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Infection process of Rhizoctonia solani on Solanum tuberosum and effects of granular nematicides

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Abstract

The infection process of *Rhizoctonia solani* AG-3 was studied on potato sprouts, cv. Bintje, in growth chamber trials at 15 °C. Initially hyphae of *R. solani* grew predominantly in the longitudinal direction of the sprouts (runner hyphae). They tended to follow the junctions between epidermis cells as was observed by SEM. The hyphae formed side-branches mainly half-way of the subterranean parts of the sprouts. They branched several times with short swollen cells to form infection cushions. Lesions developed only underneath the infection cushions and were first observed five days after inoculation. The necrotic area was proportional to the area covered with infection cushions on the sprouts. Depth of the lesions could extend up to the vascular bundle. Sprouts were colonized only in healthy tissue in the epidermal layer underneath the infection cushion and in necrotic tissue. A few days after appearance of the lesions, *R. solani* formed brown, uninfective mycelium on and in the circumference of these lesions.

Aldicarb did not influence any part of the infection process. Ethoprophos delayed the emergence of sprouts, but increased the number of sprouts per tuber. As soon as sprouts had emerged, growth was considerably promoted by ethoprophos. Ethoprophos delayed the appearance of lesions and reduced their size. Oxamyl showed the same effects to a smaller extent.

As the size of lesions appears to be proportional to the size of the infection cushions, any agents that change the size of the infection cushions, such as pesticides or antagonists, may alter the severity of the disease.

Additional keywords: potato, stem canker, disease severity, infection cushions, runner hyphae, ethoprophos, aldicarb, oxamyl, side-effects, non-target effect, scanning electron microscopy.

Introduction

Rhizoctonia solani Kühn infects stems, hypocotyls, roots or leaves of a wide range of host plants (Parmeter, 1970). The symptoms on potato are lesions on stems and stolons, and sclerotia on the tuber. During the vegetative period, a white collar of mycelium with basidia may be formed around the stem base. Different anastomosis groups (AG) can be distinguished in *R. solani*. The most common group on potato is AG-3.

Several authors (Hide and Corbett, 1974; Leach and Frank, 1982; Ruppel and Hecker, 1982; Scholte, 1987) observed in field trials an increased infection of potatoes and beet by *R. solani* after application of the nematicides aldicarb, oxamyl or ethoprophos. The increase in disease might be due to direct effects on the pathogen or on the host plant, or to indirect effects: by a decrease of the microbial antagonism or of the activity of

the mycophagous soil fauna. A previous paper showed that the enhanced incidence of the disease is not caused by an effect of the nematicides on the growth of the pathogen or on the microbial antagonism (Hofman and Bollen, 1987). This paper deals with the effects on the infection process and on the susceptibility of the host plant. The effects on the mycophagous soil fauna will be described in subsequent papers.

Although the infection process of *R. solani* has been studied on many hosts (Bateman, 1970; Dodman and Flentje, 1970; Fukutomi and Takada, 1979; Kenning and Hanchey, 1980; Marshall and Rush, 1980b; Matsuura, 1986), we did not find such a study on the infection of potato. Therefore, the assessment of the effect of granular nematicides was preceded by a detailed study of the various stages in the infection of sprouts and stolons of potato, with emphasis on the relation between mycelium development on the sprout surface and severity of the disease. Knowledge of this relation is important for understanding the effects of nematicides. With *R. solani* on rice, Marshall and Rush (1980a) found a strong correlation between size of infection cushions and lesion size. We studied whether such a correlation applies also to *R. solani* on potato.

By exposing sprouts to light, Van Emden (1965) induced resistance to infection by *R. solani*. A rapid emergence is therefore supposed to reduce the infection of the subterranean stem. Post-emergence resistance to *Rhizoctonia* has also been reported in other crops, e.g. beans (Leach and Garber, 1970). Thus, stem infection of potato is reminiscent of a seedling disease. Whereas seedlings of many crops are completely killed by *R. solani*, lesion size on potato stems is often restricted. Probably the potato plant shows some kind of locally induced resistance in reaction to stem infection.

Pesticides can affect the resistance of the host plant (Heitefuss, 1973; Sumner, 1974; Altman and Campbell, 1977). If aldicarb, oxamyl or ethoprophos influence host resistance, they may increase the size of lesions. Because of their systemic action, aldicarb and oxamyl have the highest probability of interfering with host resistance. In the literature, reduced host resistance by aldicarb is reported only once (Tisserat et al., 1977). Aldicarb applied at three times the recommended field dosage reduced resistance of beet seedlings.

Depending on the medium used for the test, ethoprophos and oxamyl showed fungitoxicity (Hofman and Bollen, 1987). This means that application of these nematicides to sterilized soil might give a reduced or delayed infection by *R. solani*. In the field, ethoprophos, applied at dosages twice as high as recommended, reduced severity of root disease in cucumbers caused by *R. solani* and *Pythium aphenidermatum* (Sumner, 1978). To investigate effects of the nematicides on the interaction between potato stems and *R. solani*, we performed all trials in sterilized soil.

Materials and methods

Plant material and growing conditions. In all trials, plants were grown from seed tubers (25-35 mm in diameter) of cv. Bintje that had been stored since the August harvest at 4 °C. Before planting, sprouting was stimulated by placing the tubers at room temperature in the light for one week. During this period, they were treated against tuber-borne *R. solani* by dipping them in a solution of 0.9 g validamycin (Solacol) per l. Tubers were planted in pots with autoclaved course sand and placed in a growth chamber at 15 °C and 16 h light per day. Most experiments were done from March till May.

Inoculation. *R. solani* was grown for 14 days at 20 °C on a perlite medium (particle diam. 1-5 mm). To one litre of perlite were added 500 ml distilled water, 16.7 g Czapek Dox liquid medium ingredients (Oxoid) and 5.0 g malt extract (Oxoid).

One week after planting, the inoculum was added to the sand (10 ml l^{-1}). Tubers and sprouts were taken carefully out of the sand and planted again after the soil had been inoculated.

Observation of the infection process. At various intervals after inoculation, sprouts were taken from the soil (at least 20 sprouts for each treatment). The fungus on and in the tissue was stained by immersing the sprouts in 0.1% trypan blue in lactophenol for 15 min, rinsed twice in water and differentiated in 30% lactic acid. The sprouts were stored in glycerol and examined for the presence and development of infection structures of *R. solani*.

SEM. Sprout parts were first stored in 35% formaldehyde. Then specimens were fixed in a glutardialdehyde solution (20 g l^{-1}) in cacodylate buffer (0.1 mol l^{-1}; 24 h; pH 7.4; 20 °C). After washing twice in cacodylate buffer, specimens were fixed in OsO_4 in the same buffer (10 g l^{-1}; 8 h; pH 7.4; 4 °C) and subsequently washed in buffer solution and distilled water. After dehydration in a graded series of aqueous ethanol up to pure ethanol, the specimens were critical-point-dried in liquid CO_2 and mounted on a specimen holder. Finally the specimens were sputter-coated with gold (about 15-20 nm thick) and examined with a Jeol 35C scanning electron microscope, operated at 15 or 25 kV.

Nematicide treatments. Before planting of seed tubers, the sand was treated with granular nematicides at dosages recommended for field application to potatoes, assuming that in the field the granulate is mixed with the upper 10 cm of soil. Ethoprophos (10 mg l^{-1}, as Mocap 20 GS), aldicarb (3 mg l^{-1}, as Temik 10 G gypsum) and oxamyl (5 mg l^{-1}, as Vydate 10 G) were mixed with the sand. To study effects at higher dosages the nematicides were also applied at double and three-fold dosages.

Post-emergence resistance. Effects of the nematicides on post-emergence resistance were tested with plants that were inoculated 23 days after planting. Before inoculation, plants were exposed to light (28 W m^{-2} from TL light sources) for about 8 days from emergence; the intensity of the light was low, which caused sprouts to become quite long. Inoculum was mixed with sand at a dosage of 30 ml l^{-1}. The mixture was placed in a layer of about 2 cm on top of the potting sand. In this trial, the effects of aldicarb and ethoprophos were tested at a dosage double the recommended dosage. The sand was irrigated as needed with Hoagland solution.

Results

Infection process. Initially, runner hyphae of *R. solani* grew mainly longitudinally along the sprout. The hyphae tended to follow the junctions of the epidermal cells (Fig. 1). At certain sites, mostly at half of the length of the subterranean part of the sprout, primary branches formed. They consisted of straight cells, but shorter than those of the longitudinal hyphae. From primary branches secondary branches developed, which

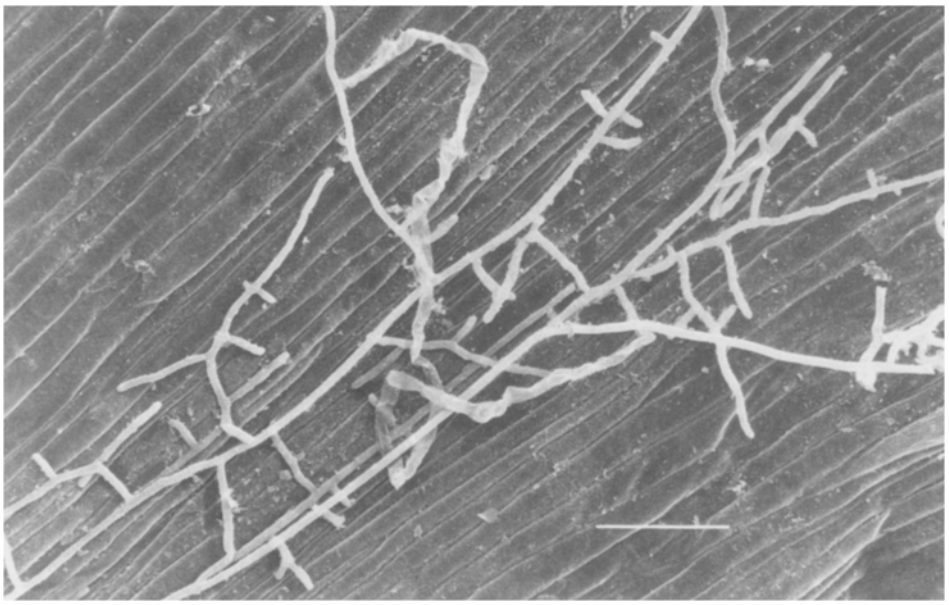

Fig. 1. Runner hyphae (and some side branches) of *R. solani* predominantly growing along the junctions of epidermal cells (three days after inoculation; bar represents 100 μm).

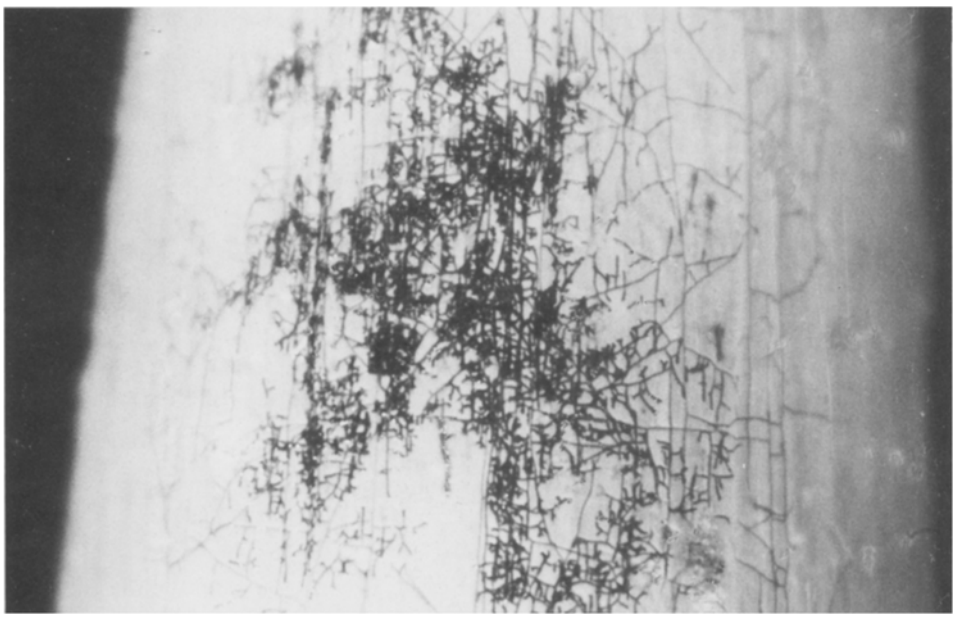

Fig. 2. At four days after inoculation short swollen hyphae of *R. solani* clump together to form cushion-shaped structures (infection cushions).

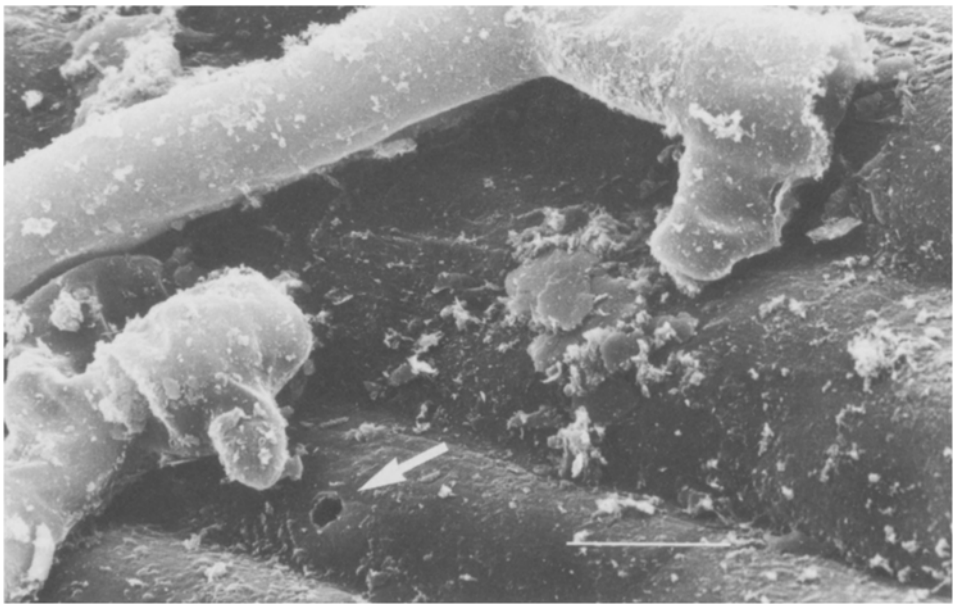

Fig. 3. Penetration peg pulled away from its penetration site (arrow) at four days after inoculation (bar represents 10 μm).

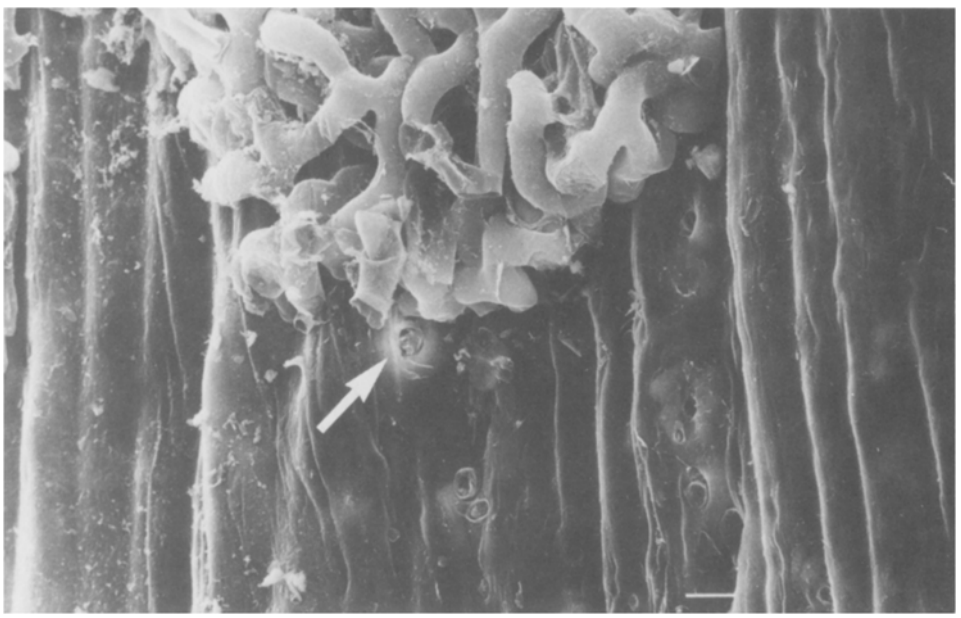

Fig. 4. Penetration sites in epidermal tissue under a partly removed infection cushion (bar represents 10 μm). The halo around the penetration site (arrow) is characteristic for a very thin tissue layer (five days after inoculation).

consisted of short swollen cells. These often branched several times to form infection cushions, i.e. dense masses of swollen cells (Fig. 2). Under the infection cushions many hyphae penetrated the epidermal cells (Figs 3 and 4). This process might be both mechanical and enzymatic. SEM showed light-colored tissue around the penetration sites (Fig. 4). Such a halo indicates that the cell walls are locally less thick (W.L. Jongebloed, personal communication). This is most likely caused by enzymatic activity.

Colonization of plant tissue was initially restricted to one or two cell layers underneath the infection cushion. Lesions developed only under infection cushions and with a margin that exceeded the infection cushion by about 2 mm. Finally, lesions were up to about 12 cell layers deep, reaching the vascular bundle of the stem. The phloem was affected and in case of a severe attack also the xylem was affected. Girdling of a sprout by this type of lesion caused death, which occurred mostly on young sprouts. Smaller lesions only slightly affected growth of stems.

R. solani colonized the dead tissue after a few days. Except during the initial colonization of epidermal cells, the mycelium was never observed in healthy tissue.

On older lesions, the mycelial mass of the infection cushions had increased, and straight-walled brown hyphae were then present on uninfected parts of the sprout. Even at this stage, colonization of plant tissue was restricted to the part directly under the infection cushion. So *R. solani* does not colonize sprout tissue progressively.

Contrary to earlier observations by Van Emden (1965), plants that had emerged healthy and were exposed to light were not found to be resistant to stem infection by *R. solani*. Even healthy-emerged sprouts can become infected so severely that they fall off.

Influence of nematicides on infection by R. solani and on sprout development. Microscopic examination of sprouts grown in sand with different dosages of aldicarb, oxamyl or ethoprophos did not demonstrate morphological changes of infection structures of *R. solani*. The relation between lesion size and sprout area covered with infection cushions was not affected by the nematicides.

Ethoprophos (Fig. 5) and, to a lesser extent, oxamyl delayed the rate of the infection process, possibly because of their fungitoxicity (Hofman and Bollen, 1987). Mycelial growth over the sprout surface was inhibited (unpublished results). Consequently initiation and appearance of infection cushions and appearance of lesions were also delayed and the lesions remained smaller. In untreated soil 50% of the sprouts showed lesions 6 days after inoculation (Fig. 5). In soil with ethoprophos at 20 mg l^{-1}, this level was reached after 15 days and with oxamyl at 10 mg l^{-1} after 9 days (these dosages are double the dosages recommended for field application).

Longitudinal growth of sprouts was initially reduced, but from the emergence of the sprouts, it was promoted by ethoprophos (Fig. 6). The number of sprouts per tuber had significantly increased from 3.40 on tubers in untreated soil to 4.04 on tubers in soil with ethoprophos at 20 mg l^{-1}. Oxamyl slightly inhibited the longitudinal development of unemerged sprouts.

When sprouts were about 40 mm long in untreated soil, their apex reached the soil surface. In untreated soil, emergence was at 15 days after planting. Ethoprophos at 20 mg l^{-1} delayed the emergence by 3 days (Fig. 6) and the infection by 9 days (Fig. 5). Oxamyl at 10 mg l^{-1} delayed the emergence by 1 day and the infection rate by 3 days.

Table 1 shows that in sterilized soil ethoprophos reduced the disease, when sprouts were inoculated after emergence. At 20 mg l^{-1}, the fraction of severely infested stems

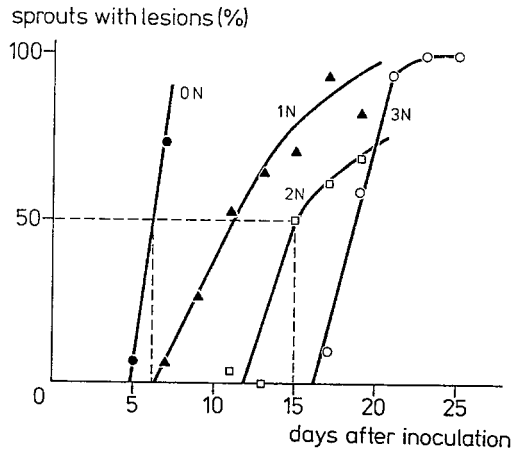

Fig. 5. Effect of three dosages of ethoprophos in the soil on the fraction of stems with lesions (%) at different times after inoculation. 0N, 1N, 2N and 3N: 0, 10, 20 and 30 mg ethoprophos per l soil.

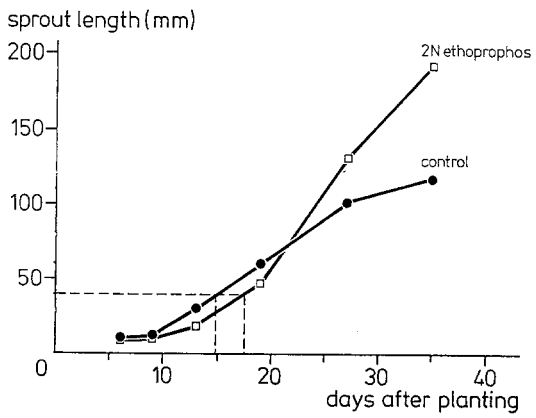

Fig. 6. Effects of ethoprophos on longitudinal growth of potato sprouts. 2N: 20 mg ethoprophos per l soil.

Table 1. Effect of nematicides on the fraction of potato stems girdled by lesions of *R. solani*, 20 days after emergence. Inoculation was at 8 days after emergence.

Treatment	Diseased stems (%)
Control	43
Aldicarb 6 mg l^{-1}	48
Ethoprophos 20 mg l^{-1}	23*
Ethoprophos 40 mg l^{-1}	17*

* Significantly different from control (Wilcoxon's test; P = 0.05).

decreased from 43% to 23%. At 40 mg l^{-1} it was as low as 17%. The presence of ethoprophos in the basal layer of uninoculated soil reduced infection in the upper soil layer. Therefore, there may be a systemic effect, because the upper soil layer (2 cm) was inoculated 23 days after planting but had not been treated with ethoprophos. This protection may be caused either by an increase in resistance of the sprouts or because of the presence of ethoprophos in or on the stem in the inoculated soil region.

Aldicarb did not affect the rate of the infection process by *R. solani*, the severity of disease or the development of sprouts.

Discussion

The size of lesions caused by infection by *R. solani* was proportional to the size of infection cushions on the sprout surface. This was similar as found by Marshall and Rush (1980a) on rice. The results indicate that lesions on potato sprouts are only formed after fungal penetration from infection cushions and not from lobate appressoria or simply through stomata or wounds as has been described for other hosts by Dodman and Flentje (1970). Progressive invasion of host tissue as has been reported for infected seedlings (Bateman, 1970) did not occur in potato sprouts.

Death of cells seemed to be caused by extracellular fungal enzymes or toxins, because the depth of the lesions exceeded the depth of the colonized tissue by many cell layers. Deterioration of cell walls was most prevalent in the third and fourth cell layer. Possibly, the first two layers were held together by mycelium of *R. solani* and deeper layers were not so seriously affected by cell wall-degrading enzymes.

The infection process was not studied on stolons, but probably proceeds in the same way as on sprouts. On roots, infection structures were never found.

The fungus can utilize nutrients released from lesions for its growth. Possibly the fungus is pathogenic as long as it grows as runner hyphae. In trials where 5 ml of perlite inoculum per litre of soil was used, stem infection rate was lower than when 0.5 ml of perlite inoculum per litre was used (Hofman, unpublished results). At the higher inoculum density the available nutrients will be used more rapidly by the colonizing fungus than at lower inoculum densities. When lesions become older a characteristic brown mycelium appears on the sprout surface. This mycelium may represent a resting stage and may survive on plant debris in soil or on the tuber surface till another growing season. The brown mycelium develops when shortage of nutrients limits further mycelial development (Boosalis and Scharen, 1959). This mycelium did not seem to be infectious, because its side-branches never formed infection cushions. Therefore at the higher inoculum densities, the sprouts had a greater chance of escaping infection than at lower inoculum densities.

Because lesion size and size of infection cushions are related to each other, any mechanism that can influence the development of mycelium on the sprout surface (such as microbial antagonism or pesticides) may have an influence on the subsequent lesion size.

Although ethoprophos is not known to be a systemic nematicide, it caused morphological changes in potato sprouts. The number of sprouts per tuber was increased and the longitudinal development of sprouts was initially inhibited and subsequently stimulated (Fig. 6). Thus ethoprophos affects the physiology, most probably the hormonal balance, of young potato plants.

In our trials, we did not find any evidence for the existence of post-emergence resistance after exposing sprouts to light as was reported previously by Van Emden (1965). Stems that had emerged healthy, became infected when inoculated eight days after emergence, but the lesions did not girdle stems to the same extent as with pre-emergence infection. The disease-reducing activity of ethoprophos was apparent both in trials with young sprouts and with older sprouts.

It cannot be expected that nematicides increase stem infection because of phytotoxicity or stimulation of the infection process in the field. Emergence of sprouts may be delayed by ethoprophos and oxamyl. In field trials on clay, ethoprophos reduced initial

development of potato plants (K. Scholte, personal communication; Hofman, unpublished results). In vitro, the relation between lesion size and surface of the infection cushions on the sprouts was not affected by any of the nematicides.

In the field, aldicarb, oxamyl and ethoprophos stimulated stem and stolon infection to the same extent (Hofman, unpublished results). The present study shows that the nematicides do not stimulate the disease by a direct effect on the interaction between the potato plant and *R. solani*. Ethoprophos, and to a lesser extent oxamyl, reduced the disease in sterilized soil, which was probably due to their fungitoxicity. From observations mentioned in this paper, it is expected that the increased disease severity in the field is associated with an increased amount of mycelium on the sprout surface. The cause of this increased development of mycelium on sprouts in nematicide-treated fields will be reported in later publications.

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Samenvatting

Het infectieproces van Rhizoctonia solani op Solanum tuberosum en de effecten van granulaire nematiciden

Het infectieproces van *Rhizoctonia solani* AG-3 werd bestudeerd op aardappelspruiten, cv. Bintje, in een klimaatcel bij 15 °C. Aanvankelijk groeide de schimmel met runnerhyfen voornamelijk in de lengterichting van de spruit. Via SEM kon waargenomen worden, dat de hyfen hierbij vooral over de begrenzingen van de epidermiscellen groeiden. Het mycelium vormde veel zijvertakkingen, bestaande uit iets gezwollen korte cellen, welke voornamelijk halverwege op het ondergrondse deel van de spruit gevormd werden. Een dichte massa van deze cellen vormde een infectiekussentje. Lesies, welke vanaf vijf dagen na inoculatie werden waargenomen, bevonden zich slechts onder spruitoppervlak bezet met infectiekussentjes. De lesiegrootte was recht evenredig met het spruitoppervlak dat bezet was met infectiekussentjes. De diepte van de lesies reikte tot aan de vaatbundels. De spruit werd alleen door de schimmel gekoloniseerd in gezond epidermisweefsel onder het infectiekussentje en in necrotisch weefsel. Enkele dagen na verschijning van lesies vormde *R. solani* bruin, niet infectieus, mycelium op en rondom de lesies.

Aldicarb had geen effect op het infectieproces. Ethoprophos vertraagde de opkomst en verhoogde het aantal tot ontwikkeling gekomen spruiten per knol in gestoomd zand. Direct na opkomst had ethoprophos echter een sterk groeistimulerend effect. Ethoprophos vertraagde de lesievorming en reduceerde de lesiegrootte, vergeleken met onbehandelde planten. Oxamyl vertoonde deze effecten in geringere mate.

Daar de lesiegrootte direct gecorreleerd blijkt met de grootte van het infectiekussentje, mag verwacht worden dat elke beïnvloeding van de ontwikkeling van het mycelium van *R. solani*, bijvoorbeeld door pesticiden of antagonisten, een verandering van de lesiegrootte ten gevolge heeft.

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