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Effect of volatile and unstable exudates from underground potato plant parts on sclerotium formation by Rhizoctonia solani AG-3 before and after haulm destruction

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Abstract

The acceleration of black scurf development after haulm destruction was mainly due to changes in the exudation of volatiles from tubers. Volatile products from decomposing potato roots and stolons and, probably, unstable substances in the tuber exudate as well, further promoted sclerotium formation.

Sclerotium production by *R. solani* AG-3 was investigated on agar media, periderm strips and harvested tubers, which were exposed to the volatile exudates from growing subterranean potato plant parts. The volatile exudate from growing tubers contained both inhibitory and stimulatory substances which were not identified definitely. Inhibition dominated during tuber growth, decreased when plants were yellowing and disappeared after the shoots were excised. When the inhibitory components were trapped by KOH, the non-trapped volatile tuber exudates from young growing plants were as stimulatory as those from plants after haulm killing. CO_2 might be an inhibitor as tuber respiration was negatively correlated to black scurf formation. Tests in vitro suggested that inhibition of sclerotium formation by CO_2 can be overcome by stimulatory nutrients. Sclerotium production on agar media was not stimulated by ethylene, although volatiles from harvested ripe apples were very stimulatory.

The results imply that after haulm killing, the increase in black scurf development may be prevented by loosening the soil and quick separation of tubers from plant residues thus preventing accumulation of the stimuli.

Additional keywords: black scurf, carbon dioxide, C/N ratio, ethylene, *Solanum tuberosum*, tuber maturation, tuber respiration, apple exudates.

Introduction

Between 3 and 17 days after haulm destruction, black scurf i.e. sclerotia produced 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 the tuber surface is needed for the initiation of sclerotium formation on tubers. Stable water-soluble tuber exudates promoted a more solid structure of 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 seemed to be governed by volatile and unstable components which might be

exudates or decomposition products thereof.

It was suggested, that after haulm destruction, alterations in stimulatory and inhibitory volatile tuber products play a major role in the stimulation of black scurf development (Dijst, 1988b). Also plant stress components and volatiles from roots and stolons might promote black scurf (Dijst, 1988a). In this study, the role of volatile products from the potato plant in stimulation of sclerotium production after haulm killing was investigated. Preliminary results were published earlier (Dijst, 1987, 1989).

Material and methods

Isolates, media, inoculation and assessment 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) were used in 50 mm diameter Petri dishes. All experiments were carried out at 18 °C.

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

Exposing cultures to volatiles.

A. Volatile exudates from harvested tubers or apples. Over a period of 18 days, plates inoculated with *R. solani*, six with WA and six with MM, were incubated together with a wet sponge in tape-sealed 4 l metal boxes. The boxes contained either a ripe apple or 25 harvested tubers with a total surface area of about 76 000 mm^2 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. Exposing cultures to ethylene. During three weeks, cultures on WA and on MM in glass dishes were incubated in glass exsiccators (content 2.1 l). Four different concentrations of ethylene were tested: 0.005 (no extra ethylene added), 0.040, 0.200 or 1.000 $\mu l\ l^{-1}$. 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 during 15 min and the initial ethylene concentrations were readjusted.

C. Exposing cultures to volatile exudates from growing tubers. The effect of volatile exudates from underground potato plant parts on the production of sclerotia on agar plates was investigated using a set-up as shown in Fig. 1. Plants were raised in 3 l pots filled with steamed potting soil. On the first day of the experiment (day 0) four groups of pots were created: (a) pots with an UNTR plant growing in potting soil, (b) pots with a COS plant in potting soil, from which the shoot was excised on day 0, (c) pots without a plant but filled with potting soil on the 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 stems collected on day 0 from extra plants. Each pot received the 'plant residues' from one potato plant. Thus, eight treatments were created and for each treatment three

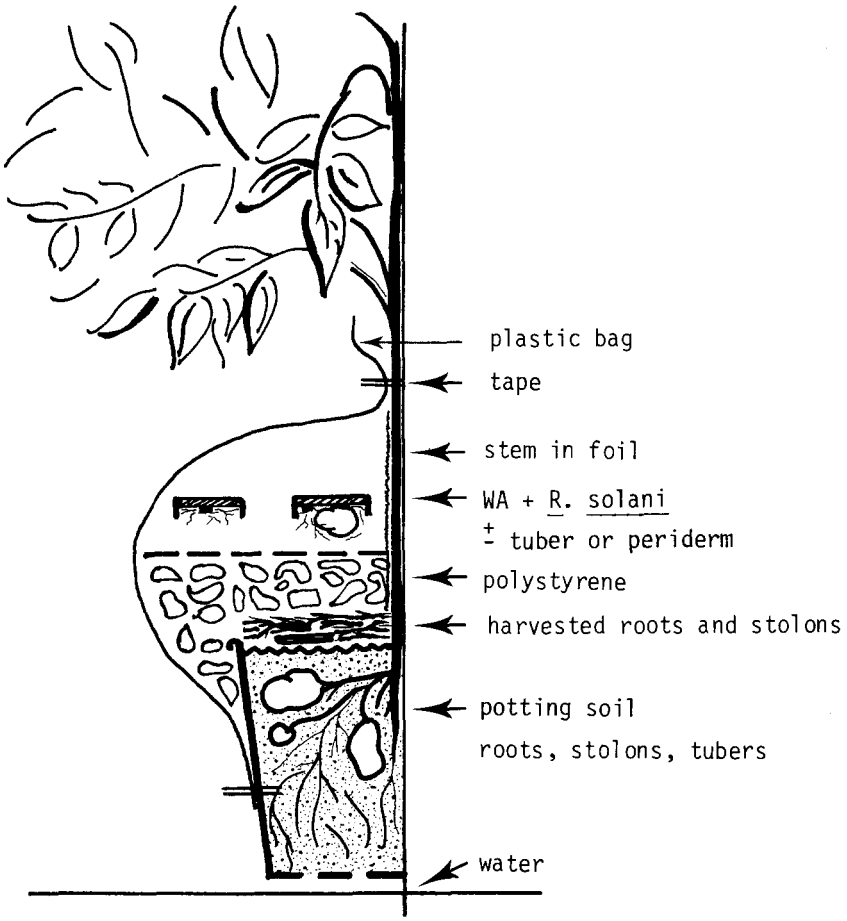

Fig. 1. Experimental design for testing the effect of volatile exudates from harvested and non-harvested underground potato plant parts on sclerotium production by *Rhizoctonia solani* AG-3. The figure shows a pot with an untreated potato plant in potting soil amended with decomposing roots and stolons.

replicates were included.

The surface of each pot was covered with a 50-mm-thick layer of large polystyrene granules and the lower 15 cm of each shoot was wrapped in aluminium, in order to prevent hyphae of exposed cultures from reaching the plant. Open petri dishes with two-day-old cultures of *R. solani* were inverted 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 cultures on WA and kept in place with an aluminium strip. Tubers and periderm strips had the same surface area as the agar, viz. 4 000 mm^2 per plate. Tubers and periderm had been collected on day 0 from the extra potato plants. Finally, each pot with plates was wrapped in grey non-air-tight plastic and tape-sealed.

The experiments were carried out with plants that were 100 days old. For experiment I the plants were grown in winter and the shoots were green during the experiment.

For experiment II the plants were grown in summer and so the plants were physiologically older and yellowing. In a third experiment again green plants were used, but now a beaker with a saturated KOH solution was enclosed beside the plates.

After three weeks of incubation, sclerotium production on the exposed agar, periderm strips and tubers was assessed. Data from tubers and periderm strips were compared directly with those of the plates, 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-l pots in order to maximise tuber production. 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 every 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 of the enclosed tubers was 0.025 m^2 and that of all enclosed tissues 0.043 m^2 per bag. On day 28, these values had increased for untreated plants only to 0.046 m^2 and 0.055 m^2 per bag, respectively. Gas bags were closed during dark periods except for night 12 when lights were accidentally kept on. Bags were closed on 1, 7, 12, 18 and 27 days after haulm destruction for 7, 17, 15, 14.7 and 20 hours, respectively. After each of these periods of time, samples were collected. For monitoring carbon dioxide and ethylene, samples were taken by syringe. For qualitative analysis of the tuber exudates, all remaining volatiles were then collected from the head space with a 100-ml Dräger hand-membrane pump and, on collection, concentrated by adsorption on 100 mg Tenax GC adsorbent (Ta 20-35 mesh, 160 × 6 mm) at 18 °C. Immediately after collection, headspace and aeration were restored.

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 FID, detector 200 °C. Components were separated on a capillary column type CP sil 19 CB, (25 m length, 0.32 mm internal diameter (i.d.), 0.2 mm 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, a rise of 2 °C min^{-1} for 15 min, isothermal at 106 °C for 15 min, a rise of 20 °C min^{-1} for 3 min and isothermal at 160 °C for the last 15 min. As a standard 0.09 mg phenanthrene gave 750 000 area counts at 160 °C and AT = 10 × 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: isothermal at 40 °C for 4 min, a rise of 2 °C min^{-1} for 20 min, a rise of 10 °C min^{-1} for the last 10 min.

Measurement of CO_2 production. Samples 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. Injection and detection temperature was 110 °C. The flow gas was H_2 at a rate of 6 ml min^{-1}. Carbon dioxide production (CP) was calculated using the formula: $CP = R \cdot (Cs - Co) \cdot (Ts \cdot As)^{-1}$ [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 = 101.3 kPa, V = 45.10 (m^3), R = 8.314 (J Mol^{-1} K^{-1}) and T = 291 °K.

In order to evaluate the effect of volatile production on black scurf development, the tissue surface area (mm^{-2}) was used as a measuring unit for both variates and not the tuber weight. 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 water and starch content.

Measurement of ethylene production. Samples were 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 (Chrompack). 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.

The statistical analysis and measurement of the C/N ratio. These have been described previously (Dijst, 1988a, 1988b). Analysed data were either not significantly (NS) or significantly different at $P = 0.05$ (*), $P = 0.01$ (**) or $P \leqslant 0.001$ (***).

Results

Exposing cultures to the volatile exudates from harvested potato tubers or apples or to ethylene. The objective of these experiments was to investigate whether the plant stress component ethylene may promote black scurf formation after haulm killing. The growth rate of mycelium and the period of time needed for completion of sclerotium maturation were not affected in any of these experiments. In boxes, sclerotium production was slightly reduced by the exudates from harvested tubers, regardless of the plant cultivar tested or shoot treatment before tuber collection (Table 1). Because the assumed ethylene production of tubers might diminish after harvest, cultures were also incubated with ripening apples which are known to produce large amounts of ethylene. In the presence of an apple, sclerotium formation significantly increased (Table 1). In exsiccators, however, sclerotium production was not increased by any ethylene supply tested (Table 2). In the boxes without an apple or tubers, sclerotium production was similar to that in aerated growth chambers. However, in exsiccators without a supply of ethylene, the production of sclerotia was reduced on WA and not on MM (Table 2). In the exsiccators the concentration of CO2 increased more than in the boxes, despite the weekly aeration. Similar results held for sclerotium production at ethylene concentrations below

Table 1. Effect of volatile exudates from apples or potato tubers on sclerotium production by *R. solani* AG-3 on agar media which contained no (WA), a low (MM) or a high amount of nutrients (MPA).

Volatile source	Sclerotium dry weight (mg/1000 mm^2)			
	WA	MM	MM	MPA
Control	0.32	0.35	0.81	32.69
Potato tubers UNTR[1]	0.15	0.29	—	—
Potato tubers COS[1]	0.17	0.24	—	—
Apple	—	—	2.16	37.26
Significances	NS	NS	***	NS
L.S.D. at $P = 0.05$			0.51	

[1] Tubers were collected from potato plants cv. Pimpernel of which the shoots were untreated (UNTR) or cut off (COS) 18 days before.

Tabel 2. Effect of ethylene and carbon dioxide on sclerotium production by *R. solani* AG-3 on water agar (WA) and on minimal medium (MM).

Experimental conditions[1]					Sclerotium dry weight (μg 10^{-3} mm^{-2})	
incubator	ethylene		CO_2			
	initial μl l^{-1}	final μl l^{-1}	final % (v/v)	final mg . l^{-1}	on WA	on MM
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					**	*
L.S.D. at $P = 0.05$					51	142

[1] The cultures had been incubated for 21 days in exsiccators that were aerated and readjusted for initial ethylene concentration each week.

5 μl l^{-1}. At 1 μl l^{-1} ethylene, however, sclerotium production was also inhibited on the weakly nutritive MM.

Exposing cultures to volatile exudates from tubers, stolons and roots. In two duplicate experiments, sclerotium production was much higher on exposed harvested tubers than on WA and it was the least on periderm strips in experiment II (Table 3). As compared to the sclerotium production near potting soil, sclerotium production on WA near plants

Table 3. Effect of volatile exudate from various sources on sclerotium production by *R. solani* AG-3 cultured on water agar (WA), on the surface of tubers or on periderm strips collected from potato plants cv. Pimpernel.

Volatile source treatment		Sclerotium dry weight ($\mu g\ 10^{-3}\ mm^{-2}$					
	plant residues added (+) or omitted (−)	Experiment I[1]			Experiment II[1]		
		WA	tuber	periderm	WA	tuber	periderm
Perlite	−	−	−	−	24 ghi[2]	43 fg	10 jk
Potting soil	−	69 cd[2]	980 a	87 bc	12 hijk	43 fg	1 lm
Plant, untreated	−	12 d	822 ab	39 d	72 efg	760 ab	8 k
Plant, shoot cut off	−	142 c	1142 a	54 c	584 bc	1225 a	21 ijk
Perlite	+	−	−	−	20 hij	74 def	0 m
Potting soil	+	−	−	−	4 kl	18 ghi	0 m
Plant, untreated	+	26 d	746 ab	104 cd	538 bcd	971 ab	33 ghi
Plant, shoot cut off	+	139 bc	1081 a	294 bc	536 cde	2349 a	42 fg

[1] The duplicate experiments were conducted with plants of the same age. During experiment I in winter, shoots remained green and during experiment II in summer, the shoots became yellow after 10 days.
[2] In eacht experiment values not followed by the same character are significantly different at $P = 0.05$ according to a three-factor analysis of variance carried out after transformation to natural logarithms. Significances: Experiment I: Pots: *; Dishes: ***; Pots × Dishes: NS. Experiment II: Pots: ***; Plant residues: NS: Dishes: ***; Pots × Plant residues: ***; Pots × Dishes: NS; Dishes × Plant residues: NS; Pots × Plant residues × Dishes: ***.

growing in potting soil was slightly inhibited by green UNTR plants (I), was slightly increased by yellowing UNTR plants (II), and was significantly increased by the volatile products from COS plants in both trials (I and II). Sclerotium production on WA was not affected by volatile exudates from perlite, potting soil or decomposing roots and stolons (plant residues) in the absence of plants. Thus, the observed effects with plants probably originated from the non-harvested tubers.

The inhibitory effect of young UNTR plants on sclerotium production on WA disappeared for the greatest part when a saturated solution of KOH was placed within the plant-soil system (Table 4). 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, the effect of inhibitory volatile tuber exudates dominates during tuber growth, masking the effect of stimulatory exudates; inhibition decreased when shoots yellowed and disappeared after haulm killing.

Volatiles from the decomposing plant residues did not affect sclerotium production in the absence of plants. In combination with growing plants, however, these decomposition products increased sclerotium production on periderm exposed to COS and on WA exposed to older UNTR in experiment II (Table 3).

The C/N ratio of subterranean potato plant parts. During normal plant maturation as well as after haulm destruction, the C/N ratio of underground potato plant parts

Table 4. The effect of volatile exudates from various sources on sclerotium production by *R. solani* AG-3 on water agar (WA) or on harvested tubers. The cultures were exposed to the volatiles with or without a saturated solution of KOH placed in the incubation headspace.

Volatile source[1] treatments	Sclerotium dry weight ($\mu g\ 10^{-3}\ mm^{-2}$)			
	culture on WA		culture on tuber	
	without KOH	with KOH	without KOH	with KOH
Plant, untreated	2 d[2]	27 c	31 bc	137 ab
Plant, shoot cut off	83 ab	54 abc	151 ab	175 a

[1] Potato plants cv. Pimpernel were used and shoots remained green.
[2] 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 logarithms. Significances: Plants: *; Cultures: *; KOH: *; Plants × Cultures: NS; Plants × KOH: *; Cultures × KOH: NS; Plants × Cultures × KOH: *.

Table 5. The C/N ratio of subterranean potato plant parts cv. Pimpernel.

Sampling date[1]	Source treatment	C/N of roots	C/N of stolons	C/N of tubers
−56	untreated	29[2]	19	–
−21 θ	untreated	18	16	–
0	untreated	30	32	31
29 †	untreated	30	32	45
10	shoot cut off	33	37	40

[1] Number of days from the day of haulm destruction; θ): tuber set; †): untreated shoots dead.
[2] Average of two replicates; standard deviations varied from 0.1 to 1.7.

increased from 16 to 29 during the early plant growth, reaching to values ranging from 30 to 45 at four weeks after haulm destruction (Table 5).

Analysis of the volatile tuber exudates. In two duplicate experiments GC and GC/MS analyses of all volatile exudates from tubers and stolons showed similar results. Regardless of COS, most components volatized 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 6). Sharp peaks appeared at RT 1.5, 2, 3, 5 and 8. Small peaks at RT 26, 30, 32, 43, and 50 seemed to be volatile derivates from the sealing and not of plant origin. Percentages of area counts measured did not appear to be affected by closing time, nor by the amount of enclosed plant tissues. Similarly, the ratio between the estimated surface area of all enclosed plant tissues and of the enclosed tubers was not related to the counts.

During the first duplicate experiment, the total area counts of samples from untreated plants remained the same but after excision of the shoots total counts decreased. In

Table 6. Percentage 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 excised.

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

[1] Days 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.

the second experiment, however, the total counts decreased for both UNTR and COS plants (Table 6). In the first experiment shoots of UNTR plants remained green but in the second trial shoots became yellow after 13 days. Data of this second experiment are shown in Tables 6, 7a, 7b and 8.

Area counts decreased at RT 0 to 4 and increased at RT 5 to 10, regardless of plant treatment (Table 7a). Compared with the UNTR, after COS, area counts were higher and increased more at RT 5 and 8 (Table 7b). In percentage terms the impact of the components at RT 2 decreased and at RT 5 and 8 increased during tuber maturation and, this process seemed slightly accelerated after COS.

MS analysis revealed that carbon dioxide and formic acid may account for the peaks at RT 0 to 4. CO_2 concentrations in the gas bags (% v/v) were measured for three weeks after COS (Table 8, Fig. 2). Compared with the UNTR, after COS, the production of CO_2 halved within three days. This result was similar when the measured concentration was ascribed to tubers only or to all enclosed tissues for calculating CO_2 production. Within 16 hours after COS, the concentration of ethylene increased to 0.040 $\mu l\ l^{-1}$ in the headspace of COS plants as compared to 0.026 $\mu l\ l^{-1}$ in the headspace of untreated plants, but these results were too variable to allow definite conclusion to be made. When the experiment ended, the insides of the gas bags of UNTR plants appeared to be dry, whereas those of COS plants had collected about seven ml of liquid exudates during the four weeks of the experiment.

Table 7a. Area counts and percentage area counts from GC analysis of volatile exudates produced by tubers and underground stem parts attached to potato plants of cv. Pimpernel with shoots untreated or cut off.

Source		Retention time (min)										
plant treatment	sampling date[2]	counts[1]						percentage counts				
		00-60	1.5	2	3	5	8	1.5	2	3	5	8
Untreated	1	43	1	12	8	1	<1	3	27	17	3	<1
	8	42	3	12	15	3	<1	8	29	36	7	1
	13[3]	9	<1	4	3	1	<1	3	41	34	10	1
	19	11	<1	2	5	2	<1	1	17	43	21	3
	28	9	<1	1	4	2	<1	4	12	43	22	3
Shoot cut off	1	46	2	12	9	3	<1	4	26	20	6	1
	8	50	2	15	19	5	1	3	29	39	9	2
	13	25	1	5	10	5	1	4	19	42	20	3
	19	20	<1	3	7	5	1	2	17	34	26	4
	28	–	–	–	–	–	–	–	–	–	–	–

[1] Counts min^{-1} 10^{-3} mm^{-2} enclosed tissue surface; values were divided by 10^6.
[2] Days after haulm destruction.
[3] First leaves turned yellow on day 13.

Table 7b. Differences between area counts and between percentage area counts presented in Table 7a.

Source		Retention time (min)										
plant treatment	sampling date[2]	counts[1]						percentage counts				
		00-60	1.5	2	3	5	8	1.5	2	3	5	8
△ (UNTR)[3]	8-1	−1	2	0	7	2	0	5	2	19	4	<1
	13-1	−34	−1	−8	−5	−0	−0	0	14	17	7	1
	19-1	−32	−13	−10	−3	1	0	−2	−10	26	18	2
△(COS)[3]	8-1	4	−0	3	10	2	1	−1	3	19	3	2
	13-1	−21	−1	−7	1	2	1	1	−7	22	14	3
	19-1	−26	−2	−9	−2	2	0	−2	−9	14	20	3
COS-UNTR	1	3	1	0	1	2	0	1	−1	3	3	0
COS-UNTR	8	5	−2	7	3	0	1	−6	9	0	−1	1
COS-UNTR	13	13	0	5	6	3	1	0	−13	5	7	2
COS-UNTR	19	6	−0	5	1	1	0	0	9	−12	2	1

[1] Counts min^{-1} 10^{-3} mm^{-2} enclosed tissue surface; values were divided by 10^6.
[2] Days after haulm destruction.
[3] △: difference between scores on different sampling dates from Table 7a;
UNTR: data compared between scores from untreated plants at the above RT;
COS: data compared between scores from plants with excised shoots at the above RT.

Table 8. Measured concentration of carbon dioxide in gas bags (headspace 4500 ml, 18 °C), and the production of carbon dioxide calculated either from the surface area of the enclosed tubers only or calculated from the surface area of all enclosed underground potato plant tissues.

Source		CO_2 concentration % (v/v)	CO_2 production (mg h^{-1} m^{-2})	
sampling[1] date	plant treatment		tubers	tubers + stem + stolons
2	Untreated	1.327 b[2]	312 ab[2]	184 ab[2]
	Shoot cut off	0.350 cd	76 def	91 de
13	Untreated	3.124 a	452 a	347 a
	Shoot cut off	0.578 c	119 cde	71 cd
19	Untreated	1.575 b	200 bc	172 ab
	Shoot cut off	0.209 d	40 f	24 e
28	Untreated	1.569 b	144 bc	122 bc
	Shoot cut off	0.212 d	35 f	21 e

[1] 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] Per column, values not followed by an identical character are significantly different (P = 0.05) according to analysis of variance after transformation to natural logarithm. Significances: Plant treatments: ***; Sampling dates: *; and Plant treatment × Sampling date: NS.

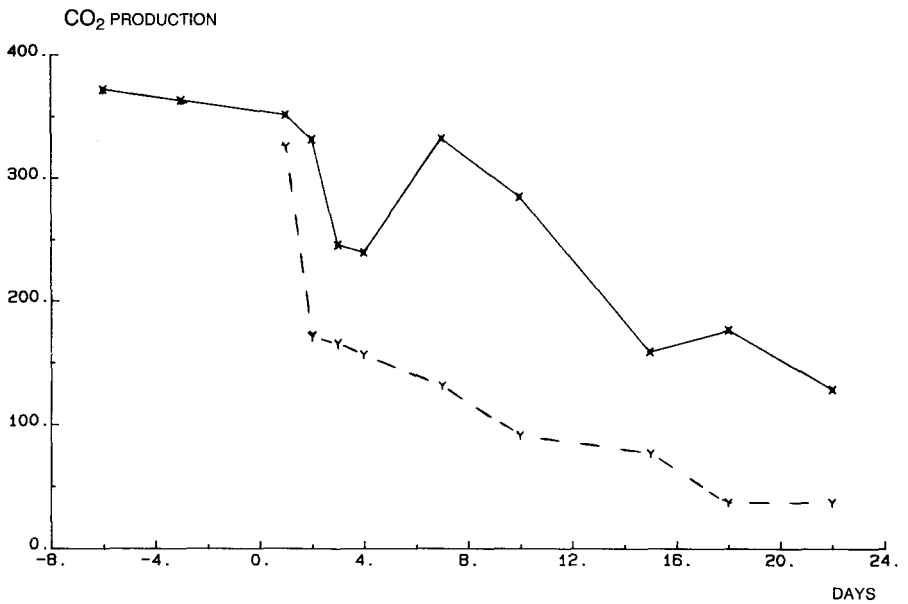

Fig. 2. The production of carbon dioxide (mg h^{-1} mm^{-2} of tuber surface area) by potato tubers cv. Pimpernel with shoots untreated (solid line) or shoots cut off (broken line), measured at several intervals before and after the day of shoot excision (day 0).

Discussion

Previous studies showed that potato tubers promote the production of mature black sclerotia by *R. solani* AG-3 on their skin (Dijst, 1985). The amount of sclerotia gradually increases during normal tuber maturation and, more rapidly, after the plant shoots are killed. On artificial media, nutrients promote sclerotium production in these AG-3 isolates, although the composition of substances is more important than the individual amounts (Townsend, 1957; Dijst, 1988a). Therefore, the hypothesis could be that the increased sclerotium production on tubers is caused by an increased exudation of nutritive substances from the tubers as no short-term trigger is involved (Dijst, 1988a). In vitro, the final mass of sclerotia was not increased by periderm components or by stable water-soluble tuber exudates (Dijst, 1988b). This study shows that the volatile tuber exudates influence sclerotium production, as suggested before (Dijst, 1988b). On agar media exposed to the volatile exudates from tubers attached to plants, the production of sclerotia was inhibited when the shoots were green and tubers were growing (Table 3). However, it was slightly stimulated when the shoots were yellowing and tuber growth stopped and, even significantly stimulated when the shoots were excised and tuber maturation was accelerated. When the inhibitory fraction was trapped by KOH, the non-trapped fraction stimulated sclerotium production always to a similar degree, regardless of tuber growth or haulm killing (Table 4). Thus, exudation of inhibitory volatiles probably decreases during tuber maturation and stops after haulm excision. In line with previous findings (Dijst, 1988a), this implies that the increase in sclerotium production on tubers depends in the first place on the decrease in exudation of inhibitory volatiles.

The stimulatory products in the volatile tuber exudates were not identified in this study. In the absence of the inhibitory substances their effect did not significantly increase after excision of the shoots (Table 4), but a slight increase in their amount was not excluded by this experiment. GC analysis of all volatile tuber exudates revealed peaks at RT 5 and 8 of which the impact increased more rapidly after haulm excision than during normal tuber maturation (Table 7b).

Also unstable tuber products may promote sclerotium production. Sclerotium production was higher on the periderm of intact tubers than on WA but less on isolated periderm strips when exposed to volatile exudates (Table 3). Thus, contact between hyphae and periderm of intact tubers further promoted sclerotium production, as observed before (Dijst, 1988a). Unstable tuber exudates may account for this result.

The inhibitory volatile tuber exudate probably contains CO_2 or other acidic gases, because it could be trapped by KOH (Table 4). MS analysis of volatile tuber exudates revealed that carbon dioxide and formic acid may account for some of the peaks found between RT 0 and 5. The impact of these peaks decreased more rapidly after haulm excision than during normal tuber maturation (Table 6). However, the separation of components below 80 °C, at RT 0 to 5, was not satisfactory. Also, no dimethylnaphthalenes were detected at RT 20 to 30 as by Meigh et al. (1973). A better analysis would require a cold-trap together with an injection temperature below 60 °C. The reports on CO_2 affecting *R. solani* are contradictory (Sherwood, 1970; Lewis, 1976). Several results from this study together indicate that CO_2 inhibits sclerotium formation by *R. solani* AG-3 under certain circumstances. In the first place, tuber respiration seems negatively correlated to black scurf development. During tuber maturation,

tuber respiration gradually decreased and black scurf gradually increased. When the plant shoots were excised and photosynthesis was stopped, tuber respiration was rapidly halved within 3 days (Fig. 2, Table 8), whereas black scurf development accelerated after 3 + 4 = 7 days. Because these AG-3 isolates produce black sclerotia within 4 days (Dijst, 1988a), this drop in tuber respiration may allow black scurf development to accelerate. These results on tuber respiration are in line with other reports (Burton 1966; Winkler, 1971). In the second place, the volatile tuber exudates from green plants slightly inhibited sclerotium production on water agar (WA), but not those from yellowing plants (Table 3). In the third place, sclerotium production on WA was inhibited in air-tight exsiccators in which the CO_2 content increased up to 1.68 mg l^{-1} but not in boxes at 0.83 mg l^{-1} (Table 2). In the same exsiccators sclerotium production was normal on the weakly nutritive medium (MM) and, therefore the inhibition of sclerotium formation on WA was not caused by a deficiency of oxygen but by the increased CO_2 content. Probably, stimulation by nutrients masked the inhibition by CO_2 on MM incubated in exsiccators and on cultured tubers exposed to the volatile exudates from growing tubers (Dijst, 1988a).

This study does not support the idea that ethylene promotes black scurf development after haulm killing. Ethylene might be produced by tubers when stressed by an increased water content after only the shoot is killed and not the roots (Yang and Hoffman, 1984; Dijst, 1988a). In gas bags containing growing tubers, the amounts of C_2H_4 seemed increased after excision of the shoots but the results were too variable. In exsiccators, sclerotium production on the weakly nutritive MM was not affected by C_2H_4 concentrations below 0.5 $\mu l\ l^{-1}$, but inhibited by 1 $\mu l\ l^{-1}$ C_2H_4 (Table 2). Stimulation by the nutrients in MM probably compensated for the inhibition by CO_2 as discussed above. Thus, the observed inhibition of sclerotium production on MM at 1 $\mu l\ l^{-1}$ C_2H_4 was probably caused by the ethylene. Furthermore, these results may imply that the significant stimulation of sclerotium production by volatile apple exudates (Table 1) was not caused by ethylene, but most likely by nutritive substances or substances that become nutritious when absorbed by the agar or liquids available (Lewis, 1976).

Decomposing potato roots and stolons produced volatile products which may further promote sclerotium production by AG-3 isolates as suggested before (Dijst, 1988b). In this study, these decomposition products did not affect sclerotium production in the absence of plants (Table 3) which is in line with Lewis and Papavizas (1974) and Lewis (1976). However, these products slightly increased the stimulation of sclerotium production by volatile tuber exudates from older intact plants (Table 3). Likewise, these products might also further increase mycelial growth and pigmentation on older tubers of intact plants. The C/N ratio of potato roots and stolons was higher than 15, increasing up to 40 after haulm killing. According to Lewis and Papavizas (1974), decomposition products from plant tissues with a C/N ratio above 15 would not stimulate mycelial growth and pigmentation in the absence of plants. However, this study showed that the effect of volatile substances from a combination of sources can differ from the effect of each source individually.

For agricultural practice, these results offer some explanation for the increased black scurf development after haulm killing. Apparently, volatile stimulatory substances are always exuded by the tubers and probably become effective after haulm killing, which stops the exudation of inhibitory volatile tuber exudates. Still, less black scurf usually

develops after haulm pulling than after chemical haulm destruction (CHD) or excision of the shoots (COS). Probably, haulm pulling reduces the effectiveness of the stimulatory exudates because this method disturbes the ridge. Increased soil aeration may prevent accumulation of the stimulatory exudates and reduce their effectiveness. In the second place, only haulm pulling breaks the stolons, terminating the contact between tubers and roots. Thereby, the tubers exude less water and precipitable substances (Dijst, 1988a, b). Less water exuding from the tubers probably limits the availability of water-soluble nutrients to the fungus and thus sclerotium formation (Blair, 1943; Lewis, 1976; Dijst, 1988a). In this study, no water was collected from gas bags which enclosed the tubers of untreated plants, whereas 7 ml was exuded during four weeks after shoot excision. It is unlikely that tubers exude so much water after haulm pulling, because they are not connected with the roots.

Furthermore, haulm pulling may cause less black scurf because it removes much of the underground plant parts from the tuber vicinity. Thereby less decomposition products are produced which may increase the stimulatory effect of volatile tuber exudates, as discussed above. It is unlikely, but not excluded by this study, that the tubers exude a significantly higher amount of stimulatory volatiles after COS than after haulm pulling. The effect of volatile tuber exudates after COS did not differ significantly from those of intact plants when the inhibitory fraction was eliminated (Table 4).

For seed potato production the results imply that in addition to virus infection an increase in black scurf development may be prevented as well without the use of chemicals. It requires the development of a new method which breaks the stolon, separates the tubers from plant residues and allows the tubers to mature in loosened soil until collection from the field.

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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, beinvloedden de produktie van sclerotiën door *Rhizoctonia solani* AG-3 op agarplaten en op geoogste knollen, die geincubeerd waren in een plant-aarde systeem. Onder dezelfde proef- omstandigheden was de sclerotiënvorming op geincubeerde losse knollen veel hoger dan op de agarplaten, maar op peridermstrips juist lager. Wellicht dragen dus naast stabiele ook instabiele knolexsudaten bij tot de vorming van lakschurft.

Het vluchtige knolexsudaat van jonge planten bleek zowel stimulerende als remmende

componenten te bevatten. Als de remmende fractie met KOH werd weggevangen, stimuleerden de resterende uitademingsprodukten van jonge groeiende knollen de vorming van sclerotiën even sterk als de uitademingsprodukten van oude afrijpende knollen na loofvernietiging. Tijdens de knolgroei overheerste de invloed van de remmende exsudaten, maar dat nam af als de plant vergeelde en verdween na loofvernietigen. Toename van lakschurft na loofdoding berust dus vooral op het wegvallen van de remmende componenten. In de praktijk zou na loofdoding de effectiviteit van de stimulerende exudaten verminderd kunnen worden door de grond van de teeltrug los te maken waardoor ze niet kunnen ophopen aan het knoloppervlak.

De remmende fractie kon worden weggevangen met KOH, wat betekent dat het gaat om koolzuur of een andere zure component. Inderdaad bleek de produktie van koolzuur door knollen geleidelijk af te nemen tijdens de veroudering en zeer snel na loofdoding. Daarnaast is bekend dat lakschurft geleidelijk toeneemt bij veroudering van de plant, en zeer snel na loofdoding. In vitro leek koolzuur de sclerotiënvorming alleen op wateragar te remmen, maar niet op een voedzamer medium. De sclerotiënvorming op agarmedia werd sterk gestimuleerd door gasvormige produkten van appels. Echter, er werd geen bevestiging gevonden voor het idee dat het 'stress'-produkt ethyleen sclerotiënvorming stimuleert. Na het loofafknippen lekte er een veel grotere hoeveelheid water uit de knollen dan uit knollen van intacte planten. Water kan het benutten van voedzame stoffen door de schimmel bevorderen. Daardoor kan een manier van loofdoden die de stolon breekt de kans op lakschurft verkleinen.

Gasvormige produkten van afstervende wortels en stolonen hadden geen invloed op de sclerotiënvorming op agarplaten. Maar wel versterkten zij de lichte stimulering die uitging van afrijpende knollen. In de praktijk zouden jonge pootaardappelen dus milieuvriendelijker beschermd kunnen worden tegen zowel virusinfectie als zware lakschurftvorming met een nieuw-te-ontwikkelen methode van 'groen-rooien' die de stolonen breekt en de knollen op het veld laat afharden in losse grond en gescheiden van de overige planteresten.

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