Neth. J. Pl. Path. 91 (1985) 153-162

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

G. DIJST

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

Accepted 6 March 1985

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:

$I = 100.(0.f + 1.vl + 2.1 + 3.m + 4.h)/(4.t)$

Where f, vl, 1, 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-

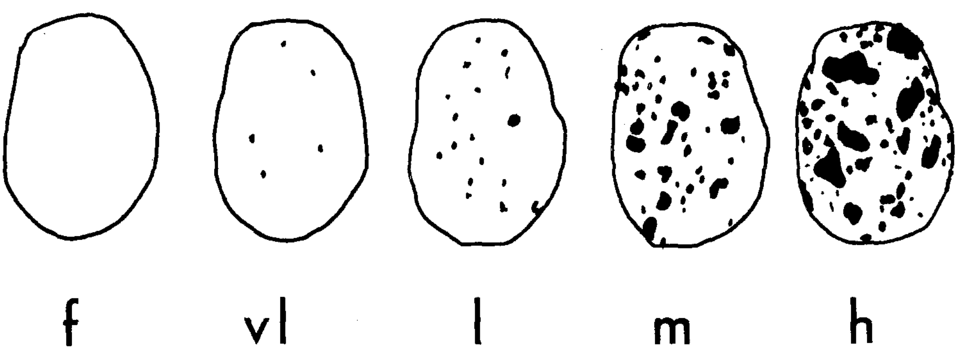

Fig. 1. Assessment key for the amount of sclerotia of *R. solani* on potato tubers: f = free, vl = very lightly, l = lightly, m = moderately and h = heavily covered with sclerotia.

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.

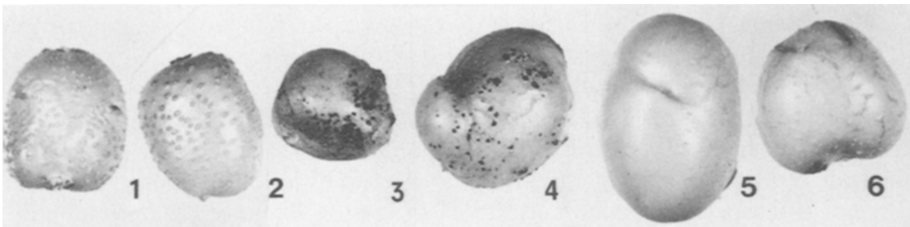

Fig. 2. Sclerotia of *Rhizoctonia solani* on potato tubers (cv. Prominent) harvested three weeks after treatment: tubers nrs. 1 and 2 from untreated plants, nrs. 3 and 4 from plants of which shoots had been cut off and nrs. 5 and 6 from plants of which stolons had been cut through.

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

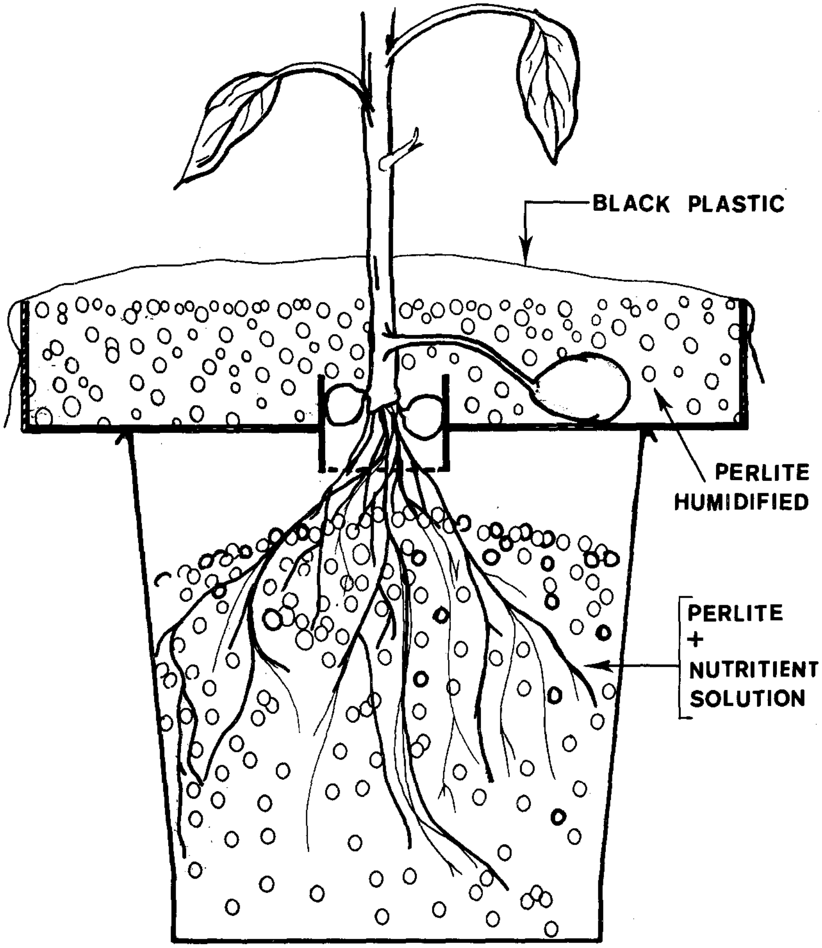

Fig. 3. The two-compartment system used for development of tubers under nutrient-free conditions.

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 aqueous tuber exudates.

Acknowledgements

The work reported here was supported by grants from the Foundation MPW (Maatschappelijke Plaats Wageningse Afgestudeerden) and seed potato growers organizations. Drs J. de Bree of the TNO Institute for Mathematics, Information, Processing and Statistics, Wageningen, assisted with statistical analyses. The meristem cultures were kindly provided by Mr D. Bakker from the NAK (Dutch Inspection Service).

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.

References

Allington, W.B., 1936. Sclerotial formation in *Rhizoctonia solani* as affected by nutritional and other factors. Phytopathology 26: 831-844.

Blair, I.D., 1943. Behaviour of the fungus *Rhizoctonia solani* Kühn in the soil. Ann. appl. Biol. 30: 118-127.

Bouman, A., Bouma, J. & Mulder, A., 1979. Rhizoctonia en ontvellingen in de pootaardappel. Landbouwmechanisatie 30: 659-661.

Bouman, A., Bouma, J., Mulder, A. & Roosjen, J., 1983. Effecten van tijdstip en wijze van loofvernietiging op de ontvellingsgevoeligheid en op de bezetting van pootaardappelen met Rhizoctonia. IMAG Publ. nr. 181, 39 pp. (with eng. summary).

Dijst, G., 1983. The production of sclerotia on potato tubers by *Rhizoctonia solani.* Acta Bot. Neerl. 32: 229.

Dodman, R.L. & Flentje, N.T., 1970. Mechanism and physiology of penetration. In: Parmeter, J.R., Jr. (Ed.), *Rhizoctonia solani:* biology and pathology. Univ. Calif. Press, Berkeley etc. p. 149-160.

Doornbos, E., 1963. Naar een snelle oplossing van het Rhizoctonia vraagstuk. Mededelingen van de NAK, Febr./March, p. 110-112.

Emden, J.H. van, 1958. Waarnemingen betreffende het parasitisme van *Pellicularia filamentosa* (Pat.) Rogers *(Rhizoctonia solani* Kühn) bij de aardappelplant. Tijdschr. Pl.Ziekt. 64: 276-281.

Parmeter, J.R., Jr. (Ed.), 1970. *Rhizoctonia solani:* biology and pathology. Univ. Calif. Press, Berkeley etc., 255 pp.

Spencer, D. & Fox, R.A., 1978. The distribution of sclerotia of *Rhizoctonia solani* Kühn on the surface of the potato. Potato Res. 21: 291-300.

Spencer, D. & Fox, R.A., 1979. Underground spread of *Rhizoctonia solani.* Potato Res. 22: 29-30.

Steiner, A.A., 1968. Soilless culture. Proc. 6th Coll. Int. Potash Inst. Florence, Italy. Publ. by Potash Inst. Bern. Switzerland. p. 324-341.

Townsend, B.B., 1957. Nutritional factors influencing the production of sclerotia by certain fungi. Ann. Bot. 21: 153-166.