their size small, but during tuber maturation both number and size increase. The sclerotia do not cause direct harm to the tuber itself, but on seed tubers they will endanger the future crop: mycelium will grow from the sclerotia and infect the young shoots. This infection leads to retarded plant growth and reduced yield. In order to prevent such infection, seed tubers are chemically disinfected. The pesticides available, however, cannot completely kill sclerotia larger than 2 mm in diameter and so tubers covered by sclerotia of that size must be graded out. In the Netherlands, the norm for the maximum amount of black scurf allowed on seed potatoes is inspected by the Dutch Seed Inspection Service (NAK). Seed potatoes are a major export commodity of the Netherlands. Grading losses due to black scurf amount to considerable financial losses to the grower.

Haulm killing prevents transmission of virus from the shoots to the tubers. The destruction may be achieved in two ways, chemically or mechanically. The chemicals used in seed potato production quickly destroy the shoots within three days. For complete coverage of the shoots by the chemicals sprayed, they are reduced in advance by pulverizing or stripping the foliage. A second spray is often required to kill newly formed shoots. Haulms are destroyed mechanically by 'haulm pulling': this method is not easy to apply but it breaks the stolons and removes most of the plant in one performance. On the mandatory date of destruction the tuber skin is usually not firm enough to withstand mechanical harvesting. In general, tubers are not collected from the field until a few weeks after haulm killing when the skin is 'set', i.e. the periderm is suberized.

To early harvested tubers, it has been observed in practice that haulm destruction aggravates black scurf: number and size of sclerotia increase more rapidly than if this measure is not employed. The stimulation of black scurf formation becomes evident during the second week after destruction. Chemical destruction enhances black scurf formation more than haulm pulling (Reestman en Schepers, 1955; Bouwman et al., 1983). Haulm pulling is the recommended measure but not under all conditions, e.g. if wet weather prevents the use of heavy machinery or if the crop is infested by *Phytophthora infestans*. However, the alternative - rapidly killing chemicals - usually results in higher grading losses due to black scurf.

The Dutch seed potato growers are constantly seeking ways to improve the quality of their products. *R. solani* AG-3 is a very common soil inhabitant and it is not likely that there are potato fields void of it. In view of environmental risks associated with the application of chemicals in

agriculture, non-chemical or integrated control measures are prefered. In seeking alternatives for black scurf control it was decided to investigate which conditions promote sclerotium formation on young tubers, and which factors cause the stimulation of black scurf after haulm killing. Spencer and Fox (1978) suggested that tuber exudates promote sclerotium formation. Because exudates might differ between cultivars, it was hoped that this study would also provide information that could be used in breeding new potato cultivars.

This thesis presents the results of an investigation into the conditions which promote black scurf after haulm destruction. Especially those factors were investigated which cause the stronger stimulation after chemical haulm killing compared to mechanical 'haulm pulling'. Different methods of haulm killing may induce different alterations in tuber exudate and periderm. Because such changes depend on the physiological state of the tuber, most of the experiments were carried out with tubers still attached to the plants. Sclerotia are considered to be mature when they are black-brown in colour and have a compact structure which makes them resistant to changes in the environment. Black scurf formation consists of three stages (sclerotial initiation, growth and maturation) (Townsend, 1957) each of which can probably be affected differently by external factors.

The thesis contains five papers. The first two treat the effect on black scurf development of the different methods of haulm killing and of root severing using different cultivars. The first paper presents results from glasshouse experiments using steamed soil or soil-less growth medium. The second describes field experiments on black scurf, skin set, flexibility of the harvest period and yield of young tubers. The following three papers present laboratory and glasshouse tests in which the alterations on tuber surface after haulm destruction were studied and their effect on sclerotium formation. The third paper describes the different stages of sclerotium formation and the distribution pattern of the sclerotia on artificial media and on tubers with regard to food supply, wounding and surface structure. In the fourth paper sclerotium production is studied on agar plates supplemented with periderm strips and precipitated water-soluble tuber exudates, sampled at different intervals after haulm killing. The fifth paper describes sclerotium formation on agar plates exposed to volatile tuber exudates and volatile decomposition products of roots and stolons from plants with intact or cut off shoots.

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Investigations on the effect of haulm destruction and additional root cutting on black scurf on potato tubers

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Abstract

In greenhouse experiments factors which are involved in the stimulation of sclerotia formation by *Rhizoctonia solani* Kühn on potato tubers after haulm destruction were investigated. Cutting off the shoot stimulated the production of sclerotia as much as chemical haulm destruction. This was also observed when potato tubers were grown away from the roots in a separate compartment filled with steamed perlite. Fewer sclerotia were produced when roots were cut in addition to haulm destruction compared to haulm destruction alone. Cutting off the roots alone often stimulated sclerotia production. The data indicate that sclerotia production was directly affected by the tuber and probably due to physiological changes in the tuber caused by elimination of the shoot.

Additional keywords: Rhizoctonia solani, sclerotia, *Solanum tuberosum.*

Introduction

During the cropping season the amount of sclerotia produced by *Rhizoctonia solani* Kühn on the surface of potato tubers increases gradually. For sanitary reasons haulm destruction is a mandatory measure when growing seed potatoes in the Netherlands. However, haulm destruction stimulates the production of sclerotia on tubers (Doornbos, 1963; Spencer and Fox, 1978), chemical destruction being more stimulating than a mechanical destruction, the so-called 'plant-pulling' (Doornbos, 1963; Bouman et al., 1979, 1983). Plant-pulling cannot be applied under all circumstances and if so, growers have to apply quickly killing chemicals, resulting in considerable grading losses due to black scurf.

Chemical destruction with DNOC kills the shoot in three days, whereas plant-pulling breaks the stolon. The extra stimulation of the sclerotium production by chemical destruction compared to that following plant-pulling thus may be caused by the continued contact between tubers and roots in the former case. The objectives of this study were (1) to compare the effect of different methods of haulm destruction in the greenhouse, trying to compare different cultivars, (2) to investigate whether this stimulation of sclerotium production is caused directly by physiological changes of the tuber or by alterations in the vicinity of the tuber, (3) to see if tuber water content affects black scurf development and (4) to examine the effect of root cutting in addition to chemical haulm destruction on the development of black scurf. A preliminary report has been published (Dijst, 1983).

Materials and methods

Potato plants were grown from rooted stem cuttings or from meristem cultures. Plants were grown in growth chambers under 16 h light per day and 18-20 °C (day) or 14-16 °C (night) for three weeks. Then they were transplanted into 150-mm pots and placed in the greenhouse where additional light was given up to 12 h per day. All soils used had been sterilized by steaming. If not mentioned otherwise the soil was inoculated with sclerotized wheat grains when the plants were two months old. A mixture of three isolates of *R. solani* was used as inoculum. These isolates had been kindly provided by Drs G. Jager (Intitute for Soil Fertility, Haren). According to an identification by Drs W.M. Loerakker (Plantenziektenkundige Dienst, Wageningen) they all belong to anastomosis group nr. 3.

The shoots were destroyed chemically by spraying with DNOC at 0.5 ml a.i. DNOC in oil (Aal omort) or metoxuron as 80% w.p. (Purivel) at 55 mg a.i. per plant.

The amount of sclerotia on the tubers was estimated visually using five classes: free, very lightly, lightly, moderately and heavily covered with sclerotia (Fig. 1). By counting the number of tubers in each class an index was calculated with the formula:

$I = 100.(0.f + 1.vl + 2.l + 3.m + 4.h)/(4.t)$

Where f, vl, l, m and h refer to the number of tubers in each of the severity classes and t is the total number of tubers.

The number of sclerotia per tuber (NSCL) was counted and the sclerotia were then collected and dried at 105 °C for 24 h. Subsequently they were weighed with an accuracy up to 0.0001 mg. In order to compare the amount of sclerotia among tubers of different sizes both number and dry weight were expressed per surface area (A in mm^2) of the tuber (NSCL/A and WSCL/A). The surface area of tubers was estimated using the fresh weight of the tuber raised to the 2/3 power. This approach is based on the assumption that the surface area of a tuber can be estimated as the surface area of a sphere with a constant specific density (sd). From the formulae of the volume of a sphere with radius r ($W/sd = 4.\pi.r^3/3$) and its surface area ($A = 4.\pi.r^2$) it is derived, that $A = a+c.(W)^{2/3}$, where W is the fresh weight and a and c are constants which can be ignored here since the formula is used for comparison only.

The dry weight of the sclerotia per surface area of the tuber (WSCL/A) is more ac-

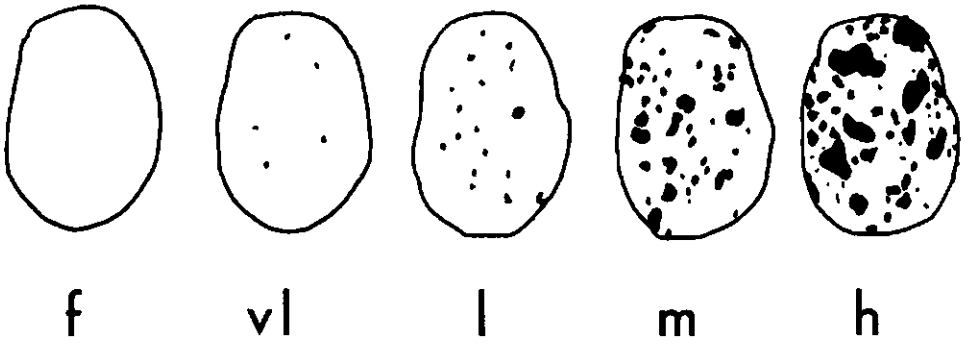

Fig. 1. Assessment key for the amount of sclerotia of *R. solani* on potato tubers: f = free, vl = very lightly, l = lightly, m = moderately and h = heavily covered with sclerotia.

curate to valuate the production of sclerotia than the black scurf index. The number of sclerotia per tuber surface area (NSCL/A) does not give information on the number of initiation points, since many small sclerotia eventually grow together to form big sclerotia. The mean dry weight of a sclerotium (SCLW) on a tuber was calculated from the total dry weight and number of sclerotia per tuber.

The water content of the tubers was calculated from the fresh (fw) and dry weights (dw): 100.(fw-dw)/fw. Dry weights were determined after tubers had been sliced and dried at 95 °C for 24 h followed by 105 °C for 24 h.

Results

Comparison of different methods of haulm destruction. Plants of cv. Astarte were grown from meristem cultures. Ten days after inoculation of the soil, plants were given different treatments. Shoots were cut off or destroyed quickly with DNOC or slowly with metoxuron. Metoxuron cannot be used for seed potatoes since it takes two weeks for complete destruction. Three weeks after plants had been treated, tubers from nine plants per treatment were harvested and the amount of sclerotia was estimated. In Table 1 the black scurf index per treatment is given. The index was calculated over all tubers harvested per treatment. Cutting off the shoots enhanced the production of sclerotia as much as chemical destruction did. Application of DNOC seemed to enhance black scurf somewhat more than that of metoxuron. Plant-pulling did not enhance sclerotia production.

The effect of cutting off the shoot. Plants were grown using the 'plaster-system' according to Van Emden (1958). So half of each pot was filled with potting soil which was covered by a layer of 10 mm plaster with a 20 mm hole in the middle. Plants of cvs Bintje and Prominent were grown from cuttings. Roots of three-week-old plants were washed free from soil and placed in the soil through the hole in the plaster. The original cut surface was held 10 mm above the plaster, the hole was filled with bee wax and the upper half of the pot was filled with steamed sand. Thus any influence from soil or roots on the vicinity of the tuber was eliminated. One week after infestation chemical haulm destruction was simulated by cutting off the shoot and plant-pulling by cutting through the stolon. Twelve and 21 days after treatment tubers of at least five plants per treatment were examined. Only cutting off shoots had stimulated sclerotial development (Table 2, Fig. 2).

Table 1. The amount of sclerotia on tubers from nine plants cv. Astarte per treatment, three weeks after chemical or mechanical haulm destruction.

Treatment	Black scurf index
None (control)	38.9
Plant-pulling	38.2
Slow chemical haulm destruction (metoxuron)	50.3
Quick chemical haulm destruction (DNOC)	62.5
Cutting off shoots	54.2

Table 2. The amount of sclerotia on tubers from plants of cvs Bintje and Prominent three weeks after shoots were cut off or stolons were cut through.

Treatment	cv. Bintje	cv. Prominent
None (control)	10[1] b[2]	0[1] b[2]
Cutting off shoots	1229 a	437 a
Cutting through stolons	–	0 b

[1] Dry weight sclerotia (μg) per tuber surface area (ca. 100 mm^2).
[2] Values followed by different characters are significantly different (P = 0.05). Cv. Bintje LSD = 751; cv. Prominent LSD = 361.

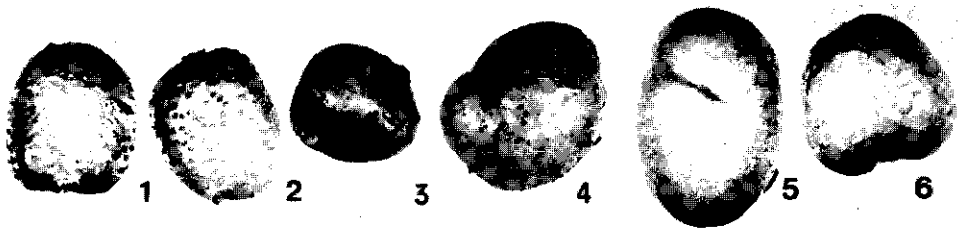

Fig. 2. Sclerotia of *Rhizoctonia solani* on potato tubers (cv. Prominent) harvested three weeks after treatment: tubers nrs. 1 and 2 from untreated plants, nrs. 3 and 4 from plants of which shoots had been cut off and nrs. 5 and 6 from plants of which stolons had been cut through.

Investigations on the effect of haulm removal. Plants of cv. Bintje were grown from meristem cultures. They were raised in a two -compartment system which allowed the roots to develop in 10 liter steamed perlite with nutrient solution (Steiner, 1968) and the tubers in an upper compartment filled with steamed perlite, humidified with demineralized water (Fig. 3). Thus any influence of biotic or abiotic components from soil or roots, on the vicinity of the tuber was eliminated. When the plants were 100 days old the perlite around the tubers was removed with a vacuum cleaner. The tubers were inoculated by placing three-day-old hyphal mats of *R. solani* on water agar on the tubers. Afterwards the tubers were covered again with humid perlite. One week later the shoots were cut off from five out of ten plants. Again one week later tubers were harvested and examined.

On tubers from untreated plants few hyphae and no sclerotia were found. Shoot removal had caused extensive hyphal growth and sclerotia had been produced moderately on the tubers and adhering perlite granules. Thus, the stimulation of sclerotia formation originated from the tuber. Roots did not turn brown until seven days after shoot removal.

The effect of root-cutting in addition to haulm destruction. In order to compare different cultivars, plants of cvs Astarte, Désiré, Doré, Ehud, Krostar, Marijke and Prominent were grown from meristem cultures in a sandy loam soil. Three weeks after soil inoculation plants were given the following treatments: root-cutting, chemical haulm destruction using DNOC, root-cutting plus spraying with DNOC, plants were pulled

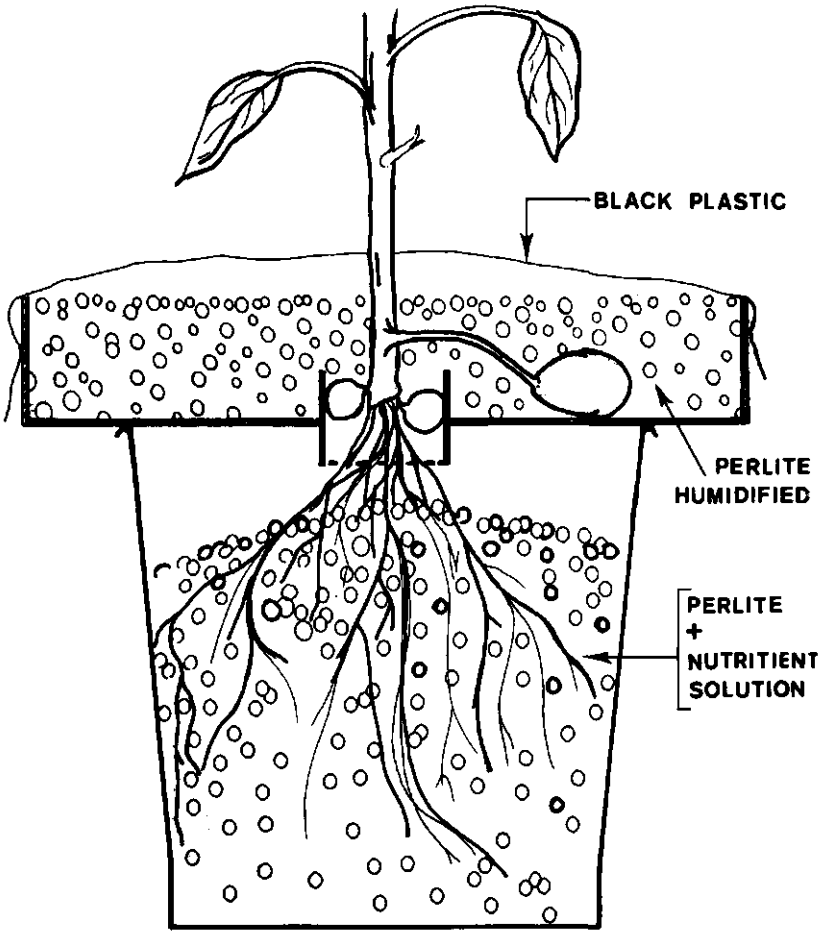

Fig. 3. The two-compartment system used for development of tubers under nutrient-free conditions.

or shoots were cut off. For root-cutting the roots were pruned by hand. At least three plants per treatment were harvested both one and three weeks after treatment. Untreated plants that had died at least one week before harvest were sampled as a separate group ('early dead control plants'). The small number of replicates per cultivar only allowed for an analysis of variance over the combined data of all cultivars tested per treatment (Table 3).

Cutting the roots alone slightly enhanced black scurf on five out of seven cultivars tested (Table 4). Root-cutting plus spraying DNOC resulted in a lower amount of sclerotia (WSCL/A) compared to spraying DNOC alone on five out of seven cultivars tested. The effect of root-cutting plus DNOC on black scurf had been almost equal to that obtained by plant-pulling. Metoxuron spraying had stimulated sclerotia production slightly less than DNOC. Tubers from 'early dead control plants' had more sclerotia than tubers from any other treatment.

Table 3. The average amount of sclerotia on potato tubers of seven cultivars three weeks after haulm destruction with or without additional root-cutting.

Treatment	Black scurf index	WSCL/A[1]	NSCL/A[2]	SCLW[3]	Water content tubers[4]
None (control)	45 bc[5]	24 f	209 a	0.12 a	77.2 d
Root-cutting	43 c	36 e	174 a	0.21 a	80.0 c
DNOC spray	57 ab	56 a	219 a	0.26 a	80.8 bc
DNOC spray + root-cutting	49 abc	40 d	209 a	0.19 a	81.9 ab
Plant-pulling	51 abc	42 c	245 a	0.17 a	83.0 a
Cutting off shoots	63 a	53 b	297 a	0.18 a	83.6 a
Significance	*	*	NS	NS	***
LSD (P = 0.05)	13.4	1.5			1.87

[1] Dry weight (μg) of sclerotia per tuber surface area (ca. 100 mm^2).
[2] Number of sclerotia per tuber surface area.
[3] Dry weight (μg) per sclerotium.
[4] Expressed as percentage of the fresh weight.
[5] Values followed by different characters in each row are significantly different (P = 0.05) according to a one-way analysis of variance with n = 21 (3 plants per cultivars) except for the analysis of the index of black scurf where n = 7 (7 cultivars) was used with n being the number of replicates per treatment.

A comparison of the data in Tables 4 and 5 learns that a high amount of sclerotia on the tubers was not always associated with a high water content. Like in the former experiment roots did not turn brown until seven days after haulm destruction. Root-cutting must have terminated the uptake of water by the existing roots. It often nihillized part of the stimulation of black scurf by haulm destruction which often coincided with a lower tuber water content. Therefore, it was to be investigated (1) whether the water content of the tuber was related to the amount of sclerotia and (2) whether these aspects differed between cultivars. A more elaborate analysis of variance showed that the treatments differed significantly with respect to both WSCL/A and the water content of the tubers. Differences between cultivars were not significant for these aspects. Changes in the amount of sclerotia were not consistently correlated with changes in tuber water content. After correction for the treatments the effect of the tuber water content was not significant with respect to the WSCL/A. However, ignoring treatments, the effect of the tuber water content was significant with respect to the WSCL/A. Thus, both differences in tuber water content and WSCL/A have originated from differences between treatments and not from differences between cultivars. Differences in tuber water content could have been only partly responsible for the differences in WSCL/A, indicating, that there are other consequences of the treatments, which affect the production of sclerotia on the skin.

Root-cutting in addition to DNOC spray had failed to reduce the stimulation by haulm destruction with the cvs Prominent and Marijke. Since this may have been due to the low number of replicates the experiment was repeated with the cultivars Promi-

Table 4. Dry weight (μg) of sclerotia per surface area (ca. 100 mm^2) of potato tubers (WSCL/A) of seven cultivars tested three weeks after haulm destruction with or without additional root-cutting.

Treatment	Astarte	Désirée	Doré	Ehud	Krostar	Marijke	Prominent
None (control)	18	22	20	25	30	43	8
Root-cutting	5	28	13	40	33	70	65
DNOC spray	37	55	25	83	63	57	70
DNOC spray + root-cutting	10	25	17	58	35	55	80
Plant-pulling	33	57	27	33	67	47	30
Cutting off shoots	23	77	30	83	57	83	25
Metoxuron spray	20	–	15	–	–	33	–
Early dead control plant	–	70	167	130	–	102	–

Table 5. The water content of potato tubers (expressed as % of the fresh weight) of seven cultivars tested three weeks after haulm destruction with or without additional root-cutting.

Treatment	Astarte	Désirée	Doré	Ehud	Krostar	Marijke	Prominent
None (control)	74.6	77.2	82.0	77.5	72.0	75.2	81.8
Root-cutting	73.6	82.8	78.9	78.0	78.1	82.8	86.0
DNOC spray	83.2	82.1	79.4	79.1	81.3	79.5	81.2
DNOC spray + root-cutting	80.0	84.5	79.5	81.1	81.2	82.2	84.9
Plant-pulling	85.5	87.3	81.5	79.1	84.3	81.1	82.5
Cutting off shoots	86.5	84.8	81.9	81.9	84.2	80.7	85.3
Metoxuron spray	80.4	–	77.6	–	–	79.9	–
Early dead control plants	–	82.3	83.0	85.2	–	80.8	–

Table 6. The amount of sclerotia on tubers of cultivars Prominent and Pimpernel two weeks after shoots had been cut off, shoots plus roots had been cut off or stolons had been cut through.

Treatment	WSCL/A[1]		SCLW[2]	
	Prominent	Pimpernel	Prominent	Pimpernel
None (control)	0 b[3]	0 b[3]	0	0
Cutting off shoots	332 a	36 a	341	539
Cutting off shoots + root-cutting	0 b	0 b	0	0
Cutting through stolons	16 b	0 b	337	0

[1] Dry weight sclerotia (μg) per surface area (ca. 100 mm^2).
[2] Dry weight (μg) per sclerotium.
[3] Values followed by different characters in each row are significantly different; cv. Prominent LSD (P = 0.001) = 251; cv. Pimpernel LSD (P = 0.001) = 33.

nent and Pimpernel. The set-up of Van Emden (1958) was modified using a sheet of plastic instead of plaster. Ten days after inoculation of the sand plants were given different treatments. The roots were cut through just underneath the plastic sheet. Fourteen days later, tubers of at least four plants per treatment were sampled. The results are summarized in Table 6. Now root-cutting in addition to haulm destruction had resulted in an amount of black scurf equal to that after breaking the stolon which was much lower than after haulm destruction alone.

Discussion

Greenhouse experiments confirmed the field observations in that haulm destruction of potato plants stimulates sclerotia production on tubers. In accordance with field observations, in the greenhouse experiments chemical haulm destruction stimulated sclerotia production to a greater extent than plant-pulling. Breaking the stolon as well as plant-pulling hardly affected sclerotia production. Cutting off the shoot enhanced the stimulation similarly as chemical haulm destruction. It was demonstrated here that stimulation of sclerotia production after haulm destruction was caused directly by the tuber.

The questions are raised as to what causes the stimulation of black scurf and whether it can be prevented. Plant-pulling and haulm elimination (chemical haulm destruction or cutting off the shoot) are similar in that the contact between the living shoot and the tuber is broken. The more quickly this happens the more quickly the tuber may mature. Spencer and Fox (1978, 1979) suggested that tuber maturation may play a role. In my experiments differences in type of cultivars and thus natural differences in stage of maturity were unlikely to be responsible for any differences in amount of sclerotia on tubers. However, the amount of black scurf per cultivar was found to be more severe on tubers from untreated plants that had died prematurely compared to green untreated plants. Also slightly more black scurf was found using the quickly killing DNOC compared to the slowly killing metoxuron. The primary cause of stimulation of black scurf is therefore thought to originate from the loss of contact between shoot and tuber and the subsequent acceleration of the rate of tuber maturation.

Haulm elimination stimulates black scurf development more than plant-pulling, indicating that additional factors are involved there. Roots did not appear to die before seven days after haulm elimination. Continued uptake of water after haulm elimination may put the tubers under stress. The extra development of sclerotia was less severe when roots had been cut in addition to haulm elimination. Therefore, this extra stimulation of black scurf is thought to originate from the remaining contact between tuber and roots which may put the tuber under stress. Another explanation for the different results of haulm elimination and plant-pulling may be found in the disturbance of the soil that goes along with the latter.

Pruning roots alone often stimulated the production of sclerotia. The sudden termination of their supply of water and nutrients may have put the tubers under stress and accelerated tuber maturation.

Cutting roots in addition to chemical haulm destruction resulted in an amount of sclerotia equal to that after plant-pulling which was lower in most cases than after DNOC spray alone. The smaller size of the sclerotia after additional root-cutting was

a second benefit which also permitted these tubers to be classified lower for black scurf than after DNOC spray alone. However, this effect of additional root-cutting was not always achieved. This may be due to differences in the number of very shallow roots. Root growth on stolons and tubers seem to depend on soil moisture. These roots also supply water to the tubers and thus may nullify any effect of root cutting. Each treatment influenced both the amount of sclerotia and the water content of the tubers regardless the cultivar tested. Since differences in water content of the tuber could have been only partly responsible for the differences in the amount of sclerotia other consequences of the treatment must be affecting the production of sclerotia. Along with root cutting goes a disturbance of the soil which may influence sclerotia production independently. Many authors noticed an effect of moisture content and aeration on the amount of sclerotia produced (Blair, 1943; Parmeter, 1970). Soil disturbance may have been different between cultivars since their roots were not equally easy to prune. Thus the effect of soil disturbance and the effect of root cutting in addition to haulm elimination in the field warrents additional study.

Sclerotia formation cannot be explained as a part of a starvation process (Allington, 1936). Sclerotia are produced most readily by conditions favouring mycelial growth. Their formation on agar media is readily initiated, but optimal supply of water and nutrients are needed for them to grow and mature (Townsend, 1957). In this study haulm destruction stimulated growth rather than initiation of sclerotia since their number did not increase visibly.

It is recognized that the surface of plant tissue can influence the development of *R. solani* both mechanically and biochemically (Dodman and Flentje, 1970). It is demonstrated here that the amount of sclerotia produced on tubers was directly influenced by factors from the tuber. These factors may be components which are thought to become available to the fungus at the tuber surface since growth in the periderm seems to be limited. Quantitative or qualitative changes in components available at the tuber surface cause the increase in the amount of sclerotia after haulm destruction. These components may be present in the periderm or may be volatiles or aquaeous tuber exudates.

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Samenvatting

Onderzoek naar de oorzaak van de invloed van loofvernietiging en wortelsnijden op de vorming van sclerotiën op aardappelknollen door Rhizoctonia solani

In kasproeven werd onderzocht welke factoren betrokken zijn bij de stimulering van de produktie van sclerotiën op aardappel door *Rhizoctonia solani* Kühn als gevolg van

loofvernietiging. Afknippen van het loof stimuleerde de vorming van sclerotiën evenzeer als chemische vernietiging. Na afknippen van het loof trad de stimulering ook op bij knollen, die zich ruimtelijk gescheiden van de wortels in gestoomd vochtig perliet konden ontwikkelen. Doodspuiten plus doorsnijden van de wortels resulteerde in minder lakschurft dan doodspuiten alleen. Wanneer alleen de wortels werden doorgesneden resulteerde dat vaak in meer lakschurft. De vorming van sclerotiën wordt dus direct door de knol beïnvloed. Dit effect lijkt voort te komen uit fysiologische veranderingen in de knol.

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Effect of haulm destruction supplemented by cutting off roots on the incidence of black scurf and skin damage, flexibility of harvest period and yield of seed potatoes in field experiments

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Abstract

In field experiments, supplementing chemical haulm destruction (CHD) with cutting off roots resulted in a lower incidence of black scurf and skin damage (ripping off the skin) at harvest date than CHD alone. The lower susceptibility to skin damage at harvest allowed harvesting to begin on an earlier date, when only a few sclerotia of *Rhizoctonia solani* had developed. Furthermore, black scurf often developed more slowly after haulm destruction if roots had been severed and this enabled harvesting to be postponed.

At harvest, gross yield was highest if roots had not been cut through prior to CHD: the extra weight existed merely of water. Weight loss during storage, however, as well as grading losses resulting from black scurf were greater after CHD alone. This resulted in an equal or even lower net yield after CHD alone than after CHD supplemented by root severing. The favourable effects of supplementing CHD with cutting off roots almost equaled those of the mechanical removal, often called 'haulm pulling' or 'plant pulling'. Factors that may affect the development of black scurf are discussed.

Additional keywords: periderm, *Rhizoctonia solani,* sclerotia, *Solanum tuberosum,* tuber.

Introduction

In the search for the fundamental causes of black scurf it appeared to be useful to investigate the stimulatory effect of haulm destruction on the development of black scurf (Dijst, 1985).

In order to prevent virus transmission by flying aphids, haulm destruction is mandatory when growing seed potatoes in the Netherlands. The date of destruction is based on local counts of winged aphids and generally coincides with the second half of tuber growth. Haulm destruction of potato plants enhances the production of sclerotia on the tubers by *Rhizoctonia solani* Kühn. Chemical haulm destruction (CHD) is usually more stimulative than mechanical haulm removal, the so-called 'haulm pulling' or 'plant pulling' (Reestman and Schepers, 1955; Bouman et al., 1983).

Therefore, haulm pulling is recommended instead of CHD. However, under certain circumstances only CHD can be used; this results in high grading losses because of black scurf. It was therefore decided to ascertain why CHD stimulates black scurf much more than haulm pulling and whether this can be prevented.

CHD differs from haulm pulling in at least two aspects, which may account for the different impact on black scurf development of these two techniques. First, tuber physiology is affected differently: haulm pulling breaks the stolon whereas after CHD the tubers are still connected to the roots, which continue to function for several days after the treatment (Dijst, 1985). Second, the two measures affect the environment around the tuber differently: haulm pulling disturbs the soil of the ridge and CHD does not. Supplementing CHD with cutting off the roots eliminates both differences, for root severing terminates sap flow from the main root system to the tubers and disturbs the soil. In greenhouse experiments, supplementing CHD with root severing was found to be fairly successful, often giving black scurf ratings equal to those after haulm pulling and lower than after CHD alone (Dijst, 1985). However, the effect of root severing on black scurf development had not been evaluated in the field. We believed that by preventing tubers from swelling with water and by stimulating soil aeration, cutting off roots in the field might reduce skin damage at harvest as well.

The objectives of this study were twofold: to evaluate the efficacy of root severing in the field as a measure for controlling black scurf after haulm destruction and to examine what factors might favour black scurf development. The field experiments reported in this paper investigated the effect of different plant treatments with or without cutting off roots on (1) the incidence of black scurf, (2) skin damage at harvest, (3) the duration of the harvest period, (4) yield at harvest, (5) water content of the tubers and (6) the decrease of fresh weight during storage. Preliminary reports on this research have been published (Dijst et al., 1985).

Material and methods

Experimental design. Details on experimental conditions are given in Table 1. Disinfected seed was used for all trials. In the first experiment with cv. Arjan, samples of tubers were taken from different sites in a grower's field. The other experiments were carried out on research farms according to block designs. In the split-split-plot trials two varieties were planted on the main plots and treatments were on subplots. The four sampling times were randomly allocated to four small 'sample-plots' within each subplot. The sample-plots consisted of 40 (in 1982) or 24 (in 1983) plants and were surrounded by two buffer rows of plants.

Plant treatments. All the treatments of one trial were applied on the same day (day 0) In some experiments the 'E' or 'A' date was chosen to be day 0. The 'E' date and 'A' date are the dates on which haulms must be killed when cropping for seed potatoes grade E or A, respectively. In the Netherlands, basic seed potatoes are classified as SE (highest quality) or E. Basic seed potatoes can produce certified seed potatoes of class A (highest), B or C. If a higher classification is decided haulm destruction must be earlier. Since E-class potatoes are thus higher qualified than A-class potatoes, the 'E' date of haulm killing is some days earlier than the 'A' date on each location.

Haulm pulling and cutting off the shoots were done by hand. In earlier work (Dijst,

Table 1. Details of the experiments.

Year	Cultivar	Location	Soil type	Planting time	Experimental block design	Number of replicates	Weather during			Date of haulm killing (day 0)
							spring	summer	experiment	
1982	Arjan	Assen	reclaimed peat	April	none	3	wet	normal	very dry	26-07
1982	Prominent	Haren	sand	April	randomized	3	wet	normal	very dry	29-07
1982	Prominent	Rolde	diluvial/ loamy sand	April	randomized	4	wet	normal	very dry	19-07 (E)
1983	Astarte	Rolde	diluvial/ loamy sand	end of April	randomized	4	very wet	dry	dry	18-07 (E)
1983	Astarte/ Prominent	Rolde	diluvial/ loamy sand	end of April	split-split-plot	4	very wet	dry	dry	25-07 (A)
1983	Astarte/ Bintje	Creil	alluvial/ loamy sand	May	split-split-plot	4	very wet	dry	rain once on day 9	12-08

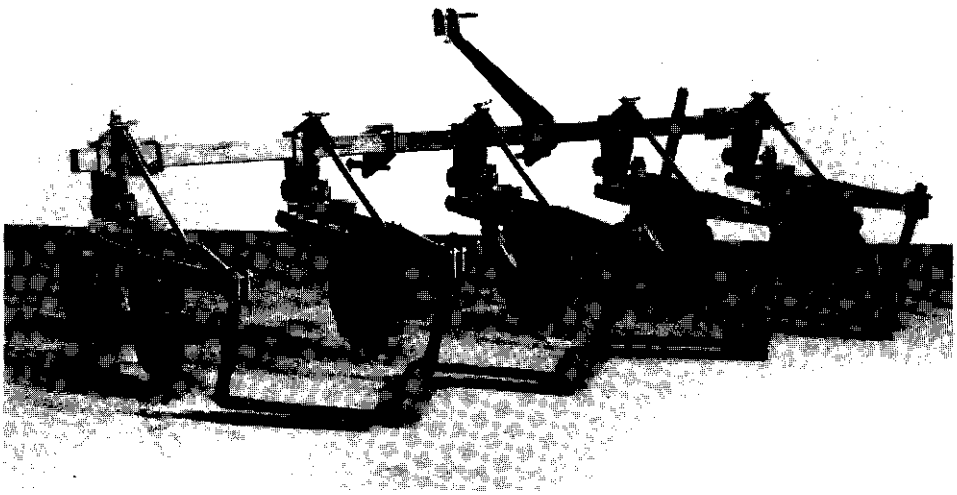

Fig. 1. The root-severing implement.

1985) the term 'plant pulling' was preferred to 'haulm pulling', because most of the plant (viz. above- and underground stems) is removed by this treatment, leaving the tubers. But since 'plant pulling' has already been used for another technique the term 'haulm pulling' has been reinstated in this report.

For CHD, dinoseb (Chimac Loofdood, 250 $g.l^{-1}$) was applied at a rate of 15 litres in 600 litres water per hectare. Where indicated, haulms were stripped prior to CHD with a flail-type haulm shredder which reduces shoots to almost leafless stems. After haulm stripping, lower amounts of chemicals are needed to kill the haulms completely and harvesting is also facilitated. In the fields at Haren the roots were cut through lifting the plant with a fork. In all other experiments a root-severing implement was used (Fig. 1). This tractor-mounted implement cuts the roots of four rows with two adjustable knives per ridge. The roots were cut through in the morning, followed by CHD either immediately or six hours later, when shoots had lost turgor.

Harvest and yield. Prior to harvest on days 3 and 10, soil water content in the centre of the ridge was determined. Tubers were harvested by hand, except for those of cv. Arjan, which were harvested by machine. Where the root-severing implement had been used, no complications occurred during mechanical harvesting, regardless of the depth of cutting. Immediately after harvest the tuber fresh weight (fw) and tuber dry weight (dw) of 32 (in 1982) or 16 (in 1983) plants per sample-plot were determinded.

Rating of black scurf. In order to estimate the amount of black scurf, samples of 100 tubers from at least eight plants were harvested per sample-plot and each sample was stored in a separate box to prevent skin damage. Three weeks later the tubers were graded. When unwashed tubers that had been classified as being free from sclerotia were subsequently washed, 10% still appeared to be lightly covered with sclerotia. Therefore, in all trials tubers were washed carefully prior to grading. The index (0-100)

was calculated from the number of tubers in each of five severity classes as described previously (Dijst, 1985).

Rating of skin damage. In order to measure skin damage at harvest, tubers from 12 plants per sample-plot were wounded in a rotating drum designed for this purpose (Bouman et al., 1983). They were then incubated for two minutes in an aqueous solution of pyrocatechol at 1.9 $g.l^{-1}$ and the damaged surface turned black within five minutes. These tubers were graded in six classes according to the amount of blackened surface area: 0% (a), 0-5% (b), 5-20% (c), 20-40% (d), 40-75% (e) and more than 75% (f). The index (I) for skin damage (0-100) was calculated using the formula:

$$I = 100.\ (0.a + 1.b + 2.c + 3.d + 4.e + 5.f)/\ (5.t)$$

where the characters a to f stand for the number of tubers in each class and t is the total number of tubers in the sample.

Yield loss. From each sample-plot at least 25 skin-damaged tubers and 25 undamaged tubers were weighed at harvest and weighed again after 30 and 90 days of storage, in order to estimate weight loss in storage. Yield loss caused by black scurf was measured by grading according to the requirements for class A seed potatoes: heavily and moderately covered tubers and some of the lightly covered tubers were eliminated until no more than 25% of those that remained were lightly covered.

Statistical analysis. All data were compared by analysis of variance using Genstat programming (V release 4.04 B, copyright 1984 Lawes Agricultural Trust, Rothansted Experimental Station). Differences between percentages (P) were analyzed after arcsinus transformation: $\arcsin (P \times 10^{-2})^{1/2} \times 57.296$.

Results

Ten days after haulm destruction the shoots were completely dead. On that date the shoots of untreated control plants were still green, and the shoots of plants where only the roots had been cut through were wilting and yellowing. There was great variation between replicates at Creil, possibly because of unequal distribution of irrigation water.

Black scurf. Analysis of variance showed a significant difference in black scurf development between cultivars: tubers of cv. Astarte being covered by sclerotia significantly less than those of cvs Prominent and Bintje (Table 2). At Creil in 1983 only few sclerotia developed on tubers of cv. Astarte and even fewer on those of cv. Bintje. Differences in incidence of black scurf between plant treatments did not become visible until 7 to 10 days after haulm destruction. The treatment effects were the same in all experiments. At Rolde in 1983 differences among treatments were more pronounced for cv. Astarte than for cv. Prominent.

During the first two experiments in 1982 black scurf increased more slowly after CHD supplemented by root severing as compared with CHD alone (Table 3). At Rolde in 1982 cv. Prominent showed only slight difference in black scurf development between the various treatments (Table 4). Nevertheless both haulm pulling, and to a lesser

Table 2. Effects of cultivars, treatments and date of sampling on severity of black scurf and skin damage in 1983, estimated by analysis of variance.

	Black scurf development		Skin removal at harvest	
	Rolde	Creil	Rolde	Creil
Cultivar[1]	*[2]	**	*	**
Treatment	***	NS	***	***
Cultivar × Treatment	NS	NS	NS	*
Day number	***	***	***	***
Cultivar × Day	NS	NS	***	**
Treatment × Day	***	*	**	***
Cultivar × Treatment × Day	NS	NS	NS	NS

[1] Rolde: cvs Astarte and Prominent haulms killed on A-date; Creil: cvs Astarte and Bintje.
[2] Significances at P = 0.05 (*), P = 0.01 (**) and P = 0.001 (***) level; NS = non-significant.

Table 3. Black scurf indices (0-100) of tubers after chemical haulm destruction (CHD) with or without cutting off the roots or after haulm pulling, in two experiments in 1982.

Method of haulm destruction	Number of days between treatment and harvest				
	cv. Prominent (1982) on sand			cv. Arjan 1982 on reclaimed peat	
	7	14	21	17	30
Haulms pulled	1	4 b[4]	9		
Shoots stripped + CHD	1	19 a	16	53 b	51
Roots cut off 250 mm deep + CHD[1]				60 b	53
Roots cut off 180 mm deep + CHD	0.1	5 b	13	35 a	36
Significance[2]	NS	**	NS	*	NS
LSD[3]	–	11	–	18	–

[1] Depth measured from the top of the ridge.
[2] Significances at P = 0.05 (*), P = 0.01 (**) level; NS = non-significant.
[3] LSD = Least Significant Difference for the above-mentioned P value.
[4] Values followed by the same characters are not significantly different.

extent, CHD supplemented by root severing gave a slightly lower incidence of black scurf than CHD alone. This also held for the experiments in 1983, but then the effects were more pronounced and differences were found to be significant.

At Rolde in 1982 when shoots were cut off either with or without root severing black scurf developed to the same extent as compared to CHD with or without root severing respectively (data not shown). An interval of six hours between root severing and CHD did not improve the effect of the supplementory root severing (data without an interval

Table 4. Incidence of black scurf (index 0-100) at different intervals after various treatments of the crop and haulm destruction on day 0.

Cultivar	Treatment[1]	Number of days between treatment and harvest					LSD[2] P = 0.05
		0	3	10	17	24	
Prominent-E	*Rolde 1982*						12.7
	S + CHD	—	11[3]	14	32 bc	42 bc	
	S + RC + CHD	—	8	19	30 bc	43 c	
	RC + 6h + CHD	—	7	20	25 abc	32 abc	
	Haulms pulled	—	10	15	15 a	31 abc	
	RC	—	13	15	20 ab	29 ab	
	Control (untreated)	10	14	14	16 a	26 a	
Astarte-E	*Rolde 1983*						12.4
	S + CHD	—	2	4	37 b	33 b	
	RC + 6h + CHD	—	1	5	10 a	28 b	
	Control (untreated)	1	2	3	3 a	13 a	
Astarte-A	*Rolde 1983*						11.5
	S + CHD	—	6	10	29 c	48 d	
	S + RC + 6h + CHD	—	2	4	23 bc	31 c	
	RC (+ 6h) + CHD	—	2	5	14 ab	27 c	
	Haulms pulled	—	2	11	13 a	18 abc	
	RC	—	4	8	10 a	9 a	
	Control (untreated)	3	3	3	12 a	15 ab	
Prominent-A	*Rolde 1983*						
	S + CHD	—	11	11	29 d	45 c	
	S + RC + 6h + CHD	—	6	12	25 cd	42 bc	
	RC (+ 6h) + CHD	—	7	9	19 abcd	31 b	
	Haulms pulled	—	12	16	22 bcd	32 b	
	RC	—	5	12	11 a	11 a	
	Control (untreated)	5	8	9	11 ab	17 a	
Astarte	*Creil 1983*						17.9
	S + CHD	—	4	4	4 a	8 b	
	RC (+ 6h) + CHD	—	2	3	9 a	15 b	
	Haulms pulled	—	3	3	1 a	5 b	
	RC	—	4	4	4 a	1 a	
	Control (untreated)	3	3	1	19 a	1 a	
Bintje	*Creil 1983*						
	S + CHD	—	7	13	27 ab	24 bc	
	RC (+ 6h) + CHD	—	12	4	25 ab	6 a	
	Haulms pulled	—	7	17	10 a	14 ab	
	RC	—	3	6	30 b	41 c	
	Control (untreated)	8	18	16	26 ab	13 ab	

[1] The treatments included: S = shoots reduced by stripping; CHD = chemical haulm destruction; RC = roots cut through; 6h = shoots destroyed chemically in the afternoon, six hours after root severing; (+ 6h) = identical results werd found when there was no time interval.
[2] LSD = least significant difference.
[3] Per date, values followed by identical characters are not significantly different. No characters per date means that there were no significant differences on that date.

Table 5. Skin damage at harvest (index 0-100) at different intervals after various treatments of the crop and haulm destruction on day 0.

Cultivar	Treatment[1]	Number of days between treatment and harvest					LSD[2] P = 0.05
		0	3	10	17	24	
Prominent-E	*Rolde 1982*						4.87
	S + CHD	—	49 d[3]	23 b	10 ab	4	
	S + RC + CHD	—	45 bcd	17 a	7 a	2	
	RC + 6h + CHD	—	41 b	15 a	9 a	3	
	Haulms pulled	—	42 b	17 a	8 a	4	
	RC	—	34 a	15 a	6 a	3	
	Control (untreated)	53	48 cd	31 c	14 b	6	
Astarte-E	*Rolde 1983*						9.98
	S + CHD	—	28 ab	24 a	13 ab		
	RC + 6h + CHD	—	20 b	8 b	5 b		
	Control (untreated)	48	33 a	32 a	21 a		
Astarte-A	*Rolde 1983*						7.60
	S + CHD	—	38 a	23 a	12 abc		
	S + RC + 6h + CHD	—	25 bc	8 b	5 c		
	RC (+ 6h) + CHD	—	20 cde	8 b	6 bcd		
	Haulms pulled	—	15 def	10 b	4 d		
	RC	—	12 f	4 b	4 d		
	Control (untreated)	24	32 ab	21 a	15 a		
Prominent-A	*Rolde 1983*						
	S + CHD	—	19 a	9 ab	4 b		
	S + RC + 6h + CHD	—	17 ab	6 b	2 b		
	RC (+ 6h) + CHD	—	11 b	4 b	3 b		
	Haulms pulled	—	11 b	3 b	1 b		
	RC	—	9 b	3 b	1 b		
	Control (untreated)	12	13 ab	14 a	15 a		
Astarte	*Creil 1983*						7.04
	S + CHD	—	48 ab	28 b	23 b		
	RC (+ 6h) + CHD	—	45 abc	17 c	11 c		
	Haulms pulled	—	40 c	19 c	13 c		
	RC	—	44 bc	13 c	14 c		
	Control (untreated)	44	52 a	44 a	52 a		
Bintje	*Creil 1983*						
	S + CHD	—	43 ab	16 b	10 b		
	RC (+ 6h) + CHD	—	43 ab	19 b	6 b		
	Haulms pulled	—	48 a	17 b	10 b		
	RC		45 ab	18 b	9 b		
	Control (untreated)	44	49 a	41 a	43 a		

[1] Legend see Table 4.
[2] LSD = least significant difference.
[3] Per date, values followed by identical characters are not significantly different. No characters per date means no significant differences on that date.

not shown in Table 3). In 1982, cutting through the roots at smaller depths resulted in lower black scurf ratings on cv. Arjan (Table 3), but no such effect was shown at Creil in 1983 on cvs Astarte and Bintje (data not shown in Table 4). Reducing shoot volume by stripping prior to root severing cancelled the effect of additional root cutting on black scurf, except in the case of cv. Astarte (A) at Rolde in 1983 (Table 4).

Skin damage at harvest. When skin set has not yet been completed the periderm rips off during harvesting and transport from the field when the tubers rub against each other. Here the amount of damage differed very little between replicates (Table 5). In 5 out of the 6 trials, skin damage at harvest decreased more quickly after haulm pulling or root severing plus CHD, than after CHD alone. This more rapid skin hardening allows harvesting to begin on an earlier date when only few sclerotia are present, thus indirectly preventing the development of black scurf.

In 1982, the skin damage at harvest of tubers cv. Prominent from plants whose shoots had been cut off appeared to be the same as that from plants that had undergone CHD. Furthermore cutting off shoots supplemented by root severing resulted in the same amount of damage as CHD supplemented by root severing (data not shown). In all split-split-plot trials in 1983 an interval of six hours between cutting roots and CHD had no effect on tuber damage at harvest (data not shown).

Table 6. The 'favourable harvest period' i.e. the number of days on which tubers could be harvested with acceptably low amounts of black scurf and skin damage at harvest (both indices below 20).

Treatment[1]	*Cultivar, location and year of the trial*					
	Prominent-E Rolde 1982	Astarte-E Rolde 1983	Astarte-A Rolde 1983	Prominent-A Rolde 1983	Astarte Creil 1983	Bintje Creil 1983
S + CHD	2 (12)[2]	2 (15)	5 (11)	11 (4)	6 (24)	11 (14)
S + RC + CHD	3 (9)	—	—	—	—	—
S + RC + 6h + CHD	—	—	13 (5)	14 (3)	—	—
RC + 6h + CHD	—	21 (4)	17 (4)	17 (3)	20 (9)	15 (10)
RC + CHD	—	—	22 (3)	19 (3)	20 (9)	12 (10)
RC deep + CHD	—	—	—	—	23 (10)	11 (12)
Haulms pulled	11 (8)	—	24 (3)	13 (4)	20 (10)	17 (11)
RC	10 (7)	—	27 (3)	27 (3)	18 (8)	8 (10)
Control (untreated)	5 (15)	4 (26)	18 (11)	24 (3)	0 (30)	0 (30)
Significance[3]	*	*	* *	* * *	*	NS
LSD[4]	9	9	11	14	6	—

[1] Legend see Table 4; deep = roots cut through 50 mm deeper than in other trials where the roots were severed just below the tubers.
[2] In brackets: first day of harvest after treatment on day 0.
[3] Significances at P = 0.05 (*), P = 0.01 (* *) and P = 0.001 (* * *) level; NS = non-significant.
[4] LSD = Least Significant Difference for the above-mentioned P value.

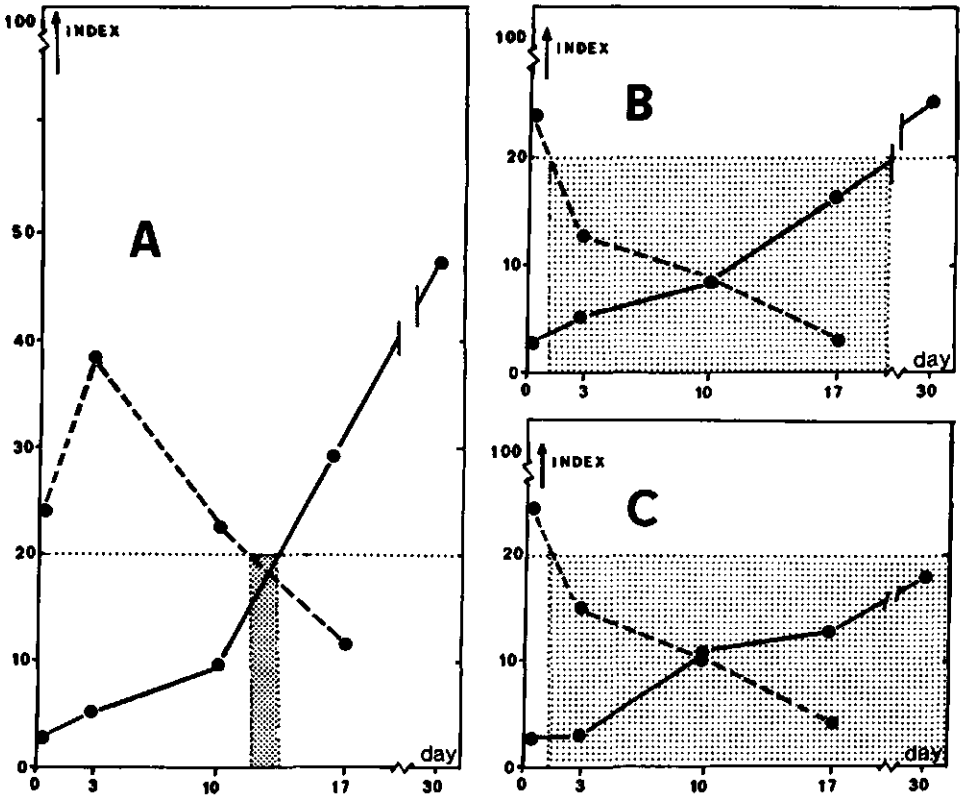

Fig. 2. The duration of the 'favourable harvest period' (shaded area) (i.e. both black scurf index (solid line) and skin damage index (broken line) below 20) as a result of haulm pulling (C), roots cut off plus chemical haulm destruction (B) as compared with shoots stripped plus chemical haulm destruction (A), for cv. Astarte in loamy sand at Rolde, 1983.

Harvest period. Tubers cannot be harvested until the skin is firm enough to withstand handling at harvest. On the other hand, tubers have to be harvested before too many and big sclerotia have developed. The mandatory limits set by the Dutch Inspection Service for the amount of sclerotia permitted on seed potatoes classes E and A are comparable with our indices 7 and 15, respectively. Thus we assumed that the skin damage and black scurf before grading were acceptably low if both indices did not exceed 20 at that moment (Fig. 2). The number of days on which both indices were below 20 were calculated and called the 'favourable harvest period' (Table 6). CHD supplemented by root severing as compared with CHD alone extended the favourable harvest period from a few days to at least two weeks, a period of time almost equal to that after haulm pulling.

Soil water content in the ridge. Soil water content in the ridge was measured at 3 and 10 days after treatment. No significant differences occurred and water content was generally very low, because the weather was very warm and dry during the experiment

Table 7. Average soil water content in the ridge, expressed as percentage of dry weight.

Year	Cultivar	Location	Soil type	Number of days between treatment and harvest			
				3		10	
				$\bar{x}$	SD[2]	$\bar{x}$	SD[2]
1982	Prominent-E	Rolde	sand	—	—	7.8	± 0.5
1983	Astarte-E	Rolde	diluvial loamy sand	3.9	± 0.2	4.2	± 0.6
1983	Astarte-A	Rolde	diluvial loamy sand	4.8	± 0.1	4.4	± 0.5
	Prominent-A	Rolde	diluvial loamy sand	5.1	± 0.3	4.4	± 0.4
1983	Astarte	Creil	alluvial loamy sand	7.4	± 0.7	15.0	± 1.0
	Bintje	Creil	alluvial loamy sand	7.0	± 0.4	15.2	± 1.4

[1] The average was calculated from the results of four treatments: (1) control (untreated), (2) chemical haulm destruction (CHD), (3) roots severed plus six hours later CHD and (4) haulms pulled (all trials, except for 1983 Astarte-E, where haulm pulling was not tested).
[2] SD = standard deviation.

(Table 7). At Creil in 1983 soil water content increased after heavy rainfall on day 9. No relation was found between soil water content and black scurf development.

Tuber water content. Tuber water content was determined for cv. Prominent at Rolde in 1982 (Table 8). Tuber dry weight of untreated control plants increased from 0.63 $kg.m^{-2}$ to about 0.85 $kg.m^{-2}$ as a result of continued photosynthesis, and their tuber water content decreased from 79% to 74% (Table 8). If only the roots had been cut through tuber dry weight increased only slightly to 0.68 $kg.m^{-2}$ in the first week. However, tuber water content decreased then because, until they died the wilting shoots continued to withdraw water from the tubers.

After haulm pulling or root severing plus CHD, both tuber dry weight (0.60 $kg.m^{-2}$) and tuber water content (79%) remained constant. After CHD alone or after cutting off the shoots, tuber dry weight remained constant (around 0.60 $kg.m^{-2}$, data not shown) but tuber fresh weight had increased from 2.91 to 3.12 $kg.m^{-2}$ by day 17 (Table 8). As a result, tuber water content slightly increased during the first 10 days. This demonstrates that the roots continued to function for some days after CHD. Roots did not visibly decay until the second week after haulm destruction. However, no relation was found between tuber water content and black scurf.

Gross yield at harvest and net yield after storage and grading. Of the various methods of haulm destruction, only two, CHD alone or cutting off the shoots, increased gross yield at harvest during the first week after destruction. Thus CHD resulted in the yield at harvest being higher than that of crops where further growth had been terminated by haulm pulling or root severing plus CHD. Most of this extra yield, however, was lost during storage (Table 9). Weight loss in storage was found to be correlated with the amount of skin damage at harvest. After root severing plus CHD as well as after

Table 8. Tuber yield (kg.m^{-2}) and tuber water content (percentage of tuber fresh weight) of cv. Prominent (1982) on loamy sand at Rolde in various treatments and when harvested at different intervals after haulm destruction or cuttung off the roots on day 0.

Treatment[1]	Number of days between treatment and harvest				
	0	3	10	17	24
Tuber yield					
S + CHD	—	2.99 b[2]	3.01 cd	3.12 bc	2.98 cd
S + RC + CHD	—	2.96 b	2.86 bc	2.74 a	2.82 bc
RC + 6h + CHD	—	2.55 a	2.67 a	2.75 a	2.63 a
Haulms pulled	—	2.86 b	2.83 ab	2.77 a	2.68 ab
RC	—	2.68 a	2.80 ab	2.76 a	2.76 b
Control (untreated)	2.91	2.92 b	3.19 d	3.19 c	3.28 e
Tuber water content					
S + CHD	—	79.5 d	80.2 d	80.6 d	80.1 e
S + RC + CHD	—	78.7 bc	78.1 b	78.9 c	79.0 d
RC + 6h + CHD	—	78.6 bc	78.4 bc	78.0 b	78.1 c
Haulms pulled	—	79.2 cd	78.4 bc	78.9 c	79.2 d
RC	—	77.1 a	75.4 a	75.3 a	75.8 b
Control (untreated)	78.9	77.2 a	74.9 a	75.3 a	74.1 a

[1] Legend see Table 4.
[2] Per date, values followed by identical characters are not significantly different: Least Significant Difference for tuber yield (P = 0.05) = 1.73 and for tuber water content (P = 0.05) = 0.7.

haulm pulling, tubers were less damaged and lost less weight during storage than after CHD alone.

Finally, tubers were graded for black scurf according to the mandatory requirements for class A seed potatoes. When harvested on day 10, both grading and weight loss in storage reduced yield after CHD to the same net yield as that obtained by haulm pulling or CHD supplemented by root severing. When harvested on day 17, grading losses became more severe and net yield after CHD alone was generally (in 4 out of 5 trials) less than net yield after haulm pulling or CHD supplemented by root severing. This result was found for all three trials at Rolde (Table 9). At Creil these results were found for cv. Bintje, but not for cv. Astarte (data not shown). There was great variation between replicates at Creil.

With respect to both yield at harvest and yield after storage, analysis of variance showed that cultivars and treatments had significant effects (P=0.001), and no interaction was found between cultivars and treatments.

Discussion

Cutting off the roots has long been known to prevent tuber cracking and to facilitate harvest in certain soils (Werner and Dutt, 1941; Finney and Findlen, 1967). Until the present research, the efficacy of root severing on black scurf had not yet been ascer-

Table 9. Gross tuber yield (kg.m^{-2}) at harvest on day 17 after haulm destruction, tuber gross weight after storage and tuber net weight after storage plus grading for black scurf, Rolde 1983.

Cultivar	Treatment[1]	Tuber yield (kg.m^{-2})					Gross yield loss (%)		
		at harvest gross	after storage				by storage		by black scurf[3]
			30 days		90 days		30 days	90 days	
			gross	net[2]	gross	net[2]			
Astarte-E	Shoots stripped + CHD	1.99	1.91	1.11	1.77	1.04	4	11	42 a[5]
	Roots cut off + 6h + CHD	1.85	1.81	1.66	1.70	1.55	2	8	8 b
	Control (untreated)	1.92	1.82	1.80	1.75	1.74	3	8	1 b
Significances[4]:	Treatments	*	*	****	NS	****	NS[5]	NS[5]	****[5]
	LSD (P = 0.05)	0.13	0.09	0.30		0.24			
Astarte-A	Shoots stripped + CHD	1.83	1.71	1.07	1.67	1.04	6 a[5]	9 a[5]	36 a[5]
	Roots cut off + 6h + CHD	1.91	1.85	1.68	1.81	1.65	3 bcd	4 abc	9 bc
	Haulms pulled	1.80	1.80	1.69	1.70	1.62	3 de	6 bc	7 bc
	Roots cut off	1.55	1.48	1.46	1.43	1.41	5 ab	8 ab	1 c
	Control (untreated)	1.88	1.78	1.72	1.73	1.67	5 ab	8 ab	3 bc
Prominent-A	Shoots stripped + CHD	2.40	2.33	1.56	2.28	1.53	3 bcde	5 bc	33 a
	Roots cut off + 6h + CHD	2.17	2.13	1.83	2.10	1.80	2 de	3 c	15 ab
	Haulms pulled	2.03	1.99	1.53	1.96	1.52	2 cde	3 c	22 ab
	Roots cut off	2.08	2.05	2.02	2.02	1.99	1 e	3 c	2 c
	Control (untreated)	2.27	2.17	2.09	2.15	2.07	4 abc	5 abc	4 bc
Significances[4]:	Treatments	**	*	*	**	*	*[5]	*[5]	**[5]
	Cultivars	****	****	**	****	**	****	****	NS
	Tms × Cvs	NS	NS	NS	NS	NS	NS	NS	NS
	LSD (P = 0.05)	0.24	0.22	0.52	0.21	0.51			

[1] CHD = chemical haulm destruction.

[2] Tuber weight (kg.m^{-2}) left after storage during 30 and 90 days and grading for black scurf in order to obtain A class seed potatoes (at least 75% free and 25% not more than lightly covered with sclerotia).

[3] Percentage of gross weight lost by grading for A-class seed potatoes.

[4] Significance at P = 0.05 (*), P = 0.01 (**), P = 0.001 (***), P ≤ 0.0005 (****); NS = non significant; LSD = Least significant difference; comparing 3 treatments for the E trial and 7 treatments for the A trial.

[5] Analysis of variance after arcsinus transformation; values followed by identical characters are not significantly different (p = 0.05).

tained. Our results show that compared with chemical haulm destruction (CHD) alone, CHD supplemented by root severing often delayed the development of black scurf and always accelerated skin hardening, confirming previous observations (Dijst, 1985). The favourable effect on skin damage at harvest time of supplementing CHD by cutting off the roots increased its positive effect on the incidence of black scurf: harvesting could be started earlier, i.e. when only small amounts of sclerotia had developed. The consequent extension of the 'favourable harvest period' may give the farmer more flexibility in planning his harvest.

Our data on tuber water content are not in line with those obtained by Werner and Dutt (1941) or Finney and Findlen (1967). However, their experimental designs differed considerably from ours in the time of haulm destruction and of harvest. In our experiments tuber water content increased slightly after CHD. This indicated that sap flow from roots continues during the first week after CHD, confirming previous findings (Dijst, 1985). The reason that gross yield (at harvest) was higher after CHD than after haulm pulling was merely the extra water content.

In most experiments, CHD supplemented by root severing prevented both an increase in tuber water content and a high incidence of black scurf. However, no relation was found between incidence of black scurf and tuber water content, confirming previous results (Dijst, 1985). The weather was very dry and warm on haulm destruction day. Nevertheless, a six-hour interval at midday between root severing and CHD did not consistently change the effects of root severing on tuber water content and black scurf. In cv. Prominent at Rolde in 1983 stripping shoots prior to cutting off the roots completely negated the effect of root severing. These results suggest changes in tuber physiology resulting from the fall-off of sap flow from shoots to be more important for the development of black scurf than changes resulting from sap flow from the roots.

When CHD was supplemented by root severing the tubers did not swell with water and skin resistance to damage at harvest may have increased, regardless of the stage of skin set. Root severing also increases soil aeration, and this may have enhanced skin set. In the literature (Spencer and Fox, 1979; Braue et al., 1983) skin set has been used as a measure of tuber maturity. This may be justifiable after haulm pulling but not after CHD, where continued water uptake may cause extra pressure on the periderm.

Supplementing CHD with cutting off the roots ultimately favoured tuber yield: first, because it often lowered grading losses because of black scurf and second, it reduced weight loss in storage. In our study, lower ratings of skin damage at harvest coincided with less weight loss in storage, confirming earlier results obtained by Meijers et al. (1982). Less skin damage also lowers the sensitivity to storage rot. When harvested at 17 days after treatment, in 4 out of 5 trials net yield after storage and grading was higher after root severing plus CHD, or after haulm pulling, than after CHD alone. Grading losses accounted for most of this result, so that differences were smaller if harvest took place earlier. Comparisons with untreated control plants showed that, contrary to what Braue and his colleagues predicted (1983), haulm killing did not reduce weight loss in store.

In this study smaller amounts of black scurf were found on cv. Astarte than on cvs Prominent or Bintje. When grown in steamed soil in the greenhouse, no differences in black scurf development were found between seven cultivars, including Astarte and Prominent (Dijst, 1985). Factors in the field may account for this ambiguity.

The second objective of this study was to examine what factors might favour black scurf. Earlier data for plants grown in steamed soil had shown that the development of black scurf was mainly influenced by the tuber itself. This suggested an effect from changes in the periderm and/or tuber exudates, probably as a result of accelerated tuber maturation (Dijst, 1985). Our results demonstrate that other factors can interfere, for although CHD usually stimulates black scurf more than haulm pulling, sometimes both methods give equally low rating of black scurf. This was even found in steamed soils (Dijst, 1985).

In order to better understand why haulm destruction stimulates black scurf development, differences in effects of CHD and haulm pulling will be discussed. These differences concern: (1) the sap flow from the main root system to the tubers, (2) the disturbance of the ridge and (3) the amount of underground stems and stolons left behind in the ridge.

(1): Tuber physiology depends on the amount and quality of the supply of water and nutrients from shoots and roots. Sap flow from the roots is terminated by haulm pulling but after CHD it still continues for a week. The effects of our various treatments on the development of black scurf suggest that the crucial causal changes in tuber physiology may be induced less by continuation of sap flow from the roots than by termination of sap flow from the shoots. Since both CHD and haulm pulling terminate sap flow from the shoots, the cessation of this flow may be responsible for the basic stimulation of black scurf but cannot explain any difference in effect between the two treatments. Previous suggestions that the extra stimulation of black scurf after CHD as compared with that after haulm pulling is merely the results of tubers swelling with water (Dijst, 1985) are not strongly supported by the results presented in this paper. No relationship was found between tuber water content and incidence of black scurf. However, even slight swelling might induce other effective alterations in tuber physiology.

(2): Haulm pulling disturbs the ridge, but CHD does not. Results of additional field experiments (Dijst et al., 1985) support the idea that increased aeration prevents black scurf development. In 1984 in three of the four experiments, haulm pulling supplemented by root severing resulted in even less black scurf than haulm pulling alone (Dijst et al., 1985). Soil disturbance affects both soil aeration and soil moisture. We found no relationship between soil water content in the vicinity of the tuber and black scurf development, but this does not prove that soil water content had no effect on black scurf: may be any visible effects were obscured by the interfering influences of volatiles. In the vicinity of the tuber the production, consumption and diffusion of volatiles will depend on moisture, soil compaction, soil disturbance and the physiological state of the underground tissue (Blair, 1943; Brown et al., 1965; Cary, 1985). Increasing concentrations of carbon dioxide have been found to affect *R. solani* both negatively and positively and these effects were not consistent for different isolates (Sherwood, 1970). Tuber respiration and periderm permeability for volatiles decrease during maturation of tubers (Burton, 1966). Tuber maturation seems to be an important factor in black scurf development, especially if accelerated somehow (Dijst, 1985). Mature tubers, whether harvested from field or greenhouse experiments, often have big sclerotia where the growing tuber was pressed against pieces of clay, plastic, stones or the pot wall, i.e. sites where minimal diffusion of volatiles can be expected. These observations contradict the suggestions that high oxygen supply and

good aeration are needed for sclerotial formation (Sherwood, 1970). Data from laboratory tests may wel differ from data in the field, where mixtures of volatiles change continuously in compositon.
(3): Supplementing CHD by root severing cancelled the two differences between CHD and haulm pulling mentioned above. However, it did not always completely eliminate the difference in black scurf rating between them. So far we have discussed a third difference between the effect of CHD and that of haulm pulling: the amount of underground plant tissue remaining after the treatment and thus the extent of possible effects during decay. Volatiles from decomposing plant tissue can often influence *R. solani* (Lewis, 1976). CHD with or without additional root severing does not affect the amount of underground plant tissue. Haulm pulling, however, eliminates underground stems and may thus reduce any effects arising from tissue decay. In these experiments, neither decay of all roots and stolons nor stimulation of black scurf could be observed until 7 to 10 days after haulm destruction. The effect of volatile exudates on black scurf therefore warrants for additional research.

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Samenvatting

De invloed van loofvernietigen en wortelsnijden op lakschurftbezetting, ontvelling, duur van de oogstperiode, opbrengst en gewichtsverlies van pootaardappelen in veldproeven

Pootaardappelen raakten minder bezet met lakschurft en werden minder ontveld bij de oogst na looftrekken of na wortelsnijden plus doodspuiten dan na loofklappen plus spuiten. Al vanaf de derde dag na trekken of wortelsnijden plus doodspuiten was de mate van ontvelling zo gering, dat met het oogsten kon worden begonnen. Op zo'n vroeg tijdstip na loofdoding konden knollen worden geoogst met nog weinig lakschurft, want de stimulering van lakschurft werd pas zichtbaar vanaf 7 à 10 dagen na loofdoding. Lakschurft ontwikkelde zich het traagst na looftrekken. Ook wortelsnijden bij het doodspuiten gaf meestal een tragere ontwikkeling van lakschurft dan klappen plus spuiten. Daardoor konden na wortelsnijden plus doodspuiten en vooral na looftrekken op nog latere tijdstippen knollen met weinig lakschurft worden geoogst.

Na klappen plus spuiten was het bruto gewicht bij de oogst het hoogst. Dat meergewicht bleek louter uit water te bestaan en het verloop in knolvochtgehalte leek erop te wijzen dat de wortels nog gedurende een week na doodspuiten blijven func-

tioneren. Dat gaf wel knollen met een hoger vochtgehalte die meer ontvelden en meer vocht en dus gewicht verloren. Dit groter gewichtsverlies bij bewaren en de hogere leesverliezen door lakschurft deden de meeropbrengst teniet: zo werd het netto knolgewicht na klappen plus spuiten, al naar gelang het moment van oogsten, meestal gelijk aan, of lager dan het netto knolgewicht na het trekken of na wortelsnijden plus doodspuiten. In dit artikel worden de factoren besproken, die mogelijk van invloed zijn op de ontwikkeling van lakschurft.

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Formation of sclerotia by Rhizoctonia solani on artificial media and potato tubers

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Abstract

Independent of the nutrient medium and the size of Petri dish, sclerotium initials of *Rhizoctonia solani* AG-3 appeared after a fixed period of time. The same held for the completion of maturation of the sclerotia. Deprivation or extra supply of nutrients reduced or increased, respectively, final mass of sclerotia but did not affect the moment of initiation and of maturation. Transfer of a mycelial mat from water agar to a nutrient-rich medium caused the formation of black solid sclerotia within four days all over the mat and not only around the site of inoculation as usually occurs on rich medium. Final sclerotial mass was higher on liquid medium than on agar. The results indicate that formation of these sclerotia is not associated with cessation of linear hyphal growth or with starvation.

On mature tubers, sclerotia and hyphae are spread over the whole surface. On young growing tubers sclerotia are rarely found in the immediate vicinity of lenticels. This suggests a release of inhibitory factors at these sites which diminishes during tuber maturation. Volatile exudates from underground plant parts seem to further promote the sclerotium formation. On all underground plant parts, even the roots, final sclerotial mass was higher after wounding.

After haulm destruction, development of black scurf was not stimulated by a short-term trigger or by roughning of tuber surface. The observations rather suggest that the stimulation results from an increased tuber exudation of stimulatory nutrients, water and stress factors and also from a reduction of as yet unknown inhibitory factors. Results indicated that in infested soils, the estimated inoculum density at the day of haulm destruction has no predictive value for black scurf development.

Additional keywords: black scurf, haulm destruction, sclerotial distribution, sclerotial initiation, sclerotial maturation, *Solanum tuberosum*, tuber maturation, tuber surface.

Introduction

Quantitative and qualitative alterations in nitritive components and pH affect production of pseudo-sclerotia by *Rhizoctonia solani* Kühn AG-3 both in their number, size and location on artificial media (Allington, 1936). the effect of nutrients on mycelial growth and sclerotial initiation differs from their effect on sclerotial growth and maturation (Townsend, 1957). Volatile compounds also affect the final mass of sclerotia pro-

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duced (Sherwood, 1970; Lewis, 1976). Spontaneous sclerotium production and effective minimum concentrations of adenosine 3',5'-cyclic monophosphate (cAMP) to induce sclerotium formation, depend on the age of mycelium (Hashiba and Ishikawa, 1978). The authors also suggested that environmental factors play a role.

Potato tubers stimulate black scurf when tuber maturation is accelerated after haulm destruction (Dijst, 1985). This effect may be caused by an increase in stimulatory factors, a reduction of inhibitory factors or by a short trigger. Armentrout et al. (1986) suggested that surface structure influences sclerotium production. Allington (1936) concluded that tubers, apart from providing surface area, offer nutrients. The objective of this study was to obtain more information about factors that stimulate black scurf formation after haulm destruction. For that purpose, sclerotial development was studied on artificial media and on tubers. An attempt was made to determine the moment of stimulus release by the tuber after haulm destruction.

Materials and methods

Isolates. Three pathogenic isolates of *R. solani* Kühn AG-3 of different origin were provided by G. Jager (Inst. of Soil Fertility, Haren, the Netherlands): 09ABa from loamy clay, pH 7; 05AHa from sand, pH 4.5; 36AG78 isolate from a symptomless plant on loamy clay, pH 7.0. Over five years of experiments these isolates gave similar results in tests on sclerotium formation, although their distribution pattern on plates differed.

Tests in vitro. Isolates were maintained on malt peptone agar (MPA) (Van den Boogert and Jager, 1984) and tested on water agar (WA). Cultures were incubated at 20 °C. Disks, 5 mm in diameter, were cut from the edge of two-day-old MPA cultures and placed on WA. After two days, small disks with hyphal tips from these WA-cultures were used to inoculate filters or plates.

Growing of plants. Cultivar Pimpernel was used for its long stolons which facilitate working with growing tubers in pot experiments. Shoot tops were cut from one-month-old plants grown in growth chambers. Growth chamber conditions were kept at 16 h light per day and 18-20 °C by day and 14-16 °C during the night. Two weeks later, axillary stem cuttings (50 mm) were taken and placed in humidified perlite for two weeks. Rooted stem cuttings were transplanted into potting soil. After three weeks, the soil was washed from the roots in order to facilitate transplanting into a soil-sand system, a slight alteration of the previously described two-compartment system (Dijst, 1985). The lower compartment was a pot (180 mm) filled with potting soil and the upper compartment was a plastic bag of the same size. The roots of the transplants were placed onto the soil through a small hole in the plastic bag and the bags were filled with coarse sand. The original cut surface of the stems was in the sand, 20 mm above the plastic bottom in order to guarantee that all stolons and tubers were formed in the sand. Potting soil, sand and perlite were steamed twice before use. Pots were placed in the glasshouse on cotton mats, which were watered dayly. Extra light was supplied for 12 h a day during the winter and temperature was 18-20 °C by day and 14-16 °C at night. Experiments were started when plants were about 100 days old, shoots were still green and tubers were 30 mm. At the day of haulm destruction, plant shoots were either left untreated (UNTR) or cut off (COS).

Plant inoculation. Per pot, either the sand or each separate tuber was inoculated. Sand was inoculated with three sclerotized wheat grains per pot, placed at 4 cm depth when plants were two months old. The wheat grains had been soaked in water for 24 h, autoclaved twice with an interval of 36 h and inoculated. However, in order to assure equal infestation of all tubers per plant, eacht tuber was inoculated separately, two weeks before haulm destruction. For that purpose the sand was removed, each tuber was placed on two-day-old WA-cultures and covered with wet perlite until assessment of sclerotium production.

Assessment of sclerotium production. Three weeks after inoculation, sclerotium production was evaluated. Black scurf index was assessed as described previously (Dijst, 1985). Sclerotia were removed from tubers by hand and from artificial media by filtration after boiling the agar in acidified water. Sclerotium production was calculated per plate or per tuber as dry weight (μg) per 1000 mm^2 of surface area (WSA).

Estimation of tuber surface. Tuber length (l), tuber diameters at the middle (m_1 and m_2), at 10 mm from the bottom (b_1 and b_2) and at 10 mm from the top (t_1 and t_2) were measured and the average diameters at each site were calculated (m, b and t, respectively). If tuber length was shorter than 30 mm, tuber surface (A) was calculated as that of a sphere: $A = 4\pi r^2$ with $r = (b + m + t)/6$. If the tuber was longer, its surface was estimated as that of a cylinder in the middle (M) with half a sphere at each end (B and T):

$A = A_B + A_M + A_T$ with:

$A_B = 2\pi r_B^2$ with $r_B = (b_1 + b_2 + 20)/6$

$A_M = 2\pi r_M^2 (l - 20)$ with $r_M = (b + m + t)/6$

$A_T = 2\pi r_T^2$ with $r_T = (t_1 + t_2 + 20)/6$

Statistical analysis. Analyses of variance were carried out using GENSTAT-4 (Dijst et al., 1986). Differences between amounts of sclerotia produced (WSA) were analyzed after transformation to natural logarithm: ln(WSA + 1).

Results

Sclerotium production in vitro. The objective of this investigation was to repeat the study of Allington (1936) who suggested that sclerotium production starts at termination of hyphal linear growth. For this purpose cultures were compared on nutrient-richt agar (MPA), nutrient-poor agar (WA) and on liquid malt peptone (MP) in Petri dishes, 50, 90 and 150 mm in diameter. Isolates were similar in radial growth rate, viz. 10 mm per day on WA and 11 mm on MPA, which decreased slightly after five days. On WA a few hyphae developed with some branches (ca. seven hyphae per 10 mm, measured 10 mm from the site of inoculation). On MPA, dense colonies developed with concentric zones of heavily branched mycelium. All three isolates formed a few tiny sclerotia of about 1 mm in diameter on WA scattered over the plates. Isolates on MPA differed in distribution pattern of sclerotia. Isolate 09ABa produced many sclerotia as an almost solid mass extending not farther than 20 mm from the site of inoculation. Isolate 05AHa formed many sclerotia in a wide circle at 16 to 50 mm from the centre. Isolate 36AG78 produced many small sclerotia scattered over the plates, but never

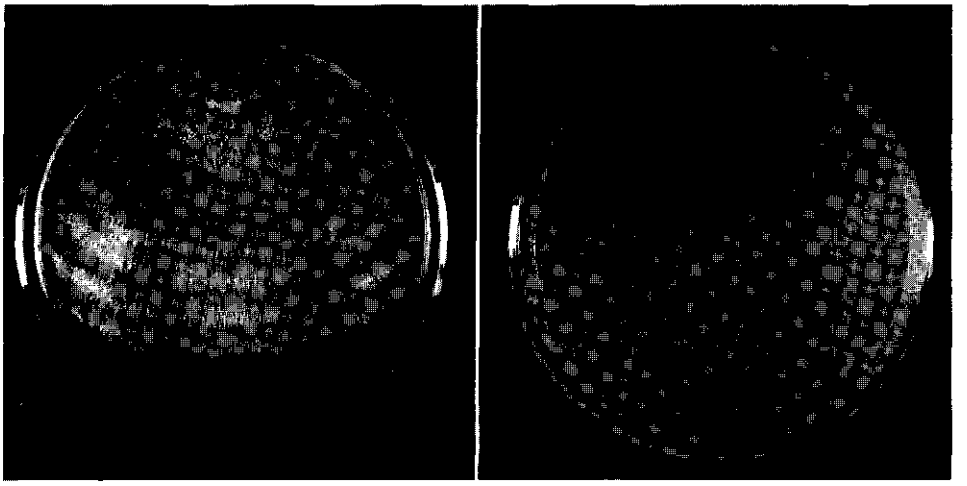

Fig. 1. Sclerotium formation by *R. solani* AG-3 on a 90-mm malt peptone agar plate at 20 °C: the same culture with immature light brown sclerotia at 6 days after inoculation (left) and with black mature sclerotia at 20 days after inoculation (right).

farther than 80 mm from the inoculation site. After 4 days of growth on agar media, white sclerotial initials were visible and at day 8 all sclerotia were black and solid regardless of dish size, type of medium or isolate tested (Fig. 1). Sclerotium production on MPA stopped at day 9, even when the colony had not yet reached the edge of the plate in the 150-mm dishes. Thus, on agar media, initiation and maturation of sclerotia was not associated with linear growth.

In addition to providing a surface, tubers probably offer nutrients that facilitate sclerotium production (Allington, 1936). Since black scurf stimulation is not visible until 7 to 10 days after haulm destruction, the time it takes before a response of *R. solani* can be observed on agar media after a sudden supply of nutrients was determined. On day 0, 90-mm Petri dishes with WA or MPA, were covered with a hydrophylic membrane filter (Sartorius SM 11107, cellulose acetate, 0.2 μm pore diameter) and inoculated with isolate 05AHa. On day 4 the filter with mycelium was transferred to either WA or MPA. Growth and sclerotium production were evaluated on day 8. Mycelium transferred from WA to WA or from MPA to MPA developed and produced sclerotia as described above. An increase in nutrient supply (transfer from WA to MPA induced formation of many hyphal branches and many sclerotia within the next four days. The mycelium formed a dense mat and sclerotia were scattered all over the colony and not restricted to the site of inoculation as is usally the case on MPA (Fig. 2: dish 5 and 6). Therefore, it is improbable that a local check of mycelial growth induces sclerotium formation on agar plates, as suggested by Townsend (1957). A reduction in nutrient supply (transfer from MPA to WA) reduced final sclerotium dry weight, but did not affect their location within the colony (Fig. 2: dish 7 and 10). Neither the time of initiation nor the period of time needed for maturation of the sclerotia was affected by these alterations in nutrient supply. Thus, on agar media, stimulation of sclerotium production occurred within 4 days after an increase in nutrient supply.

On liquid MP, a large black sclerotial crust, surrounded by smaller crusts, was form-

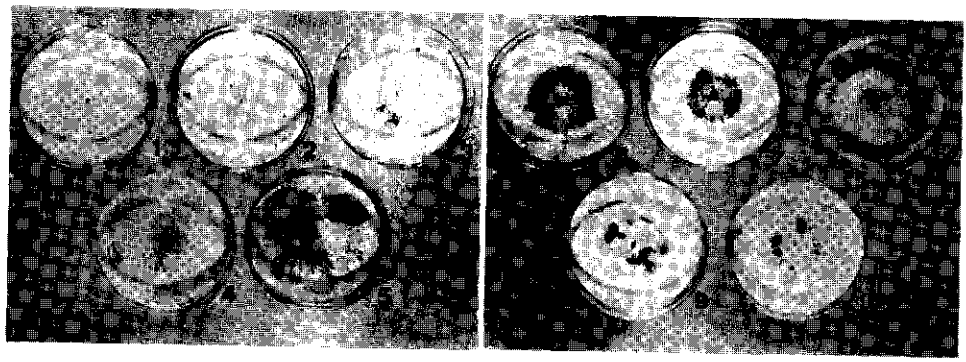

Fig. 2. Sclerotium formation by *R. solani* AG-3 isolate 05AHa (Rs) at 12 days after inoculation on day 0 on acetate cellulose membrane filters placed on either water agar (WA) or malt peptone agar (MPA) and transferred on day 4 to another plate. History of the plates:
1) inoculated on WA and kept on the same plate;
2) inoculated on WA and transferred to an empty dish;
3) inoculated on WA and transferred to a fresh WA plate;
4) inoculated on WA and transferred to an 'old'* MPA plate;
5) inoculated on WA and transferred to a fresh MPA plate;
6) inoculated on MPA and kept on the same plate;
7) inoculated on MPA and transferred to an empty dish;
8) inoculated on MPA and transferred to a fresh MPA plate;
9) inoculated on MPA and transferred to an 'old'* WA plate;
10) inoculated on MPA and transferred to a fresh WA plate.
* 'old' = previously used for 4 days by another Rs culture.

ed at the site of inoculation. These sclerotial crusts exuded large brown droplets and continued to grow for at least two weeks longer than those on agar media. Thus, availability of water also increased final sclerotium dry weight, altered sclerotial distribution pattern within the colony and did not affect the moment of sclerotial initiation nor the period of time needed for maturation.

In order to investigate possible effects by degeneration products, filter plus mycelium were removed from the agar plates after 10 days and the plates were re-inoculated. In general, the appearance of the second culture on the same plate, did not differ from the first one (see also Fig. 2: dish 4 and 5 as compared to 9 and 10). Continuous growth on the same plate resulted in sclerotia on and around the first dense zone, whereas transfer to fresh medium on day 4 produced sclerotia in the second dense zone (Fig. 2: dish 6 and 8). Therefore, sclerotium production does not seem to be induced by a local check of mycelial growth as suggested by Townsend (1957) nor by degeneration products.

Sclerotium formation on tubers. When growing tubers were inoculated with mycelium on WA, white initials became visible within 4 days and these became mature sclerotia within the next 4 days, similar to the pattern obseved on agar plates. In infested sand, black sclerotia gradually developed on the growing tubers and white initials were seldomly observed suggesting that maturation occurs more quickly in vivo than in vitro.

Cutting-off shoots (COS) stimulated not only sclerotium formation (Dijst, 1985), but also growth of mycelium on the tubers. At the time of haulm destruction, some

hyaline hyphae and a few tiny black sclerotia were found on infested tubers, which became embedded in a mass of mycelium in which the first brown hyphae appeared after 8 to 10 days. With each following day, fewer hyaline hyphae and more brown hyphae and black sclerotia appeared. The sudden production of hyphae and sclerotia after COS seemed analogous to the sudden production of sclerotia on agar all over existing mycelium after a supply of nutrients.

On young growing tubers, sclerotia were never observed on or beside lenticels. However, on older mature tubers and after COS, sclerotia were found all over the tubers, showing no definite preference or aversion for lenticels; and lenticels seemed to be 'explored' by runner hyphae (Fig. 3). This indicates that inhibitory factors may be reduced when tubers mature.

Furthermore, the study examined whether the stimulation was initiated by a single signal from the tubers shortly after COS. In a glasshouse experiment, tubers were inoculated at 0, 9 or 13 days after COS and were either left attached to the plant or their stolon was broken. The inoculated tubers were covered by wet coarse perlite with a plastic sheet folded loosely on top to keep the perlite moist. Data of two experiments were similar and are shown in Table 1. Sclerotium production on both attached and detached tubers was very high when incubated in pots with COS plants, low in pots with untreated plants, but very low in boxes without plant material. At harvest, water content

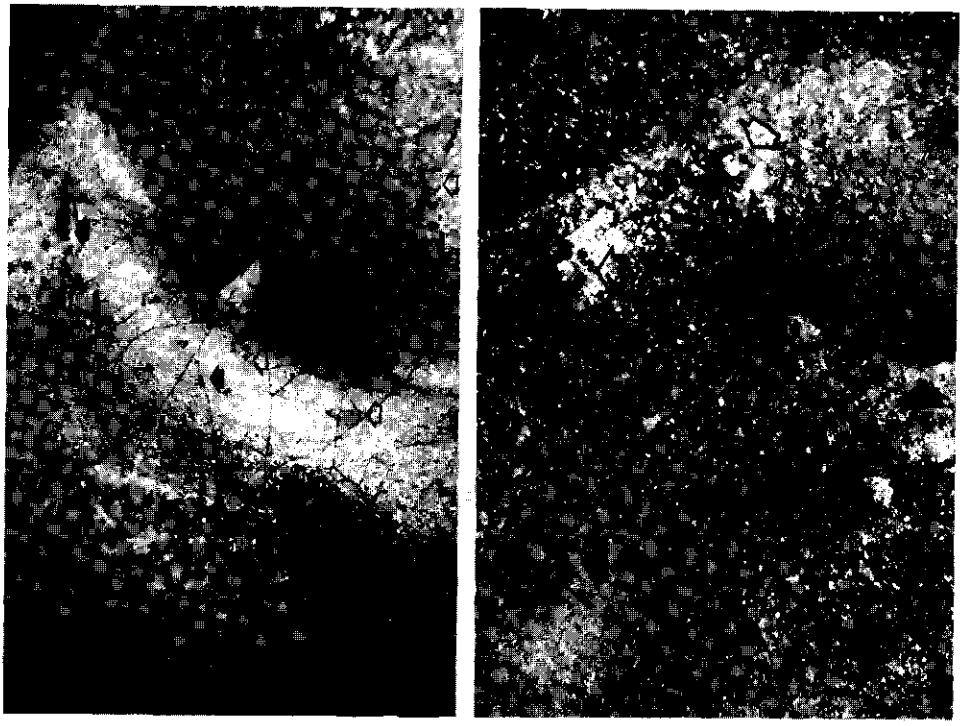

Fig. 3. Surface of mature tubers with lenticels (arrow) fully or partially sclerotized (black arrow) by *R. solani* AG-3 and brown runner hyphae around an eye (left) and, at higher magnification, elswhere at tuber surface (right).

Table 1. Final sclerotium dry weight of *R. solani* on potato tubers (ng mm^{-2}) after three weeks of incubation in boxes or in pots with plants with shoots either untreated or cut off; tubers remained attached to the plants or stolons were broken.

Plant treatment	Inoculation time[1]							
	tubers incubated (still attached or loose) in pots with plants						in a box without plants	
	one tuber/pot				two tubers/pot			
	0	9	13	13	13		9	13
	att.	att.	att.	loose	att.	loose[2]	loose	loose
Experiment 1[4]								
Shoots untreated	473 b[3,4]	154 b	—	—	—	—	112 b	—
Shoots cut off	6969 a	10329 a	—	—	—	—	166 b	—
Experiment 2[5]								
Shoots untreated	—	—	701 cd[3,5]	710 bc	661 cd	1183 bc	—	282 d
Shoots cut off	—	—	3052 a	2271 a	1435 ab	1851 ab	—	76 e

[1] Number of days after the day of haulm destruction.
[2] Loose tuber taken from plants of same age where shoots had been cut off.
[3] Analysis of variance was carried out per experiment over ln(WSA + 1) and indicated differences were either not significant (NS) or significant at P = 0.05 (*), P = 0.01 (**) or P < 0.01 (***).
[4] Per 'Tuber treatment' (incubation in plant pot from day 0 or 9, or in box from day 9) values followed by the same characters are not significantly different at P = 0.05, significances being **, *** and NS, when testing 14, 10 and 25 tubers, respectively.
[5] Split-plot design, comparing 15 tubers per plant treatment × tuber treatment combination. Significances: plant treatment = NS; tuber treatment = ***; plant treatment × tuber treatment = ***. Values followed by the same characters are not significantly different at P = 0.05.

of the perlite was the same in the plant pots and in the separate boxes suggesting that volatile exudates of underground plant parts affect black scurf development. On a loose COS tuber incubated near an attached UNTR tuber less sclerotia developed (1183) then when incubated in a separate pot (2271). Black scurf development on the UNTR tuber was not affected by the near presence of a loose COS tuber. Thus, growing tubers seem to exude a volatile inhibitor which can suppress the stimulation of black scurf on COS tubers. All results were identical regardless of the time of inoculation. Thus, the possibility that a short term trigger is involved seems unlikely.

Influence of damage and wounding on black scurf formation. When grown in infested perlite, enormous amounts of sclerotia were produced on all underground potato plant parts, including roots. Tuber periderm appeared to be damaged by the sharp granules. In order to investigate the effect of alterations in surface structure on black scurf development, tubers were partly wounded by stripping off the periderm while they were still attached to the plant. Wound periderm is produced within a few days where periderm is removed (Artschwager, 1927). Sclerotia remained immature on wounded sites regardless of plant treatment. On UNTR tubers, dry weight of sclerotia was less on wounded than on unwounded sites. On COS tubers, wounded sites often became completely

covered by a solid layer of immature sclerotia. This shows that growth and maturation of sclerotia are affected differently. For mature sclerotia only, black scurf index on unwounded and on partly wounded tubers was 4 and 13, respectively, with UNTR tubers and 6 and 33, respectively, with COS tubers. Thus, on damaged tubers the production of mature sclerotia is enhanced.

Discussion

Sclerotium production in vitro. Allington's observation (1936) that sclerotium production starts after termination of linear hyphal growth does not apply to the isolates used in my experiments. No effect of degeneration products was observed on agar media, which confirmed the results of Allen and Haenseler (1935). Neither restriction of linear growth nor local inhibition of hyphal growth nor deprivation of nutrients stimulated sclerotium formation by *R. solani* AG-3. Thus, sclerotium production is not associated with starvation as has been reported for other sclerotium-forming fungi (Townsend, 1957; Chet and Henis, 1975).

By others (Wheeler and Walker, 1965; Christias and Lockwood, 1973) deprivation of nutrients has been reported to induce sclerotium formation on liquid media both in *R. solani* and in other sclerotium-forming fungi, while a supply of nutrients delayed it. In my experiments, however, a decrease of nutrients reduced the amount of sclerotia and a supply enhanced it; the time of initiation and the speed of maturation were not affected.

It is not yet possible to explain the discrepancies in the results observed by the various research workers. Christias and Lockwood (1973) used a different medium, namely potato dextrose broth, which may be less suitable for studying the development of sclerotia by *R. solani*. In my experiments, isolates kept on MPA still produced mature sclerotia after five years, but not if kept on PDA. Furthermore, my isolates behaved differently on liquid than on agar media. Townsend (1957) reported that it is not the total amount, but the composition of nutrients which plays a role.

Sclerotium formation on tubers. Tuber physiology and environmental conditions influence development of black scurf (Dijst, 1985). During tuber maturation, several factors influencing black scurf development may change. The production of sclerotia on wound periderm and on artificial medium indicates that the surface structure is not a major critical factor, as suggested by Armentrout et al. (1986), although skin roughening may facilitate it. On young growing tubers, inhibiting exudates may reduce the formation of sclerotia around lenticels. Stimulatory circumstances may be created by mature tubers. This can be inferred from the fact that black scurf was not significantly reduced on loose mature tubers that were placed close to growing tubers, which seem to produce inhibitors. Finally, volatiles from underground plant parts may facilitate sclerotium formation, since more black scurf developed on detached tubers when incubated in pots with plants than on those in boxes without plant material.

The higher incidence of black scurf after chemical haulm destruction than after haulm pulling may be due to increased exudation of nutrients, water and factors caused by stress. The increased formation of sclerotia after tissue damage or nutrient supply to the agar medium is in agreement with this view. The results from in vitro tests suggest that slight increases in exudation of water might facilitate uptake of nutrients and thus

enhance black scurf, but no evidence for such a relation with water was found in the field (Dijst, 1985; Dijst et al., 1986).

Stimulation of black scurf probably starts within 3 to 7 days after halum destruction. This can be inferred from the fact that a sudden increase in mycelium and black sclerotia appears on growing tubers between 7 and 10 days after haulm destruction but on agar plates a similar increase occurred within 4 days after a supply of nutrients. After COS, more black scurf developed than on untreated plants regardless whether tubers had been inoculated at 0, 9 or 13 days after the day of COS. Thus, no evidence was found for a short-term trigger to initiate the stimulation of black scurf formation after haulm destruction. Furthermore, this result indicates that an estimation of the inoculum density at the time of haulm destruction does not allow a prediction of black scurf development.

Haulm killing probably affects two different processes. First, initiation of sclerotia may be stimulated by an increase in the amount of a 'hyphal extension inhibitor', a fungal product reported to repress hyphal extension but not the total hyphal growth (Shibata et al., 1980b) or cAMP (Hashiba and Ishikawa, 1978). Secondly, haulm killing may increase the availability of nutrients or stress components which stimulate the growth and maturation of sclerotia. The second process is suggested by the experiments in vitro with nutrient supplements and in vivo by wounding of tubers.

Hyphal extension and hyphal growth are two separate processes influenced by different factors as appeared from studies with various carbon sources (Armentrout et al., 1986) and with validamycin on inhibition of hyphal extension (Shibata et al., 1980a). From these reports it may be concluded that tests with nutrients may serve to study sclerotial growth, but are useless to investigate sclerotial initiation.

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Samenvatting

Vorming van sclerotiën door Rhizoctonia solani op kunstmatige voedingsbodems en op aardappelknollen

Op voedingsbodems werd de sclerotiënvorming door *Rhizoctonia solani* Kühn AG-3 niet beïnvloed door een beperking van de hyfengroei. Wegnemen of toedienen van een voedingsbron verminderde resp. vermeerderde de totale myceliumgroei en sclerotiumvorming en de mate van vertakking van de hyfen, maar had geen effect op het tempo van initiatie en afrijping van de sclerotiën. De vorming van sclerotiën houdt dus geen verband met het afsterven van de kolonie. Het pas later toedienen van voeding aan mycelium gaf een ander verspreidingspatroon van de sclerotiën dan een continu voedingsaanbod.

Bij jonge groeiende knollen lijken, vooral bij de lenticellen, lakschurft-remmende factoren aanwezig te zijn. Op afrijpende knollen lijkt de sclerotiënvorming vergemakkelijkt te worden doordat die remming afneemt en door stimulerende factoren. Vervolgens lijken vluchtige exsudaten van ondergrondse delen van de plant die toename in lakschurft verder te bevorderen.

Vanaf zeven tot tien dagen na loofvernietiging is een versnelde toename van hyfen

en sclerotiën op de knollen zichtbaar. In een myceliummat op wateragar ontstond een dergelijke snelle toename binnen vier dagen na een voedselgift. Dit doet vermoeden, dat stimulering van lakschurft op knollen binnen drie tot zeven dagen na loofdoding begint. De stimulering lijkt niet in gang te worden gezet door een kort signaal of schilverruwing, maar lijkt te berusten op een samenspel tussen verschillende veranderingen: afname in remmende factoren en toename in exsudatie van voedingscomponenten, water en/of stressfactoren. Stressfactoren zouden van invloed kunnen zijn, want na verwonding van knollen nam de sclerotiënvorming toe. Exsudatie van water en nutriënten kunnen mede van invloed zijn gezien de sterkere sclerotiënvorming op vloeibare dan op agarbodems en na een extra voedingsgift. De resultaten geven aan dat de ontwikkeling van lakschurft na loofdoding niet kan worden voorspeld uit de lakschurftindex of inoculumdichtheid op de dag waarop het loof gedood wordt.

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Effect of periderm and water-soluble exudates of potato tubers on black scurf formation before and after haulm destruction

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Abstract

Tuber components which may account for the acceleration of black scurf formation after haulm destruction were investigated.

Non-water-soluble components of the tuber periderm (NWSPC) seemed to promote the initiation of sclerotia and the pigmentation of hyphae of *Rhizoctonia solani* AG-3, but they did not induce maturation or affect growth of the sclerotia on tubers, regardless whether plants were untreated or shoots had been cut off (COS). After COS, hyphae became pigmented, but no sclerotia were initiated on hydrophilic filters which prevented hyphae to touch the skin of non-harvested tubers. No evidence was found that these NWSPC, skin surface structure, or residues of water-soluble exudate on skin play a major role in the stimulation of black scurf formation after COS.

Precipitated water-soluble tuber exudate (PWSTE) did not promote the formation of sclerotia on agar plates or on periderm strips, even if sampled after COS. On plates with PWSTE sclerotia formed were of a more solid structure but no real black sclerotia or brown hyphae developed. After COS, PWSTE became more light in colour and higher in osmotic value, but did not significantly change in pH, C/N ratio, amino acid content, content of some sugars or its cotton wool-like appearance. After COS, but not after haulm pulling, which breaks the stolons, PWSTE gave rise to a higher yield in dry weight and, more sand sticked to the tubers. This suggests that PWSTE may act as a glue which keeps more sand and sclerotia firmly attached to the skin at harvest after COS.

From these observations it can be inferred that volatile or instable components (VIC) probably govern hyphal pigmentation and growth of sclerotia and play a role in sclerotial pigmentation. After COS, alterations in VIC seem to play a major role in the stimulation of black scurf. Within three days after COS, skin set was significantly increased, which may reduce exudation of components that inhibit the formation of black scurf.

Additional keywords: Rhizoctonia solani AG-3, skin set, *Solanum tubersum*, tuber maturation.

Introduction

The production of sclerotia on the surface of potato tubers by *Rhizoctonia solani* Kühn AG-3 (black scurf) is accelerated by changes in the tubers after haulm destruction (Dijst, 1985). Haulm destruction also accelerates tuber maturation, which causes skin set, alters the surface structure and changes exudation (Burton, 1966). Black scurf formation is stimulated more by chemical haulm destruction (CHD) or cutting off shoots (COS)

than by 'haulm pulling', which breaks the stolons (Dijst et al., 1986). This difference seems to be caused by differences in tuber exudation (Dijst, 1988). It was the objective of the present study to investigate the possible role of tuber periderm and of water-soluble tuber exudates in the stimulation of black scurf after haulm killing. A preliminary report has been published (Dijst, 1987).

Material and methods

Isolates, media, inoculation. As 'minimal medium' (MM) a fourfold dilution was used of a medium described by Townsend (1957); it contained per litre demineralized water: 0.875 g KNO_3, 0.438 g KH_2PO_4, 0.188 g $MgSO_4$, 0.255 glucose and 15 g agar. Other media, isolates of *Rhizoctonia solani* AG-3, inoculum and inoculation thechniques have been described before (Dijst, 1988).

Assessment of sclerotium production. The production of sclerotia by *R. solani* on tubers was measured as described previously (Dijst, 1988); sclerotium production on agar was measured after 10 days of incubation at 20 °C in 50-mm Petri dishes.

Growth and sampling of plants. Potato plants cv. Pimpernel were grown from stem cuttings in a two-compartment system which allows access to tubers without disturbing root growth. Details have been described previously (Dijst, 1988). Samples were collected from 10 plants (i.e. about 12 tubers) per treatment, using new plants on each sampling date, comparing plants with shoots untreated (UNTR) and shoots cut off (COS) of the same age.

Assessment of skin set. Immediately after harvest, sections taken from the middle of the tubers, were stained for 10 min in Sudan 4, saturated in 70% ethanol and washed in 70% ethanol. The number of red suberized cell layers was counted.

Sampling periderm and sub-periderm. Periderm was added onto agar media as strips, either unwashed or washed three times in 100 ml demineralized water using ultrasonic vibration. Wash water, concentrated by evaporation at 60 °C, was added on plates as small droplets. The 2-mm-thick layer of sub-periderm tissue was ground in liquid nitrogen and placed on agar, either unfractionated or after fractionation by centrifugation for 15 min at 27000 *g*. To plates with non-liquid samples 0.5 ml demineralized water was added. The amount of each sample added per plate was equivalent to 1000 mm^2 tuber surface.

Collection of water-soluble exudates. Tubers still attached to the plant, were incubated for 30 min at 10 °C in enough demineralized water to completely cover the tuber surface in a glass beaker of minimal size. Control solutions were produced using sand in order to correct for the effect of the sand adhering to the tubers. Exudates were filtered through paper and cellulose acetate filters (Sartorius, SM11107, with 0.45 μm pore diameter) and frozen; freeze-dried precipitates were kept at −20 °C under vacuum until use. In order to obtain enough exudate for reliable tests samples from several sampling dates were merged, taking into account the tuber surface area sampled.

Determination of amino acids, proteins and sugar content. For analysis of amino acids an analyser was used, based on ion exchange chromatography (Waters Chromatography Div., Milford, MA, USA) using post column OPA detection. Prior to analysis samples were purified on SEP-PAK C-18 (Waters Chr. Div.). Protein content was measured spectrophotometrically according to Bradford (1976). Sugar content was measured with the single reagent tests of Boehringer Mannheim (Anonymus, 1980).

Measurement of C/N ratio. Carbon and nitrogen contents of dry precipitates were measured by gas chromatography. Helium was used as flow gas at a flow rate of 60 ml min^{-1}. For destruction at 1000 °C oxygen was added at a flow rate of 20 ml min^{-1} in a reduction column (Cu, 0.6 mm i.d.) of quartz wool (Merck). Components were separated on a Porapack QS column, 2 m length, 5 mm i.d., 50-80 mesh, at 110 °C oven temperature. As a reference 5-chloro-4-hyroxy-3-methoxy-benzyl isothioureum phosphate was used.

Results

Tuber periderm. In order to investigate how quick skin set is accelerated by haulm killing, periderm suberization was assessed at several intervals after COS or haulm pulling. If skin set had started prior to the day of haulm destruction, no acceleration was observed thereafter. In two experiments skin set had not started yet at the day of haulm destruction and an acceleration of skin set appeared within three days after COS or haulm pulling. This result is shown for one experiment in Table 1.

It was then investigated whether black scurf formation is stimulated because of alterations in exudation, skin surface structure or chemical composition of the skin as a result of this quick skin set. Therefore, tubers, periderm strips, ground sub-periderm or droplets of wash water from periderm, sampled at 3, 14 or 21 days after COS, were placed on inoculated agar plates. These samples gave similar results on water agar (WA) and on MM. Regardless of COS, hyphae pigmented only on the surface of tubers and periderm strips, and sclerotia only matured on tubers. On the periderm strips sclerotia remained

Table 1. Tuber skin set after haulm destruction, expressed as the number of suberized cell layers in the tuber periderm of cv. Pimpernel.

Plant treatment	Number of days after haulm destruction						
	1	2	3	4	6	8	10
Shoots untreated	6.2[1]	5.5	6.3	6.1	5.8	7.6	7.2
Shoots cut off	5.7	5.5	7.6	7.9	8.0	6.9	8.8
Significances[2]	NS	NS	*	**	**	NS	**

[1] Means of four tubers from two plants per treatment. Per tuber the mean value was used from four sections taken from the middle of the tubers.

[2] Per date differences were either not significant (NS) or significant at P = 0.05 (*) or P = 0.01 (**). For all dates: Plant treatment = **, Sampling date = **, Plant treatment × Sampling date = NS, with LSD (5%) = 1.15, LSD (1%) = 1.53.

light brown and of a loose structure. On plates with periderm strips the sclerotia were located only on the strips and equally often on both sides, regardless of the date of sampling or plant treatment before sampling. Thus, regardless of COS, non-water-soluble periderm components seemed to promote sclerotial initiation and hyphal pigmentation, but not the maturation or growth of sclerotia. In Petri dishes, final sclerotium dry weight on agar plates was higher when tubers were placed on the agar, but not if washed or unwashed periderm strips or periderm wash water from untreated plants was added. Data of one out of three replicate trials are shown in Table 2. When sampled after COS, tubers, periderm and periderm wash water sometimes induced a higher amount of sclerotia on the plates as compared to the UNTR. However, this result was found at a different sampling date in each trial. Supernatant of centrifuged sub-periderm, which may contain residues of water-soluble exudate, never increased sclerotium formation, regardless of COS. Thus, no major role in the stimulation of black scurf after COS seems to be played by alterations in surface structure or chemical composition of the periderm or in water-soluble components from the periderm.

In order to further investigate the effects at the tuber surface, cellulose acetate filters

Table 2. Sclerotium dry weight (mg) produced by *R. solani* AG-3 on water agar supplied with tuber tissue, periderm or sub-peridermal tissue sampled from 1000 mm^2 tuber surface of untreated control plants (UNTR) or plants with cut off shoots (COS).

Sample	Number of days after COS					
	3		14		20	
	UNTR	COS	UNTR	COS	UNTR	COS
None (Control)	0.4 f[3]	0.4 f	0.1 d[3]	0.1 d	0.1 d[3]	0.3 cd
Tuber	2.7 e	2.8 de	5.7 b	7.8 b	2.3 b	40.5 a
Periderm untreated	0.5 f	0.5 f	0.6 cd	0.4 cd	—	—
Periderm washed[1]	0.4 f	3.5 d	0.4 cd	0.4 cd	0.4 cd	0.3 cd
Wash water periderm[2]	0.6 f	3.1 de	0.3 d	0.2 d	—	—
Sub-peridermal layer untreated	40.3 b	13.9 c	34.1 a	26.8 a	—	—
Pellet of sub-peridermal layer	12.3 c	105.7 a	16.9 ab	14.0 ab	—	—
Supernatant sub-peridermal layer	3.5 d	0.8 f	1.0 c	0.2 d	0.7 bc	0.4 cd
Periderm washed + supernatant of the sub-peridermal layer	0.8 f	2.9 de	6.7 b	0.2 d	0.6 c	0.2 cd

[1] Periderm was washed in demineralized water with ultrasone vibration.
[2] Wash water was concentrated by evaporation at 60 °C.
[3] Per sampling date values followed by identical characters are not significantly different at $P = 0.05$, according to analysis of variance after transformation to natural logarithm.
Differences were found to be: NS = non significant or *** = significant at $P < 0.01$.
Significances: Plant treatment = *** (at dates 3 and 14) or NS (at date 20), Sample type = *** and Plant treatment × Sample type = *** at all dates.
Data are averages from five replicates tested on water agar.

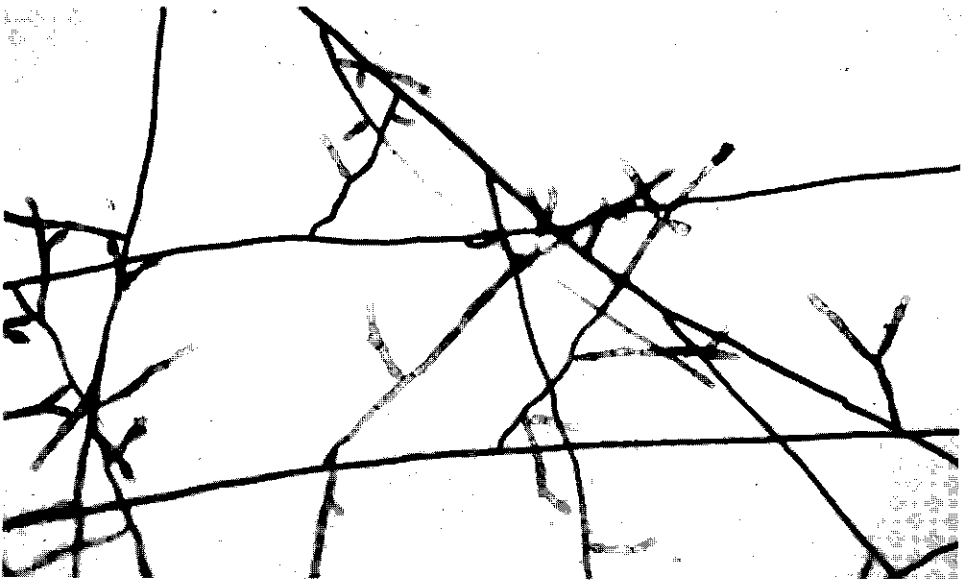

Fig. 1. Brown, sometimes shortly branched hyphae of *R. solani* AG-3 developed on cellulose acetate filters wrapped around tubers still attached to potato plants after shoots were cut off.

(Sartorius, SM11107, pore diameter 0.2 μm, 150 mm in diameter) with fungal inoculum on the outside, were wrapped around tubers that were still attached to the plant. The filters never covered more than 60% of the total tuber surface in order not to hinder tuber respiration and create stress. The tubers were covered up with perlite or sand and the filters were sampled after two weeks. Perlite or sand gave similar results even if the experiments started at 3, 7 or 10 days after COS. Hyaline hyphae without sclerotia developed all over the filters, and only after COS also brown hyphae with both long and short internodes were formed at the sites of close contact between filter and tuber surface (Fig. 1). After COS, significantly more brown hyphae developed when there was a 2-mm-thin layer of WA between filter and tuber, but sclerotia never developed. In contrast, black sclerotia were always formed on filters incubated on WA without plant material. This leads to the conclusion that components, exuded from UNTR tubers, inhibted pigmentation of mycelium and initiation of sclerotia and that after COS only sclerotial initiation is still inhibited. Contact between hyphae and periderm seems to be required to overcome these inhibitive effects.

Water-soluble tuber exudates. In order to investigate whether stable water-soluble exudates from harvested tubers may stimulate sclerotium formation after COS, harvested tubers were placed on WA for some days. After they had been removed, the agar was inoculated, but mycelium and sclerotia developed as usually. Then, the effect of freeze-dried precipitated water-soluble tuber exudate (PWSTE) on sclerotium formation was investigated. The PWSTE obtained, resembled cotton wool and did not stick to the glass. As compared to UNTR, after COS, the PWSTE was more difficult to redissolve in water, lighter in colour, slightly lower in pH and higher in osmotic value at days 0-9,

Table 3. Dry weight (μg) and carbon and nitrogen content of precipitated water-soluble exudates and dry weight (mg) of sand adhering to the tubers, expressed per 1000 m^2 tuber surface.

Exudate source, Plant treatment	Number of days after haulm destruction			
	9	13	17	13-17
Dry weight (μg):				
Tubers, Shoots untreated	661	381	407	546
Tubers, Shoots cut off	570	434	560	734
Sand (500 mg)	—	15	22	—
% N: (means of two sub-samples)				
Tubers, Shoots untreated	1.17	1.10	0.87	—
Tubers, Shoots cut off	1.19	1.12	0.89	—
Sand (500 mg)	—	1.11	1.02	—
(sd)	(0.04)	(0.66)	(0.06)	
% C: (means of two sub-samples)				
Tubers, Shoots untreated	18.95	15.93	13.30	—
Tubers, Soots cut off	16.00	14.60	12.60	—
Sand (500 mg)	—	10.30	8.30	—
(sd)	(2.50)	(2.94)	(3.06)	
C/N: (means of two sub-samples)				
Tubers, Shoots untreated	16.04	14.56	15.28	—
Tubers, Shoots cut off	13.49	13.29	14.24	—
Sand (500 mg)	—	9.28	8.08	—
(sd)	(2.39)	(3.23)	(4.59)	
Dry weight (mg) of sand adhering to tubers:				
Tubers, Shoots untreated	672	260	187	245
Tubers, Shoots cut off	700	513	494	522
(sd of five day-samples)				(205)

slightly lower in carbon content and C/N ratio. After COS, the PWSTE was significantly higher in dry weight and significantly more sand adhered to the tubers than after haulm pulling or when plants were untreated. No differences were detected in nitrogen, protein and sucrose content and no fructose, glucose or amino acids were detected (Table 3 and 4).

When PWSTE was added onto inoculated artificial media or periderm strips, neither growth or pigmentation of mycelium, nor the initiation, growth or pigmentation of sclerotia was affected (Table 5). However, the few, on WA normally wool-like-soft sclerotia, became of a more solid structure when PWSTE was added to the agar plates.

Discussion

Pigmentation of mycelium was induced on agar plates by washed and unwashed periderm strips but not by precipitated water-soluble tuber exudates (PWSTE), regardless of plant treatment before sampling. When contact between hyphae and the periderm

Table 4. Colour, pH, osmotic value (mosmol), and content of protein (mg), amino acids (ng) and sucrose (μg) in precipitated water-soluble tuber exudate[1] sampled at different intervals after haulm destruction.

Exudate source, Plant treatment	Colour	Number of days after haulm destruction					
		0-7		0-9		13-17	
		amino acids	sucrose	mosmol	pH	pH	protein
Tubers, Shoots untreated	brown	0	13.33	71	8.7	9.4	12.2
Tubers, Shoots cut off	white	0	8.05	105	8.3	9.5	14.6
Sand (500 mg/1000 mm^2	—	0	0	0	7.2	8.8	0
(sd)				(17)	(0.15)	(0.15)	

[1] Exudates sampled represented 2000, 16000, 1000, 1000, 2000 and 8000 mm^2 tuber surface for estimating amino acid, sucrose, osmotic pressure, pH (day 0-7), pH (day 13-17) and protein content, respectively, and were redissolved in 0.5 ml water for measuring pH and mosmol (equivalent to g NaCl per kg water).

Table 5. The amount of sclerotia (μg) produced by *R. solani* AG-3 on water agar, without or with tuber periderm strips (c. 750 mm^2), supplemented with 0.5 ml redissolved water-soluble exudates (from 2000 mm^2 tuber surface area) sampled between 13 and 17 days after haulm destruction.

Exudate source, Plant treatment	Exudate redissolved in phosphate buffer (pH 7)		Exudate redissolved in water	
	without periderm	with periderm	without periderm	with periderm
Tubers, Shoots untreated	90 a[1]	4 b	200 a	6 c
Tubers, Shoots cut off	90 a	2 bc	55 b	2 c
Sand (500 mg)	74 a	2 bc	181 a	3 c
Water	134 a	1 c	330 a	1 c

[1] Per experiment values followed by identical characters are not significantly different. Analysis of variance were done after transformation to natural logarithms. Differences were either not significant (NS) or very significant ($P < 0.01$ = * * *). Comparing 6 replicates, Significances: Exudate = NS, Periderm = * * * and Exudate × Periderm = NS.

of growing tubers was prevented by filters, hyphae only pigmentated after COS. This leads to the conclusion, that pigmentation of mycelium is induced by non-water-soluble periderm components, regardless of plant treatment and that it is inhibited by tuber exudates both of UNTR and COS plants.

In line with previous results (Dijst, 1988), the data suggest that non-water-soluble periderm components, rather than surface structure, make tubers a place of preference

for sclerotium production. This was indicated by the fact that on agar plates supplemented with washed or unwashed periderm strips, sclerotia formed only on the strips and equally on both sides. Addition of PWSTE did not affect the distribution pattern, regardless of COS. Location of sclerotia can be affected by C source, pH and amino acids (Allington, 1936; Moromizato et al., 1980a, b) but after COS, PWSTE had not changed in these aspects.

For initiation of sclerotium formation on growing tubers, the hyphae need to make contact with the periderm, seemingly to overcome inhibitory effects of exudates. On plates, the number of sclerotia did not seem to be affected by periderm strips or by PWSTE. Therefore, initiation may be regulated by volatile or instable components (VIC) that become available at tuber surface.

Growth of sclerotia on agar media was not improved by periderm strips or by PWSTE. As suggested by Allington (1936), no stable stimulating compounds diffused from tubers into agar. On agar plates, maturation of the sclerotia seemed somewhat improved by PWSTE, for it induced sclerotia of a more solid structure, regardless of COS. On isolated periderm strips, growth and maturation of sclerotia were slightly inhibited and addition of PWSTE to the strips gave no improvement. This inhibition may have been caused by fungistatic compounds (Allen and Kuç, 1968; Shih and Kuç, 1973); Kannaiyan and Prasad, 1980). On wound periderm sclerotia also developed badly (Dijst, 1988), which may indicate that periderm is a nutrient source of sub-optimal composition (Townsend, 1957) to be supplemented by exudates. The results suggest that VIC stimulate growth and pigmentation of mycelium and of sclerotia. These VIC may be substances exuded by the tuber, but lost by the used procedure of sampling and precipitation, or may be substances produced at the tuber surface by decomposition of periderm and exudate components. After COS, PWSTE gave rise to a higher yield in dry weight than PWSTE after haulm pulling or from untreated plants. Because of the high pH and low C/N, the volatile decomposition products of PWSTE may promote survival of *R. solani* (Lewis, 1976) and thus a higher dry weight of PWSTE after COS may cause a higher production of the stimulatory VIC after COS than after haulm pulling.

The results support the idea (Dijst, 1988) that stimulation of black scurf after COS is caused by an alteration in a ratio of inhibitory and stimulatory factors. The periderm might regulate this ratio. Crucial changes probably occur between 3 and 17 days after haulm destruction (Dijst, 1988). Skin set had increased at three days after haulm killing, which may then reduce exudation of inhibitory components. On growing tubers, sclerotia are readily formed in sunken eyes, having a single-layered epidermis with vascular tissue close underneath (Lyshede, 1977) where nutrients are more likely to exude than through the multilayered periderm (Dijst, 1988).

On young tubers sclerotia are not as strongly attached to the skin as on older tubers. After COS, significantly more sand kept attached to the skin. The cotton wool-like appearance of PWSTE indicates that it contains large molecules which might act as a 'gluing' factor. Large molecules are unlikely to have passed the suberized periderm and may be residues of disrupted outer cells.

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Samenvatting

De invloed van periderm en waterig exsudaat van aardappelknollen op de ontwikkeling van lakschurft voor en na loofvernietiging

Met het oog op de versnelde vorming van lakschurft na loofdoding werd de invloed onderzocht van periderm en waterig knolexsudaat op de sclerotiënvorming door *Rhizoctonia solani* Kühn AG-3.

Niet in water oplosbare componenten van epidermiscellen spelen waarschijnlijk wel een rol bij de initiatie van sclerotiën en bruine hyfen op de knollen, maar niet bij de groei en afrijping van de sclerotiën. Dit geldt zowel voor onbehandelde planten als voor planten waarvan het loof afgeknipt is (COS = cutting off shoots). De sterkere toename van lakschurft na COS kan evenwel niet verklaard worden uit veranderingen in de niet in water oplosbare schilcomponenten. Immers, wanneer filters waren aangebracht om de nog aan de plant vastzittende knollen, zodat direct kontakt tussen de hyfen en de schil was verhinderd, dan ontstonden er op die filters nooit sclerotiën en alleen na loofafknippen bruine hyfen. Verder werden er geen aanwijzingen gevonden dat veranderingen in de structuur van het schiloppervlak of in residuen van waterige exsudaten op de schil een rol spelen bij de stimulering van de lakschurftvorming na loofvernietiging.

De totale produktie van sclerotiën op kunstmatige media en op periderm strips werd niet gestimuleerd door de toevoeging van geprecipiteerd waterig knolexudaat (PWSTE = precipitated water soluble tuber exudates), ongeacht of de monsters waren genomen van onbehandelde planten dan wel COS-planten. Op agarplaten met PWSTE werden de sclerotiën wel compacter in bouw, maar ze werden niet echt zwart en er ontstonden geen bruine hyfen. Na COS was PWSTE lichter van kleur en hoger in osmotische waarde, maar er traden geen significante veranderingen op in C/N-quotiënt, pH, gehalte aan aminozuren, eiwitten en sommige suikers, en in het wollige uiterlijk van PWSTE. Na COS, maar niet na looftrekken, dat de stolon breekt, nam het drooggewicht van PWSTE per eenheid van knoloppervlak toe en bleef er bij de oogst meer zand aan de knollen plakken. Dit wijst erop, dat PWSTE als een hechtmiddel kan functioneren, waardoor, vooral na COS, bij de oogst meer zand en sclerotiën aan de knollen blijven zitten.

De resultaten doen vermoeden dat vluchtige of instabiele componenten (VIC) de pigmentatie van hyfen en de groei van sclerotiën induceren en een rol spelen bij de pigmentatie van de sclerotiën. Tevens lijken veranderingen in VIC een hoofdrol te spelen bij de stimulering van lakschurft na loofdoding. De schilverkurking, die binnen drie dagen na loofdoding significant was toegenomen, zou de exsudatie kunnen verminderen van lakschurftremmende componenten.

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6

EFFECT OF VOLATILE AND UNSTABLE EXUDATES FROM UNDERGROUND POTATO PLANT PARTS ON BLACK SCURF FORMATION BEFORE AND AFTER HAULM DESTRUCTION

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Abstract

Volatile exudates of non-harvested tubers affected the production of sclerotia by *Rhizoctonia solani* AG-3 on agar plates and on harvested tubers that were incubated within a plant-soil system with growing potato plants. Sclerotium production on incubated harvested tubers was much higher than that on agar plates and was least on periderm strips. This indicates that unstable substances in tuber exudate may also promote sclerotium formation.

The volatile tuber exudate from young plants contained inhibitory and stimulatory components as well. Inhibition dominated during the period of tuber growth, decreased when plants were yellowing and disappeared after haulm destruction. On agar plates or tubers incubated beside a beaker containing KOH, the non-trapped tuber volatiles from untreated plants stimulated sclerotium formation to the same extent as those from plants after haulm destruction. CO_2 might be an inhibitor as tuber respiration gradually decreased during tuber maturation and halved within two days after haulm killing, while black scurf increased gradually during tuber maturation and rapidly after haulm destruction. Volatiles from ripe apples significantly stimulated sclerotium production on agar media, but ethylene or volatiles from harvested tubers did not. The concentration of CO_2 and the food base available may have interfered with the effect of ethylene.

Volatile products of decomposing underground potato plant parts slightly increased the stimulatory effect of tuber volatiles from older control plants on sclerotium formation. For agricultural practice, this implies that after haulm destruction, loosening the soil for better aeration of the tuber vicinity as well as a quick separation of tubers from plant residues may prevent accumulation of the stimuli and thereby prevent extra black scurf development.

Additional keywords: carbon dioxide, C/N ratio, ethylene, *Rhizoctonia solani* AG-3, *Solanum tuberosum*, tuber respiration, tuber maturation, volatile apple exudates.

Introduction

Between 3 and 17 days after haulm destruction, the production of sclerotia by *Rhizoctonia solani* Kühn anastomosis group 3 (AG-3) on potato tubers is stimulated by alterations in the tuber (Dijst, 1988a). Previous observations (Dijst, 1988b) showed that contact between hyphae and a tuber surface is needed for the initiation of sclerotium formation on tubers. Stable water-soluble tuber exudate promoted a more solid structure in the sclerotia formed on agar plates, but did not affect their number, pigmentation or final dry weight. Pigmentation of hyphae and sclerotial growth on tubers appeared to be governed by volatile and unstable components which might be exudates or decomposition products thereof.
After haulm destruction, alterations in stimulatory and inhibitory volatile and unstable tuber products were suggested as playing a major role in the stimulation of sclerotium formation on tubers (Dijst, 1988b). Volatiles from other underground plant parts may also promote black scurf (Dijst, 1988a). The role of volatile products from the potato plant in stimulation of black scurf formation after haulm killing was investigated in this study. A preliminary report was published earlier (Dijst, 1987).

Material and methods

Isolates, media, inoculation and asssessment of sclerotium production. These have been described previously (Dijst, 1988b). For all tests, two-day-old cultures of *R. solani* AG-3 grown on water agar (WA) or on minimal medium (MM) in 50 mm diameter glass Petri dishes were used. All experiments were carried out at 18 °C.

Plants. Potato plants cv. Pimpernel were grown from stem cuttings as described previously (Dijst, 1988a). Glasshouse temperature was 18 ± 2 °C. Comparisons were made between untreated control plants (UNTR) and plants of which the shoots were cut off (COS) of the same age. The experiments were carried out with 10 to 12 weeks-old plants which had green shoots and 20 to 30 mm-long tubers.

Exposing cultures to volatile exudates of potato tubers.
A. Volatile exudates from harvested tubers and apples. Over a period of 18 days, inoculated plates, six with WA and six with MM, together with a wet sponge were incubated in 4 l metal boxes that were tape-sealed. Boxes contained either a ripe apple or 25 harvested tubers with a total surface area of about 76 000 mm² except for the control boxes which contained no apple or tubers. The tubers were either of cv. Bintje which had been stored for seven months, or of cv. Pimpernel, harvested on the first day of the experiment from UNTR and COS plants of which the shoots had been excised 18 days previously.
B. Volatile exudates from growing tubers. In order to test the effect of volatile exudates from underground potato plant parts on the production of sclerotia on agar plates, a set-up was used as shown in Fig. 1 for one out of eight 'treatments'. Plants were raised in 3-litre pots filled with steamed potting soil. On the first day of the experiment (day 0) four groups of pots were made: (a) pots with an UNTR

plant growing in potting soil, (b) pots with a COS plant in potting soil, of which the shoot was cut off on day 0, (c) pots without a plant but filled with potting soil on planting date and (d) pots without a plant but filled with wet coarse perlite on day 0. Half the pots in each of these four groups were then covered with the 'plant residues' i.e. roots, stolons and underground stem parts collected from extra plants on day 0. Per pot the 'plant residues' were used from one potato plant. Thus, eight 'treatments' were created, each with three replicates.
The surface of each pot was covered with a 50 mm thick layer of large polystyrene granules and the lower 15 cm of the shoots wrapped in aluminium, in order to prevent hyphae of exposed cultures reaching any biotic surface. Petri dishes with two-day-old cultures of *R. solani* were placed upside down on top of the polystyrene granules. Sclerotium formation on inoculated WA, periderm strips and harvested tubers were compared, using three replicates of each. Periderm strips and tubers were placed on inoculated WA and kept in place with an aluminium strip. Per plate, tubers and periderm strips had the same surface area as the agar, viz. 4 000 mm^2. Tubers and periderm had been collected on day 0 from the same plants as the 'plant residues'. Finally, non-airtight plastic bags were taped around each pot with plates.
In a second experiment a beaker with a saturated KOH solution was placed beside the plates. After three weeks of incubation, final sclerotium dry weight on the exposed agar, periderms strips and tubers was measured. Data from tubers and periderm strips were compared with those of the plates without recalculation, because the sclerotia had been formed only on the surface of the exposed tubers and periderm and not on the underlaying agar.

Collection of volatile tuber exudates. Plants were grown in a two-compartment system (Dijst, 1988a), using 10-litre pots in order to obtain many tubers per plant. After three months of plant growth the sand was removed from the upper compartment. Tubers, stolons and underground stems were enclosed in 10-l-Teco-polyethylene gas bags (PEt:Al:PE= 12:12:75) (Tesseraux, West Germany). Bags, provided with a gas septum and with a tap at both ends, were made airtight with Xantopren light body (ADA Spec.no.19 type II low viscosity, Bayer Dental), polymerized with Elastomer Activator Liquid. Around the stolons and tubers the headspace volume was 4.5 litres. The headspace was aerated for 20 min each 12 h at a flow rate of 65 ml min^{-1}. Six days later (day 0) shoots of two out of four plants were cut off. On day 0, the total surface (m^2) of the enclosed tubers was 0.025 and that of all enclosed tissues 0.043 per bag, increasing only for untreated plants to 0.046 and 0.055 per bag, respectively, on day 28. Gas bags were closed on 1, 7, 12, 18 and 27 days after haulm destruction for 840, 1 020, 900, 880 and 1 200 minutes, respectively. Bags were closed during dark periods except for night 12 when lights were left on accidentally. Volatiles were collected with a 100-ml Dräger hand-membrane pump. Immediately after collection, headspace and aeration were restored. On collection, volatiles were concentrated by adsorption on 100 mg Tenax GC adsorbent (Ta 20-35 mesh, 160 x 6 mm) at 18 °C.

Qualitative analysis of volatile tuber exudates. The procedure was based on previous descriptions (Varns, 1982; Waterer and Pritchard,

1984). Volatiles were removed from the Tenax adsorbent with a Thermodesorption Cold Trap injector (300 °C for 20 min in a lead block, desorption flow 35 ml min^{-1}; trapped at -70 °C. After heating at 200 °C for 5 min, injection took place. Samples were analysed on a gas chromatograph unit (GC) equipped with a Flame Ionization Detector (FID) (detector 200 °C), coupled with an electronic digital integrator. Components were separated on a capillary column type CP sil 19 CB, 25 m length, 0.32 mm internal diameter (i.d.), 0.2 μm film thickness. Helium was used as flow gas at a flow rate of 1.4 ml min^{-1}. The oven temperature programme was: 76 °C for 1 min, rise 2 °C min^{-1} for 15 min, iso 106 °C for 15 min, rise 20 °C min^{-1} for 3 min and iso 160 °C for the last 15 min. As a standard 0.09 μg phenanthrene gave 750 000 area counts at 160 °C and AT = 10 x 64.
In a second experiment samples were further analysed on a GS/MS unit. At collection, volatiles were concentrated on either 100 mg charcoal or on 100 mg Tenax (20-25 mesh). The adsorbent was transferred to 4 ml septum vials for desorption at 130 °C for 1 h. Subsamples of 0.5 ml were removed by syringe and analysed according to Varns and Glynn (1979): chrompack column 120, CP Sil 5 CB, 25 m length, 0.22 mm i.d., 1.3 μm df, carrier gas He, flow rate 250 mm sec^{-1}; temperature programme: iso 40 °C for 4 min, rise 2 °C min^{-1} for 20 min, rise 10 °C min^{-1} for the last 10 min.

Measurement of CO_2 production. Samples were taken by syringe and analysed on an Intersmat GC unit with Thermal Conductivity Detector, equipped with a stainless steel column, (external diameter 1/8 Inch, 3 m length, 80-100 mesh) filled with Porapack S at 85 °C oven temperature. Temperature of injection and of detection was 110 °C. H_2 was used as a flow gas at a flow rate of 6 ml min^{-1}.
Carbon dioxide production (Ps) was calculated using the formula:
$Ps = R.(Cs-Co).(Ts.As)^{-1}$ [mg l^{-1} h^{-1} m^{-2}] with As = tissue surface area (m^2) per bag, Co = concentration CO_2 (% v/v) of the blank, Cs = concentration CO_2 (% v/v) of the sample, Ts = period of time (hours) gas bag was closed and R = 82.92 = transformation factor from % (v/v) to mg l^{-1} at P = 101 320 Pascal, V = 45.10 (m^3), R = 8.314 (J Mol^{-1} K^{-1}) and T = 291 °K.
For comparison of volatile production, the tissue surface area (mm^{-2}) was used as a unit and not the tuber weight, because tuber surface area is also the unit for measuring black scurf formation and is thus adequate for evaluating the effects of exudate production on black scurf development. Moreover, tuber fresh and dry weights are not constant references, because tubers of equal size from the same plant can differ in age and thus in both water and starch content.

Measurement of ethylene production. Samples were taken by syringe and analysed on an Intersmat GC unit equipped with a FID and a stainless steel column (i.d. 4 mm, length 2.4 m) filled with Alumia GC (Chromopack, Middelburg). N_2 was used as a flow gas at a flow rate of 85 ml min^{-1}. The temperature of the oven was 105 °C, and that of injection and detection 140 °C.

Measurement of the C/N ratio and *statistical analysis* have been described previously (Dijst, 1988a, 1988b).

Results

Exposing cultures to volatile exudate from harvested potato tubers or apples or to ethylene. The object of this experiment was to investigate whether plant stress components promote black scurf formation after haulm killing as suggested previously (Dijst, 1988a). Ethylene is produced readily by any type of tissue under stress, e.g. by damage (Yang and Hoffman, 1984). It might therefore be produced by tubers when the plant shoot is destroyed while the underground plant parts are unaffected (Dijst et al., 1986). Therefore, cultures on WA and on MM were incubated in tape-sealed metal boxes together with tubers harvested from UNTR or COS plants. Because the assumed ethylene production of tubers might diminish after harvest, cultures on MM were also incubated with ripening apples that produce a large amount of ethylene.

In boxes with tubers, final sclerotium dry weight was 50 % lower than in control boxes without tubers. This inhibition appeared both on WA and on MM. In boxes containing an apple final sclerotium dry weight was significantly increased. On MM, sclerotia yielded 0.8 mg per plate in the control boxes and 2.2 mg per plate in boxes containing an apple (Least significant difference = 0.7 at $P<0.01$). The growth rate of mycelium and the period of time needed for the completion of sclerotium maturation were not affected by volatile exudates of apple.

In the next experiment, plates were incubated in 2.1-litre glass exsiccators with four different concentrations of ethylene between 0.005 and 1 ppm. The CO_2 concentration in all exsiccators increased within a week from 0.04 to 0.81 % (v/v). Therefore, each week the exsiccators were opened under the hood for 15 min and the ethylene concentrations readjusted. During three weeks of incubation sclerotium dry weight was not stimulated by any of the concentrations tested (Table 1). The sclerotium production, however, had been inhibited on MM by 1 ppm C_2H_4 and on WA by all concentrations tested. In exsiccators the sclerotium production on WA was inhibited even when no extra ethylene was added, whereas in tape-sealed metal boxes without extra ethylene no inhibition was observed. This difference may originate from an increase in the concentration of CO_2 which occurred in the exsiccators, despite the weekly aeration, and not in the boxes (Table 1). From these results with ethylene it may be inferred that the observed inhibition of sclerotium formation in boxes with potato tubers might be caused by an increased concentration of carbon dioxide. Thus, sclerotium production seems to be affected by the ratio between the concentrations of the several volatiles around in combination with the food base available, rather than by alterations in the concentration of one component alone.

Exposing cultures to the volatile exudate from growing tubers. In order to investigate the effect of volatile exudates from non-disturbed growing tubers in soil on the production of sclerotia, inoculated plates, periderm strips and harvested tubers were incubated in a soil or a plant-soil system as described under 'material and methods'.

Some of the results of two replicate experiments are shown in Table 2. Final sclerotium dry weight was significantly higher when incubated with COS plants than with UNTR control plants. A slight inhibitory effect appeared on WA when the plates were incubated with young green UNTR plants, whereas incubation with yellowing UNTR plants induced

Table 1. Final concentrations of ethylene and carbon dioxide and final sclerotium dry weight (μg) on wateragar (WA) and minimal medium (MM), after 21 days of incubation at 20 °C in exsiccators that were aerated and readjusted for initial ethylene concentration each week.

Experimental conditions					Final sclerotium dry weight on incubated agar plates	
Incubator	*Ethylene*		*CO_2 final*			
	initial	*final*			on WA	on MM
	ppm	*ppm*	*%(v/v)*	*mg/l*	μg	μg
Box	*0.005*	0.003	0.045	0.83	272 a	208 a
Exsiccator	*0.005*	0.002	0.091	1.68	26 b	201 a
Exsiccator	*0.040*	0.020	0.099	1.83	0 b	160 ab
Exsiccator	*0.200*	0.057	0.093	1.72	0 b	275 a
Exsiccator	*1.000*	0.511	0.101	1.86	35 b	49 b
Significances					*P=0.01*	*P=0.05*
Least significant difference					*78*	*142*

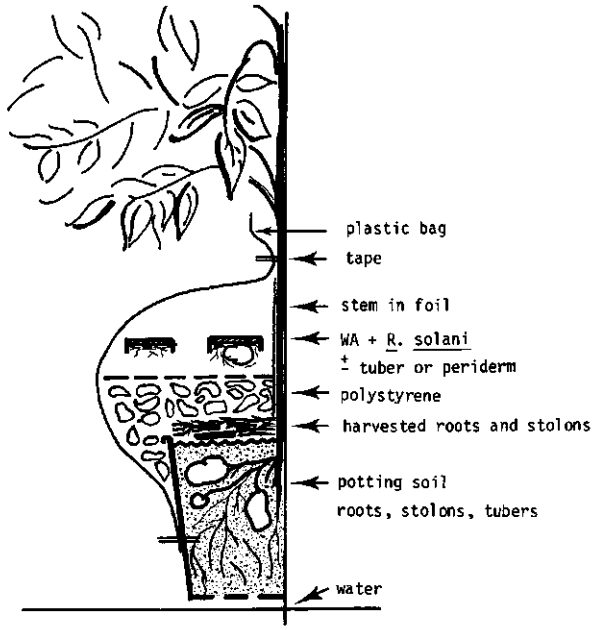


Fig. 1. The experimental design for testing the effect of volatile exudates from harvested and non-harvested underground potato plant parts on sclerotium formation by *Rhizoctonia solani;* here shown for a pot with an untreated potato plant in potting soil amended with decomposing roots and stolons.

Table 2. Sclerotium dry weight (μg per 1000 mm^2) on water agar (WA) or on the surface of harvested tubers or periderm strips placed on WA, measured after three weeks of exposure to volatile exudates from potting soil, perlite, or growing roots , stolons and tubers of potato plants cv. Pimpernel (with shoots untreated or cut off) and to volatile decomposition products from roots and stolons ('plant residues').

Volatile source in pots		Sclerotium dry weight in Petri dishes			
	Plant residues added	Experiment I[1]	Experiment II[1]		
		on WA	on WA	Tuber	Periderm
Perlite	-	-	24 ghi[2]	43 fg	10 jk
Potting soil	-	69 ab[2]	12 hijk	43 fg	1 lm
Plant, shoot intact	-	12 b	72 efg	760 ab	8 k
Plant, shoot cut off	-	142 a	584 bc	1225 a	21 ijk
Perlite	+	-	20 hij	74 def	0 m
Potting soil	+	-	8 kl	18 ghi	0 m
Plant, shoot intact	+	26 b	538 bcd	971 ab	33 ghi
Plant, shoot cut off	+	139 a	536 cde	2349 a	42 fg

Significances:
*Experiment I: Pots: *, Dishes: ***, Pots x Dishes: NS*
*Experiment II: Pots: ***, Plant residues: NS, Dishes: ***, Pots x Plant residues: ***, Pots x Dishes: NS, Dishes x Plant residues:NS Pots x Plant residues x Dishes: ****

[1] Two replicate glasshouse experiments with plants of same age, but different in physiological age: the first with 'young' green plants grown in winter and the second with 'older' plants grown in summer of which the shoots started to yellow 10 days after haulm destruction.
In each trial sclerotium production was evaluated on both WA, WA plus a tuber as well as WA plus periderm strips, but not all data are shown for experiment I because of the similarity in results with experiment II.

[2] Analysis of variance carried out after transformation to natural logarithm, indicating differences were either not significant, or significant at P = 0.05 (*), P = 0.01 (**) or P < 0.01 (***). Per experiment values not followed by an identical character are significantly different at P = 0.05.

Table 3. Sclerotium dry weight (μg/1000 mm^2) on water agar (WA) or on harvested tubers, measured after three weeks exposure with or without KOH to volatiles from underground plant parts of potato plants cv. Pimpernel of which the shoots were either green and intact or cut off.

Volatile source	Culture on WA		Culture on tuber	
	without KOH	with KOH	without KOH	with KOH
Plant, shoot intact	2 d[1]	27 c	31 bc	137 ab
Plant, shoot cut off	83 ab	54 abc	151 ab	175 a

[1] Values not followed by an identical character are significantly different at P = 0.05 (*) according to a three-factor analysis of variance after transformation to natural logarithm. Significances: Plants: *, Cultures: *, KOH: *, Plants x Cultures: NS, Plants x KOH: *, Cultures x KOH: NS, Plants x Cultures x KOH: *.

slight stimulation of the sclerotium production. Because incubation with perlite, potting soil or decomposing roots and stolons ('plant residues') had not affected sclerotium production, the observed effects seem to originate from the non-harvested tubers.

Final sclerotium dry weight was lower on exposed periderm strips than on WA, whereas it was higher on exposed tubers than on WA, regardless of the type of 'treatment' with which they had been incubated. Thus, contact with the surface of intact tubers provides an extra stimulation of black scurf formation, possibly caused by unstable substances in the tuber exudate.

Volatiles from the decomposing underground 'plant residues' did not alter sclerotium production in cultures incubated with potting soil, perlite or COS plants. However, these volatile decomposition products increased the slight stimulatory effect of yellowing UNTR plants. The inhibitive effect from young UNTR plants disappeared when a saturated solution of KOH was placed within the plant-soil system (Table 3). In the presence of KOH, volatile tuber exudates from young green UNTR plants were as stimulatory for sclerotium production as those from COS plants. Thus, when tubers are growing, stimulation of black scurf is overruled by inhibitory substances in the volatile tuber exudate. Furthermore, the production of black scurf inhibiting volatiles by tubers appears to diminish during tuber maturation and seems to stop after haulm killing.

The C/N ratio of underground potato plant parts. The above mentioned results showed a different effect of decomposition products incubated with UNTR plants than with COS plants. This difference may originate from other decomposition products which are normally produced at the surface of the growing underground plant parts. Such decomposition products may differ between UNTR and COS plants because of the difference in physiological state of these plants. Volatile decomposition products originating from plant tissue with a C/N ratio of less than 15 have been reported to stimulate growth and pigmentation of the mycelium whereas decomposition products from tissue with a higher ratio had the opposite or no effect at all (Papavizas and Lewis, 1974). Therefore, it was investigated whether the C/N ratio of underground potato plant parts alters during plant growth and after haulm killing by COS. The test plants formed tubers at 21 days before COS. Shoots of UNTR plants were dead at 28 days after COS.

The C/N ratio of roots was about 30 at 56 days before COS, 17 at 21 days before COS, 30 on the day of COS and still 30 at 10 and 28 days after COS, both for the COS plants and the UNTR plants. The C/N ratio of stolons and underground stems was 16 at 21 days before COS, 32 on the day of COS; it had increased for UNTR plants to 37 at 10 days after COS but was still 32 for COS plants at 28 days after COS. The C/N ratio of tubers was 31 on the day of COS, increased for UNTR plants to 40 and 42 at 10 and 28 days after COS and for COS plants to 44. The standard deviations of these results varied between 0.2 and 1.0. Thus, during plant maturation black scurf increases and the C/N ratio of underground potato plant parts increases from 16 or higher reaching 30 to 44. After haulm destruction, an acceleration appears both in the formation of black scurf and in the increase in the C/N ratio of underground potato plant parts.

Table 4. Percentage of area counts registered by gas chromatographic analysis of volatile exudates from tubers that were still attached to potato plants of cv. Pimpernel; shoots were either intact or cut off.

Retention time (min):		0- 60	00-05	05-10	10-20	20-30	30-40	40-60
Oven temperature °C :		76-160	76-86	86-96	96-106	106	160	160
Sampling date								
1[1]	*Shoot intact*	100	90	10	0.1	0	<.1	0.1
	Shoot cut off	100	85	15	0.1	<.1	<.1	0.2
8	*Shoot intact*	100	81	11	1	<.1	0.4	7
	Shoot cut off	100	76	17	2	0.2	0.3	4
13	*Shoot intact*[2]	100	81	14	1	0.1	0.4	3
	Shoot cut off	100	66	28	2	0.3	<.1	3
19	*Shoot intact*	100	66	23	5	1	2	3
	Shoot cut off	100	58	32	3	2	1	4
28	*Shoot intact*	100	65	27	1	0.4	0.4	6

[1] Samples taken at different intervals after haulm destruction collecting head space of 4.5 l after gas bags were closed for 900 min. Tuber surface enclosed was 0.025 m^2 on day 0 and increased for untreated plants to 0.046 m^2 on day 28.
[2] First leaves turned yellow on day 13.

Table 5. Area counts and percentage area counts from GC analysis of volatile exudates produced by tubers and underground stem parts attached to plants of cv. Pimpernel with shoots untreated or cut off.

		Counts[1] [2]						Percentage counts				
Oven temperature °C		76-160	76	78	80	84	90	76	78	80	84	90
Retention time (min)		0- 60	1.5	2	3	5	8	1.5	2	3	5	8
Plant treatment	*Sampling date*											
Untreated	1[1]	43	1.4	12	8	1.4	0.3	3	27	17	3	0
	8	42	3.2	12	15	3.0	0.4	8	29	36	7	1
	13[3]	9	0.3	4	3	1.0	0.1	3	41	34	10	1
	19	11	0.1	2	5	2.4	0.4	1	17	43	21	3
	28	9	0.4	1	4	1.9	0.2	4	12	43	22	2
Shoot cut off	1	46	1.9	8	9	2.9	0.3	4	18	20	6	0
	8	50	1.7	15	19	4.6	1.1	3	29	39	9	2
	13	25	0.9	5	10	5.0	0.9	4	19	42	20	3
	18	20	0.3	3	7	5.2	0.7	2	17	34	26	3

[1] Legend see Table 4.
[2] Counts per min per 1000 mm^2 enclosed tissue surface; values were divided by 1
[3] First leaves turned yellow on day 13.

The volatile exudates fron non-harvested underground plant parts. In order to investigate which alterations occur in the volatile tuber exudate after COS, the production of carbon dioxide, ethylene and other unknown volatiles were measured at several intervals after COS.

GC and GC/MS analyses of all volatile exudates from tubers and stolons showed that, regardless of COS, most components volatize below 80 °C, eluting in the first ten minutes of retention time (RT); very few components eluted at RT 10 to 40 and few at RT 40 to 60 (Table 4). Sharp peaks appeared at RT 1.5, 2, 3, 5 and 8 (Table 5). Small peaks at RT 26, 30, 32, 43, and 50 seemed to be volatile derivates of the silicon sealing and not of plant origin.

The total counts of COS samples reduced in the first experiment, whereas those of UNTR samples remained the same during the first four weeks after COS. In a second experiment, however, the total counts reduced as much for UNTR as for COS plants (Table 5). Total counts or percentages of counts measured did not appear to be affected by closing time, amount of enclosed plant tissues or the ratio between the estimated surface area of all enclosed plant tissues and of the enclosed tubers.

Compared to the UNTR, after COS, the percentage of counts decreased at RT 0 to 5 and increased at RT 5 to 10, mainly because of alterations in counts at RT 2, 5 and 8. Percentage-wise the impact of the components at RT 5 and 8 increased during tuber maturation and this increase seemed slightly accelerated when the shoots were cut off. After COS, actual counts only increased at RT 5. The exudation of components eluting at RT 1.5, 2 and 3 was not affected by haulm destruction. This was inferred from the fact, that during the three-week period of sampling, similar changes occurred in their individual as well as in the total counts of each sample, both from UNTR and COS plants.

Regardless of COS, no dimethylnaphtalenes were detected, although Meigh et al. (1973) reported that they are detectable in tuber exudate at RT 20 to 30. MS analysis showed that carbon dioxide and formic acid may belong to the peaks at RT 0 to 5. For a better total analysis, a cold-trap concentration of samples seems to be required at injection as well as a temperature below 60 °C.

Ethylene concentrations were measured separately during the first days after COS. After the gas bags had been closed for 16 hours, ethylene concentration in the head space was about 0.026 ppm for UNTR plants and 0.040 for COS plants, but the results varied too much between replicates to allow any conclusion to be made.

CO_2 concentrations in the gas bags (% v/v) was measured for three weeks after COS (Table 6, Fig. 2). Compared to the UNTR, after COS, the production of CO_2 halved within three days. This result was similar when the production was ascribed to tubers only or to all enclosed tissue.

Thus, most of the gaseous tuber exudates volatilized below 60 °C. Separation and identification warrant further chemical study. The sharp decline in tuber respiration within the first three days after COS might then allow black scurf formation to accelerate. When the experiment was terminated the inside of the gas bags of UNTR plants appeared to be dry, whereas those of COS plants had collected about 7 ml of liquid exudates during the four weeks of the experiment.

Table 6. Production of carbon dioxide per bag (headspace 4500 ml, 18 °C), either ascribed to only the surface of tubers or to the surface area of al underground potato plant tissues enclosed in each gas bags; plants wer untreated or shoots cut off on day 0.

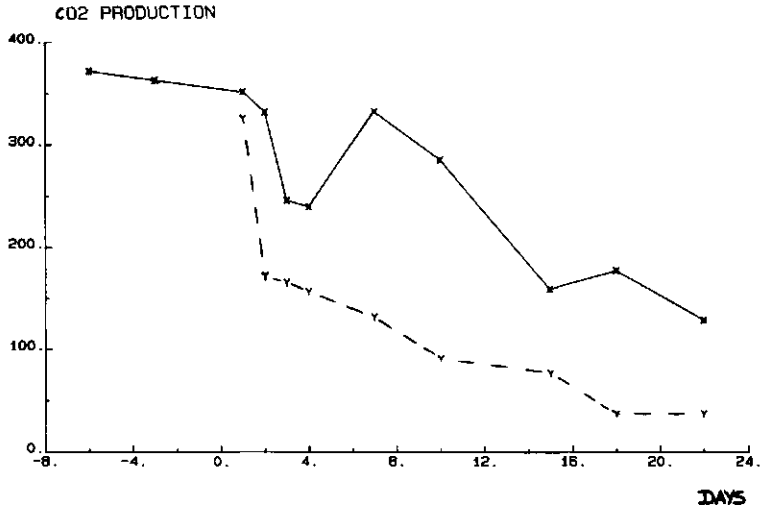
Source		CO_2 concen-	CO_2 production (mg h^{-1} m^{-2})			
Sampling[1] *date*	*Plant treatment*	tration % (v/v)	Tubers		Tubers+stem+stolons	
2	*Shoot untreated*	1.327[2]	312[2]	ab[3]	184	ab
	Shoot cut off	0.350	76	def	91	de
13	*Shoot untreated*	3.124	452	a	347	a
	Shoot cut off	0.578	119	cde	71	cd
19	*Shoot untreated*	1.575	200	bc	172	ab
	Shoot cut off	0.209	40	f	24	e
28	*Shoot untreated*	1.569	144	bc	122	bc
	Shoot cut off	0.212	35	f	21	e

[1] Number of days after haulm destruction; first leaves yellowed on day 13. Bags closed during dark period except for day 13; closing time 840, 900, 880 and 1 200 min on days 2, 13, 19, and 28 respectively.

[2] Means of two bags per plant treatment.

[3] Values not followed by an identical character are significantly different (P = 0.05) according to analysis of variance after transformation to natural logarithm. Significes: Plant treatments: P< 0.01, Sampling dates P = 0.05, and Plant treatment x Sampling date: not significant.

Fig. 2. The production of carbon dioxide (mg h^{-1} mm^{-2} of tuber surface area) by potato tubers cv. Pimpernel with shoots untreated (solid line) or shoots cut off (broken line), measured at several intervals before and after the day of haulm destruction (day 0).



Discussion

The results mentioned above, showed that black scurf formation is governed in the first place by a ratio between inhibitory and stimulatory volatile tuber exudates as was suggested previously (Dijst, 1988a, b). In contrast to the general assumption, tubers always stimulate black scurf, regardless of haulm destruction. During tuber growth the effect of the stimulatory substances seems to be masked by inhibitory substances. The inhibition decreased when the plants yellowed and tubers matured and it disappeared after COS. This result may explain why tests with harvested tubers were non-conclusive.

The inhibitive volatile tuber exudate could be trapped by KOH, indicating that it contains carbon dioxide or other acid-forming gasses. Although reports on CO_2 affecting *R. solani* are contradictory (Sherwood, 1970; Lewis, 1976), CO_2 may be an inhibitor of black scurf formation. In line with previous reports (Burton, 1966; Winkler, 1971), tuber respiration reduced gradually during tuber maturation, but halved within three days after the shoots had been cut off and photosynthesis had stopped. It has already been reported that the production of black sclerotia visibly increases within four days after conditions had become favourable (Dijst, 1988a). Therefore, this sudden drop in tuber respiration may be the reason why the increase in black scurf formation becomes visible at about 7 to 10 days after haulm destruction. Inhibition of sclerotium formation on growing tubers around lenticels (Dijst, 1988a) and on agar plates incubated in air-tight exsiccators (this paper) support the idea that carbon dioxide may inhibit black scurf formation under certain conditions.

Contact between the hyphae and the periderm of intact tubers appeared to further promote black scurf. This result is in line with previous observations (Dijst, 1988a). Contact between hyphae and isolated periderm strips did not promote sclerotium formation. So, instable tuber exudates or decomposition products of exudates which only become available on intact tubers may account for this result.

The results of the experiments with volatile tuber exudates do not explain why chemical haulm destruction (CHD) or cutting-off the shoots (COS) stimulate black scurf so much more than haulm pulling, which breaks the stolons.

It has been suggested that plant stress components might promote black scurf after CHD (Dijst, 1985, 1988a). However, the above-mentioned experiments with ethylene were not conclusive. Inhibition by CO_2, dependent on the food base available, may have masked any effect of ethylene in these tests. The observed stimulation of the sclerotium production on agar by volatile apple exudate may be caused by ethylene but also by substances that serve as a nutrient after absorption by the agar (Lewis, 1976).

The results in this paper support the idea that CHD and COS may be more stimulating than haulm pulling because of extra exudation of water and nutrients. In the gas bags around tubers and stolons of plants with cut-off shoots 7 ml of liquid exudate was collected and nothing in those of plants with intact shoots. Because haulm pulling breaks the stolons and thus terminates the contact between tubers and both roots

and shoot, it is improbable that after haulm pulling tubers exude such a large amount of liquid as after COS. Soil water content around the fungus probably influences its development because of the indirect effect on soil aeration and thus pH and availability of water-soluble nutrients (Blair, 1943; Lewis, 1976). Further, the precipitated water-soluble tuber exudates, that may serve as a nutrient source, gave rise to a higher yield in dry weight after COS than after haulm pulling (Dijst, 1988b). The different results with WA and the slight nutritive MM in this paper indicate that the food base available may interfere with the effect of volatile substances.

After CHD or COS, more plant residues remain in the vicinity of the tubers than after haulm pulling. This may contribute to the higher stimulation of black scurf formation after CHD and COS because the volatile products from decomposing roots and stolons slightly increased the stimulatory effect of tuber volatiles. The decomposing plant residues had a C/N ratio above 15 and their volatile products increased the formation of both mycelium and sclerotia. This is not completely in line with the results of Papavizas and Lewis (1974) and Lewis (1976), possibly because they did not test isolates of the AG-3. These authors reported that decomposing plant material with a C/N ratio below 15 produce volatiles, mainly ammonia, that alter the pH of exposed media, might function as a nutrient, stimulate mycelial growth and pigmentation on artificial media, but reduce colonization of buck wheat stems in soil; plant residues of C/N above 15 had the opposite or no effect. These authors found no correlation between sclerotium production on artificial media and decomposition of any kind of plant tissue. In this paper decomposing potato plant residues did not have an effect of their own on the formation of sclerotia by the AG-3 isolates tested, but merely added to the stimulatory effect of tuber volatiles. In line with Townsend (1957) this confirms that it is the composition of substances and not just their individual amounts which influences sclerotium formation in isolates of the AG-3.

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Samenvatting

De invloed van vluchtige en instabiele exsudaten van de ondergrondse delen van de aardappelplant op de ontwikkeling van lakschurft voor en na loofvernietiging

De vluchtige exsudaten van aardappelknollen, die nog aan de plant bevestigd zaten, beïnvloedden de productie van sclerotiën door *Rhizoctonia solani* AG-3 op agar platen en op geöogste knollen, die geïncubeerd waren in een plant-aarde systeem. In dezelfde proefopzet was de sclerotiënvorming op geïncubeerde losse knollen veel hoger dan op de platen, maar op peridermstrips juist veel geringer. Wellicht bevorderen dus ook instabiele knolexsudaten de vorming van lakschurft.

Het vluchtige knolexsudaat van jonge planten lijkt zowel stimulerende als remmende componenten te bevatten. De remming overheerste tijdens de knolgroei, nam af als de plant vergeelde en verdween na loofvernietigen. Als de remmende fractie met KOH werd weggevangen bleek het vluchtig knolexsudaat van onbehandelde planten evenzeer sclerotiënvorming op agar media te stimuleren als afrijpende knollen na loofvernietiging. Toename van lakschurft na loofdoding berust dus waarschijnlijk vooral op het wegvallen van de remmende componenten. Losmaken van de grond bij of na het loofdoden kan wellicht helpen om ophoping van de niet meer geremde stimulerende fractie aan het knoloppervlak te voorkomen.

Vluchtig knolexsudaat bleek voornamelijk te bestaan uit laag moleculaire componenten die in de gaschromatograaf beneden de 60 °C vervluchtigen. Alleen de remmende fractie kon worden weggevangen met KOH, wat betekent, dat het gaat om koolzuur of een zuurvormende component. Het is inderdaad niet onwaarschijnlijk, dat koolzuur deel uitmaakt van de remmende fractie. Koolzuurproduktie van de knollen nam geleidelijk af tijdens de veroudering van de plant, maar nam abrupt af na loofdoding, terwijl we weten dat lakschurft geleidelijk toeneemt bij veroudering van de plant, maar sterk na loofdoding. De sclerotiënvorming op agarmedia werd sterk gestimuleerd door gasvormige producten van appels, maar niet door ethyleen. Het effect van ethyleen leek beïnvloed door de concentratie koolzuur en het type voedingsbodem.

Gasvormige producten van afstervende wortels en stolonen hadden geen invloed op de sclerotiënvorming op agar platen. Echter, de lichte stimulering, die uitgaat van knollen aan oudere planten veranderde in een wat sterkere stimulering in de aanwezigheid van afstervende wortels en stolonen. De sterke toename van lakschurft na loofdoding zou daarom waarschijnlijk ook verminderd kunnen worden door zo snel mogelijk na loofdoding de ondergrondse planteresten weg te halen bij de afhardende knollen.

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GENERAL DISCUSSION

Sclerotium formation was studied in vitro and on tubers. In contrast to the original expectation, the formation of sclerotia by *Rhizoctonia solani* AG-3 did not appear to be induced by a starvation process. On tubers, both mycelial growth and sclerotium formation were stimulated after haulm destruction. On agar plates, production of mycelium and sclerotia increased through a supply of nutrients but was restricted by nutrient deprivation. Independent of hyphal growth, sclerotium formation on agar plates stopped 8 days after inoculation but on liquid medium it continued beyond that time. Since staling products had no impact, a solution in water apparently facilitates the uptake of nutrients, changing sclerotial development.

Both on artificial media and on tubers, small sclerotia often grew together forming one large sclerotium. On agar plates, initiation points always appeared macroscopically 4 days after inoculation, regardless of the size of Petri dish and nutrient medium. The same independence held for sclerotial maturation which was completed within 8 days after inoculation. In mycelial mats transferred from water agar to a nutrient-rich plate, many extra mature sclerotia were formed within the next 4 days. Such rapid maturation also seemed to take place on tubers, where the white initials were seldom observed.

Alterations on the tuber surface associated with tuber maturation appeared to have a major effect on black scurf formation. On young growing tubers only a few hyphae were observed and occasionally some tiny sclerotia, but never close to lenticels. When the tubers stopped growing and matured, sclerotia were found more often near lenticels and increased in both number and size. When tuber maturation accelerated as a result of premature plant death, the number and size of the sclerotia increased much more rapidly than during normal tuber maturation. After haulm killing, stimulation of black scurf formation became evident within 7 to 10 days, regardless of the method used. Black scurf formation was not stimulated visibly when the shoots were destroyed after tuber maturation, but always appeared when the shoots were killed during tuber growth. The rate of stimulation differed much between the methods applied.

Several plant factors affect black scurf formation. After haulm killing, the factors that stimulate black scurf formation probably become effective within 3 to 6 days. Volatile tuber exudates appeared to play a major role. Periderm components as well as unstable substances, water and water-soluble components exuding from the tubers also had some effect.

The ratio between inhibitory and stimulatory volatile tuber exudates seem to have a major effect on the final mass of sclerotia. Stimulatory volatile substances were exuded as much by tubers of green intact plants as by tubers after haulm destruction. However, during tuber growth, the stimulus was concealed by inhibitory volatiles. The inhibition decreased slowly during normal tuber maturation but disappeared very fast after haulm killing. As the inhibitors could be trapped by KOH carbon dioxide may be an inhibitor. This may also be inferred from the tuber respiration which halved within 3 days after haulm destruction.

Non-water-soluble periderm components appeared to promote initiation of sclerotium formation on growing tubers but not to a higher extent after haulm destruction. This process has not yet been elucidated. On inoculated filters in dishes some large sclerotia developed but none on filters wrapped around growing tubers with the inoculum on the outside. After haulm destruction, some brown hyphae with short internodes developed on

these filters, but no sclerotial initials. Tuber surface structure did not appear to affect sclerotial initiation. On isolated strips of periderm brown hyphae were formed but very few sclerotia, regardless of the date of sampling. The amount of sclerotia on the periderm strips was much lower than on harvested tubers. It is unlikely that this difference in sclerotial mass is caused by inhibitory components from broken cells in the strips. For, on wounded sites of tubers sclerotia remained immature, but their final mass was increased when the plant shoots were cut off. Thus, unstable tuber exudates or decomposition products of tuber exudates only available on intact tubers, may have a stimulatory effect.

Water and non-volatile substances exuding from the tubers may further promote black scurf. The tubers exuded more water after the shoots had been cut off, and the laboratory tests mentioned above suggest that solution of nutrients in water promoted sclerotial growth. Precipitable water-soluble tuber exudates (PWSTE) added to agar plates promoted sclerotium maturation. Although PWSTE was expected to serve as a nutrient, it did not affect initiation and growth of sclerotia on agar plates or on isolated strips of periderm. However, this result does not exclude an effect of water-soluble exudates on black scurf formation in vivo. First, because these components may interfere with the effect of volatiles and second, because they may be altered during precipitation. In vivo, PWSTE might also act as a glue which attaches sclerotia firmly to the tuber skin.

Volatile products from decomposing roots and stolons and from potting soil did not affect sclerotium production on exposed agar plates. However, in a whole-plant-system, sclerotium production on agar plates was stimulated more by volatile tuber exudates from yellowing intact plants if these volatiles were mixed with the volatile products from decomposing roots and stolons. These results with plants are in line with laboratory experiments which indicated that the effect of a gaseous product on sclerotium production can depend on other volatiles in the vicinity and the food base available.

Haulm pulling (HP) accelerates black scurf formation less than quick chemical haulm destruction (CHD). It seems most important that HP breaks the stolons and disturbs the soil, two effects not achieved by CHD. The chemicals used did not seem to play a role, because cutting off the shoots at soil level or 10 cm above soil level (COS) stimulated black scurf formation as much as CHD.

The breaking of the stolons by HP stops the sap flow from roots to tubers and reduces exudation of liquids from the tubers. After CHD and COS the sap flow from roots to tubers continued for a week, and more liquid and precipitable substances exuded from the tubers than after HP. It has further been investigated whether the release of stress factors, such as ethylene, could also play a role. However, no definite conclusions could be drawn from these experiments.

Disturbing the soil by HP provides a better aeration which probably prevents the accumulation of stimulatory volatile exudates at tuber surface. Root severing in addition to CHD or COS reduced root mass in contact with tubers, distrubed the soil and gave rise to lower amounts of black scurf than after CHD or COS alone. The same effect has been reported for HP (pers. comm. Bouwman), indicating the importance of increased soil aeration.

In the third place only HP removes underground stems, stolons and roots from the ridge. This removal reduces the amount of decomposition products in the vicinity of the tuber which can slightly increase the stimulatory effect of volatile tuber exudates on black scurf formation.

Perspectives for Rhizoctonia control. Haulm destruction has a major effect on the final mass of black scurf at harvest time. So, in the first place there is a great need for an alternative method to prevent virus infection of the tuber without the increase of black scurf. If haulm pulling is not recommanded, e.g. because of wet weather conditions or Phytophthora infection, root severing in combination with chemical haulm destruction can be recommended. Root severing can be carried out by an inexpensive but functional implement. It moderated black scurf formation and increased the period of time in which tubers can be harvested with a firm skin and limited black scurf. However, because of the environmental risks involved with chemicals in agriculture, a fully non-chemical alternative is preferred. Stimulation of black scurf can be avoided by breaking the stolons and separating the tubers from the plant residues. Until the tubers have been collected from the field, a good aeration is needed at the tuber surface to prevent black scurf development during skin set, but infection by other pathogens should be avoided. The Research Institute for Plant Protection (IPO) and the Institute for Agricultural Engineering (IMAG) have taken the initiative to develop such a new method. Removal of plant residues from the field on the day of stolon breaking would reduce soil infestation by Rhizoctonia sclerotia from stolons and probably also by Verticillium microsclerotia from stems.

There is not much reason to believe that cultivars differ significantly with regard to the factor that can promote black scurf. Even if such factors existed in the materials tested, screening for them would be of little use because of the many other factors involved which can mask their effect. No differences in black scurf formation on intact plants were found between seven cultivars which differed naturally in tuber maturation. The same holds for black scurf formation after haulm destruction: the strong effect of the accelerated tuber matuarion seems to veil any difference between cultivars. Therefore, screening cultivars for resistance to acceleration of tuber maturation in the field seems more appropriate. Furthermore, because breaking the stolon on the day of haulm killing moderates black scurf formation, cultivars with an easily breaking stolon on that date would be useful.

There is an increasing interest in ways to predict the increase of damage. The amount of black scurf at harvest time depended on the method of haulm destruction and not on the degree of tuber infestation on the day of haulm killing or during the first 13 days afterwards. This implies that in infested soils and starting with low levels of tuber infestation, the estimated inoculum density on the day of haulm destruction has no predictive value for further black scurf formation.

It is important to further investigate the fungal developments that cause damage to seed potato production. Both sclerotium initials on tubers and infection cushions on stems and stolons develop from hyphae with short internodes. The fungus infects only potato plants, but produces its sclerotia on several plant species although potato tubers and stolons seem to be preferred. An investigation into the mechanisms that control the initiation of sclerotium formation as well as the initiation of infection may clear why potato plants are preferred by isolates of AG-3. On seed potato tubers, however, also few initiation points are unfavourable, as they can give rise to very large sclerotia. Therefore, it is also important to analyse the factors that promote a higher final mass of sclerotia on tubers, i.e. the inhibitory and stimulatory volatile exudates.

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SUMMARY

Factors influencing black scurf formation in untreated crops and after haulm destruction were investigated. As potato tubers mature they may gradually become covered with black scurf, the sclerotia of the fungus *Rhizoctonia solani* AG-3. After haulm destruction, black scurf formation is stimulated by changes at the tuber surface due to accelerated tuber maturation. These changes probably start within 3 to 6 days.

The final amount of black scurf at harvesting depended on the method of haulm destruction and not on the cultivars tested or on the degree of tuber infestation. Mechanical haulm destruction by 'haulm pulling' (HP) stimulates black scurf formation less than chemical haulm destruction (CHD) or cutting of the shoots (COS). This is probably because it breaks the stolons, disturbs the soil and removes most plant residues from the vicinity of the tuber. After CHD or COS, the sap flow from roots to tubers continued for a week, and more liquid and precipitable substances exuded from the tubers after HP. This increased exudation may promote black scurf formation: also on agar media sclerotial growth was increased within 4 days after a nutrient supply and sclerotial growth continued over a longer period of time when the medium was liquid. In field and pot trials, severing the roots in addition to CHD or COS moderated black scurf formation and extended the period of time in which tubers could be harvested with a firm skin and limited black scurf.

The initiation of sclerotium formation on tubers is probably promoted by non-water-soluble components of the tuber periderm. Sclerotial maturation was promoted by precipitated water-soluble tuber exudates. Volatile and unstable components which become available on the surface of intact tubers promote sclerotium formation. The final mass of sclerotia, however, is probably influenced most by stimulatory and inhibitory volatile tuber exudates. When the inhibitors were trapped by KOH, sclerotium formation on exposed agar plates was stimulated just as much by tuber volatiles from green intact plants as by tubers after haulm destruction. Inhibition dominated during tuber growth, decreased only gradually during tuber maturation and disappeared rapidly after haulm killing. Carbon dioxide may be an inhibitor because tuber respiration was halved within 3 days after haulm killing. Volatile products from decomposing roots and stolons had no effect of their own, but they increased the slight stimulation of sclerotium formation by naturally maturing tubers. The effect of a gaseous product on sclerotium production may depend on other volatiles in the vicinity as well as on the food base available. Perspectives for black scurf control were discussed.

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SAMENVATTING

Ten behoeve van de pootaardappelteelt werden de factoren onderzocht die de ontwikkeling van lakschurft beïnvloeden al dan niet na loofvernietigen. Ook werd onderzocht waarom chemisch loofvernietigen de vorming van lakschurft meer stimuleert dan looftrekken. Lakschurft bestaat uit ruststructuren of 'sclerotiën', die de ziekteverwekkende schimmel *Rhizoctonia solani* AG-3 vormt op de schil van aardappelen. Tijdens het afrijpen kunnen jonge knollen geleidelijk bezet raken met sclerotiën. Die sclerotiën nemen echter veel sneller toe in aantal en grootte als de knol versneld afrijpt. Een dergelijke stimulering wordt binnen 7 tot 10 dagen na loofdoding zichtbaar. Zowel na looftrekken als na chemisch loofvernietigen is de stimulering het gevolg van veranderingen aan het knoloppervlak, die waarschijnlijk vanaf 3 tot 6 dagen na loofdoding van invloed zijn.

De uiteindelijke hoeveelheid lakschurft bij de oogst hing in kasproeven af van de methode van loofdoding en niet van de getoetste cultivar of de mate van knolbesmetting in de eerste 13 dagen na loofdoding. Looftrekken (HP) stimuleerde de vorming van lakschurft in mindere mate dan chemische loofvernietiging (CHD) of het afknippen van het loof (COS). Dit is waarschijnlijk het geval omdat alleen looftrekken de stolon breekt, de aarde van de rug losmaakt en het grootste deel van de ondergrondse stengeldelen uit de rug verwijdert. Na chemische vernietiging of afknippen van het loof lekte uit de knollen meer vocht en precipiteerbare waterige exsudaten dan na looftrekken, wellicht omdat de sapstroom van wortels naar knollen nog een week in stand bleef. Deze sterkere exsudatie kan lakschurft stimuleren: ook op agar platen ontstonden meer sclerotiën binnen 4 dagen na een extra voedselgift en de sclerotiënvorming ging langer door als het medium vloeibaar was. Zowel in het veld als in kasproeven was de stimulering van de lakschurftvorming na chemisch vernietigen minder sterk indien ook de wortels waren doorgesneden. Wortelsnijden bij het chemisch loofvernietigen verlengde ook de periode waarin de knollen geoogst konden worden met een afgeharde schil en geringe lakschurftbezetting.

De initiatie van sclerotiën lijkt bevorderd te worden door periderm componenten, die niet in water oplossen (NWSTC). De afrijping van de sclerotiën werd gedeeltelijk bevorderd door geprecipiteerde waterige knolexsudaten (PWSTE). Deze effecten van NWSTC en PWSTE werden niet versterkt door loofvernietiging. De totale massa aan sclerotiën werd gestimuleerd door vluchtige en instabiele producten die vrijkomen aan het oppervlak van knollen. De hoeveelheid sclerotiën die op de knollen ontstaat wordt waarschijnlijk vooral bepaald door de verhouding tussen remmende en stimulerende vluchtige knolexsudaten. De remmende componenten werden weggevangen met KOH. Knollen van groene planten en knollen van planten met vernietigd loof produceren een even sterk stimulerend vluchtig exsudaat. In de eerste fase van de knolgroei domineerde echter het effect van de remmende exsudaten. Die remming nam geleidelijk af tijdens normale knolafrijping, maar verdween binnen drie dagen na loofdoding. Koolzuur kan een remmende factor zijn, aangezien de knolademhaling binnen drie dagen na loofdoding gehalveerd werd. Vluchtige producten die vrijkomen bij het afsterven van de wortels en stolonen hadden geen invloed op de sclerotiënvorming. Wel versterkten zij het stimulerende effect van natuurlijke afrijpende knollen. In cultures op agar media bleek het effect van een gasvormige stof op de sclerotiënvorming af te hangen van de overige vluchtige componenten en van de voedingsbodem. Perspectieven voor lakschurftbeheersing werden besproken.

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CURRICULUM VITAE

Gerda Dijst werd 16 juni 1953 geboren in Amsterdam. Na het doorlopen van de Montessorischool behaalde zij in 1972 het eindexamen-β aan het Barlaeus gymnasium te Amsterdam. Aan de toenmalige Landbouwhogeschool te Wageningen behaalde zij in juni 1977 het kandidaatsexamen plantenziektenkunde, met het accent op de fytopathologie, virologie, microbiologie en plantefysiologie. In januari 1981 studeerde zij af aan de toenmalige Landbouwhogeschool met fytopathologie als hoofdvak. Daartoe waren vier leeronderzoeken uitgevoerd: virologie (identificatie, dr G. Thottappilly, Michigan State Univ., USA), bodemmycologie (VAM en biologische bestrijding, prof dr N.C. Schenck, Univ. of Florida, USA), plantefysiologie (vruchtafrijping, dr E. Knegt, LU Wageningen) en fytopathologie (fysiologie van het parasitisme, dr ir L.C. Davidse, LU Wageningen). Zij assisteerde verschillende practica van de afdelingen Fytopathologie, Entomologie, Nematologie, Plantkunde en Virologie.

Vanaf juni 1981 is zij als bodemmycoloog verbonden aan het Insituut voor Plantenziektenkundig Onderzoek (IPO) te Wageningen. Tot eind 1985 deed zij daar in tijdelijke dienst eco-fysiologisch onderzoek naar de oorzaak van de stimulering van lakschurft na loofdoding, speciaal gelet op de rol van knolexsudaten. Daarna was zij voor twee jaar gedetacheerd op het Proefstation voor de Akkerbouw en Groenteteelt in de Vollegrond (PAGV) te Lelystad. Daar bekeek zij de mogelijkheden voor biologische of geïntegreerde ziektebeheersing m.b.t. pathogene bodemschimmels in de vollegrondsteelten. In 1988 werd zij in vaste dienst aangesteld op het IPO voor onderzoek naar de beheersing van bodemreceptiviteit, de ontvankelijkheid van een perceelsgrond voor een bodemziekte.