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

G. DIJST

Research Institute for Plant Protection (IPO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands*

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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.

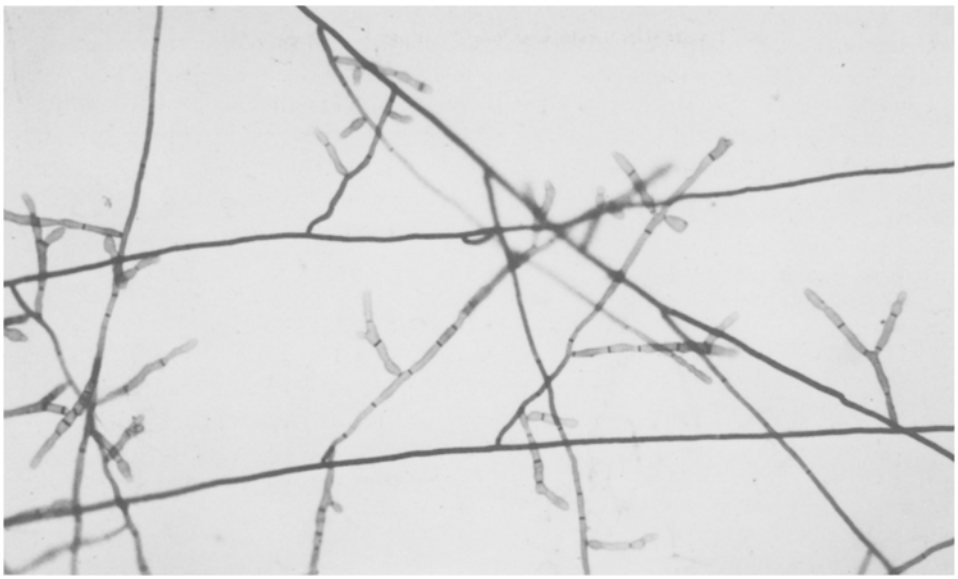

Fig. 1. Brown, sometimes shortly branched hyphae of *R. solani* AG-3 developed on cellulose acetate filters wrapped around tubers still attached to potato plants after shoots were cut off.

(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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