Neth. J. Pl. Path. 72 (1966) : 279–283

ROTTING BY SPREAD OF MYCELIUM FROM ASCOSPORE LESIONS OF *SCLEROTINIA TRIFOLIORUM*[1]

Rotting door een massale ontwikkeling van mycelium uit ascospore-lesies van Sclerotinia trifoliorum

J. DIJKSTRA
Foundation for Agricultural Plant Breeding (S.V.P.), Wageningen

Brown lesions on red clover, resulting from infection by ascospores of *Sclerotinia trifoliorum*, were sometimes surrounded by a light grey ring. Clover rot developed rapidly from these rings under moist conditions. The beginning of rot in the field was often observed in the joints of the trifoliate leaves. Freezing plants at −6° or −8 °C or freezing small parts of plants with carbon dioxide snow resulted in a quick spread of mycelium, with rotting, from the ascospore lesions. Weakening the plants by bruising did not promote this transition to active rotting. Very heavy infection by ascospores, however, may cause immediate rotting in the plants. The information obtained indicates that in the field clover rot originates mainly from ascospore lesions.

INTRODUCTION

For breeding red clover resistant to *Sclerotinia trifoliorum* Eriksson use is generally made of inoculation with mycelium from a culture on agar or cereal products. The results with this inoculum are very often disappointing. Since infection with ascospores as a start for mycelial growth and rotting in the field is considered important by some investigators (VALLEAU *et al.*, 1933; DILLON WESTON, 1950; LOVELESS, 1951) it was considered worth while to investigate whether ascospore inoculation could be developed for use in breeding resistant clover. Some comparisons of ascospore with mycelial inoculation for breeding resistant clover and the methods for production and inoculation of ascospores have already been described by the author (1964).

Ascospore infection causes lesions in the leaves of the plants, which usually show no extension to rotting during a greater or lesser period. Sometimes there is no rotting at all in plants densely covered with brown spots of ascospores. For breeding purposes it was therefore desirable to know more about the transition from the limited ascospore lesions to active spread of mycelium with rotting and how this transition can be induced. It was also considered important to investigate the significance of ascospore infection in the field. The observations and experiments made about these last mentioned phenomena are described in this article.

LITERATURE

Some 1–2 months after ascospore inoculation of red clover LOVELESS (1951) obtained active rotting originating from the brown spots caused by the infection, when the leaves had been subjected to very moist conditions or when they had been exposed to night frosts. After the frost the infection points were first

[1] Accepted for publication 30 December, 1965.

surrounded by a yellow ring before the actual rotting set in. In the field he observed that the fungus did not begin to develop, from leaves infected in the autumn, before mid-April, while the non-infected crop showed no rotting at all. COLEMAN (1907) writes that in a clover field with many apothecia in the autumn, rotting did not occur before January, when after frost the thaw and rain set in. This observation agrees with that of LOVELESS. However, he does not mention details about the way in which the rotting had been produced.

In his experiments PURDY (1949) found that on detached clover leaves water-soaked by a vacuum treatment, ascospore infection was followed by mycelium and rot over a considerable portion of the leaf surface. On the other hand, non-water-soaked leaves inoculated with ascospores, showed only slight signs of penetration.

LOVELESS (1951) experimented with leaves of beans *(Vicia faba)*, applying a more and a less intensive ascospore infection. With a heavy infection he observed such a mass of lesions that they flowed together and in that case an agressive mycelial growth occurred over the entire leaf surface.

From this literature it is evident that prolonged high air humidity, frost damage and presumably also the water-soaking of leaves under vacuum, promote the development of rotting from ascospore lesions.

OBSERVATIONS

In the field and in the laboratory it was observed that ascospore infection in red clover can give lesions in one or two days. These may be limited for a long time to brown spots. Fig. 1 shows ascospore lesions and rotting. In a field naturally infected with clover rot some of the brown spots were found in the autumn to be encircled by narrow light grey rings. When leaves carrying these symptoms were laid in moist petri dishes the mycelium of *Sclerotinia* developed from these spots after a few days. The fungus could be identified on agar culture. Similar encircled spots were observed on red clover plants infected with ascospores in a chamber with high air-humidity and a temperature of 10—14 °C. Again, from the light ringed spots rot and mould developed.

The initial rotting in the field and in the laboratory was found especially in and around the joints of the trifoliate leaves of the plants. These places remain moist for a long time, providing favourable conditions for growth of the fungus.

In October 1959 many apothecia together with numerous and widespread brown spots in clover leaves of many varieties and families appeared in a trial field. Rotting in this field developed only after a frost and snow period in January. The abundant mycelial growth on nearly all the plants made it very obvious that the mycelium originated from ascospore lesions. Apothecia had become very scarce by that time (at a rough estimate, 1 per 50 one-meter rows of clover plants) so that it may be assumed that the development of rot started from the brown spots and not from fresh ascospores. Transitional stages as described above were not observed, but these could have occurred under the snow cover.

On this field sclerotia had been laid out during the preceding summer at the end of every row and the apothecia developed at these places. If sclerotia acted as the direct source of infection, as is stated by some authors, it would be expected that most of the plants at the end of the rows adjacent to the buried selection would be attacked by mycelium early in the season with more killing of

these plants as a consequence. This was not the case, which makes the development of mycelial infection from sclerotia unlikely. In no case did mycelial development on the plants clearly start from sclerotia in the field.

Stimulation of mycelial spread from ascospore lesions

Seven hundred and twenty rows of clover plants in the field mentioned above were all covered with brown spots as a result of ascospore infection in October 1959. In this stage of infection differences of resistance in red clover cannot be detected (DIJKSTRA, 1964). In order to discover such differences, it is necessary in some way to weaken the plants, so that mycelial growth and rotting from ascospore lesions is stimulated. The normal winter climate could act in this way, but it was considered very desirable to develop a standard artificial method, in order to be sure of mycelial spread.

Artificial application in the field of approximately normal frost conditions would be very expensive. We therefore tried freezing parts of the leaves of the plants by using pieces of carbon dioxide snow. Damage was also obtained by bruising the plants. The same treatments were applied to plants grown in seed boxes under plastic, the leaves again being covered with ascospore infection spots. The results of these experiments in the field and in the seed boxes are presented in Table 1.

When rotting started the typical pattern of attack by clover rot was produced in all cases. The carbon dioxide snow treatment clearly paved the way for the transition from ascospore lesions to rotting, while this was not the case with bruising.

In other experiments plants of different varieties raised in seed boxes were frozen by placing them in a freezing chamber at -6 °C or -8 °C. This procedure

TABLE 1. Rotting of clover leaves infected with ascospores, following different methods of inflicting damage. Treatment in the field on 20 October. Assessment one month later. Treatment in seed boxes on 20 November. Assessment 14 days later.

Rotting van met ascosporen geïnfecteerde klaverbladeren na verschillende wijzen van beschadiging. Behandeling op het veld op 20 oktober. Beoordeling een maand later. Behandeling in zaaipannen op 20 november. Beoordeling 14 dagen later.

	In the field			In seed boxes	
	Total number of treated rows	Rotting by mycelium; number of rows		Number of plants	Number of spots with rotting by mycelium
Freezing with carbon dioxide snow / *Bevriezing met koolzuursneeuw*	12	12	100%	60	149
Bruising / *Kneuzen*	8	0	0%	60	3
Untreated / *Onbehandeld*	700	16	2%	60	1
	Totale aantal behandelde rijen	*Rotting door mycelium; aantal rijen*		*Aantal planten*	*Aantal plekken met myceliumrot*
	In het veld			*In zaaipannen*	

comes closer to natural conditions than either of the methods just mentioned. Inoculation with ascospores was carried out both before and after freezing of the plants. A considerable degree of rotting by *Sclerotinia* was observed about a fortnight after the freezing operation. The result of the attacks is evident from the percentages of surviving plants, which for the unfrozen and the frozen plants at –6 °C and –8 °C were on the average 72.9, 43.1 and 18.5% respectively. A similar experiment in another year had comparable results. A photograph of this trial is given in Fig. 2. In both trials there was also some rotting in non-frozen plants. This originated from portions of leaf blades so densely infected with ascospores that they turned completely brown. Apparently the leaves were so weakened at these places that rotting by spread of mycelium could develop easily. This result agrees with the experience of LOVELESS (1951) after heavy ascospore infection of bean leaves.

DISCUSSION

For answering the question: "Does rotting in the field mainly originate from ascospore lesions?" the following observations are important.

1. Grey rings surrounding the brown spots of ascospore infection quickly developed into rotting in moist conditions and could therefore be considered as a transitional stage between the ascospore lesions and spread of mycelium over the entire leaflet.
2. Commencement of rotting from the ascospore lesions was favoured by freezing the plants at –6 °C or –8 °C. These temperatures can be considered as quite normal in winter periods in the Netherlands.
3. Rotting often started in the joints of the trifoliate leaves. At such places rotting is more likely to have resulted from ascospore infection than from mycelial infection. The plants were already covered with spots arising from ascospore infection; as against this, mycelium, starting directly from sclerotia, would have had to be carried upwards from the soil by dust or insects.
4. In the neighbourhood of sclerotia placed in the field there was no more killing of the plants than at other parts. After melting of the snow cover the grey-white mycelium was seen on nearly all the plants. Ascospore lesions were widespread, but as compared to these sclerotia were rather sparsely distributed. Development of rotting directly from the mycelium of sclerotia seems in this case also most unlikely.

These four points taken together make it very probable that ascospore lesions are important in initiating the aggressive mycelial spread with rotting in the fields.

In this connection another question arose, viz. whether ascospore inoculation could be used in testing red clover plants for resistance to *Sclerotinia trifoliorum*. From our results it is evident that freezing at –6 °C or –8 °C gives a quick transition from the limited ascospore lesions to the desired spread of mycelium with rotting and it can therefore be used for breeding experiments. Rotting originating from ascospore infection can be induced without freezing, but for sufficient rotting in this case the infection with spores has to be so intensive that the method is impraticable.

The entirely different results obtained from freezing (carbon dioxide snow) and from bruising parts of plants occupied with ascospore lesions was most re-

markable. The biochemical products in leaves following freezing may be better suited to the development of the fungus than those resulting from bruising or an other possibility is that the resistance mechanism of the healthy cells surrounding the bruise may be in some way changed so that it no longer functions properly and the fungus can advance.

SAMENVATTING

Bruine puntjes van rode klaver, veroorzaakt door ascosporeninfectie van *Sclerotinia trifoliorum*, waren soms omgeven door een lichtgrijze ring. Uit deze ringen kon onder vochtige omstandigheden gemakkelijk klaverkanker worden verkregen.

Het begin van de rotting in het veld werd vaak waargenomen in het gewricht van de drie klaverblaadjes.

Na bevriezing van de planten bij –6 °C of –8 °C of behandeling van delen van planten met koolzuursneeuw ontstond er een massale ontwikkeling met rotting uit de lesies van ascosporen. Deze overgang trad niet op na verzwakking van de planten door kneuzen. Bij zeer zware infectie met ascosporen trad dikwijls direct rotting in de planten op.

De waargenomen verschijnselen maken het waarschijnlijk dat de klaverkanker in het veld hoofdzakelijk uitgaat van de ascospore-lesies.

REFERENCES

COLEMAN, L. C., – 1907. Über Sclerotinia trifoliorum Erikss., einen Erreger von Kleekrebs. Arb. K. biol. Anst. Land- u. Forstwirtsch. 5: 469–488.

DIJKSTRA, J., – 1964. Inoculation with ascospores of Sclerotinia trifoliorum for detection of clover rot resistant red clover. Euphytica 13: 314–329.

DILLON WESTON, W. A. R., – 1950. Clover sickness. Ann. appl. Biol. 37: 320–323.

LOVELESS, A. R., – 1951. Observations on the biology of clover rot. Ann. appl. Biol. 38: 642–664.

PURDY, L. H., – 1949. Studies in the genus Sclerotinia. Diss. Univ. California.

VALLEAU, W. D., E. N. FERGUS & L. HENSON, – 1933. Resistance of red clovers to Sclerotinia trifoliorum., Erikss. and infection studies. Bull. Ky agric. Exp. Stn 341: 115–131.

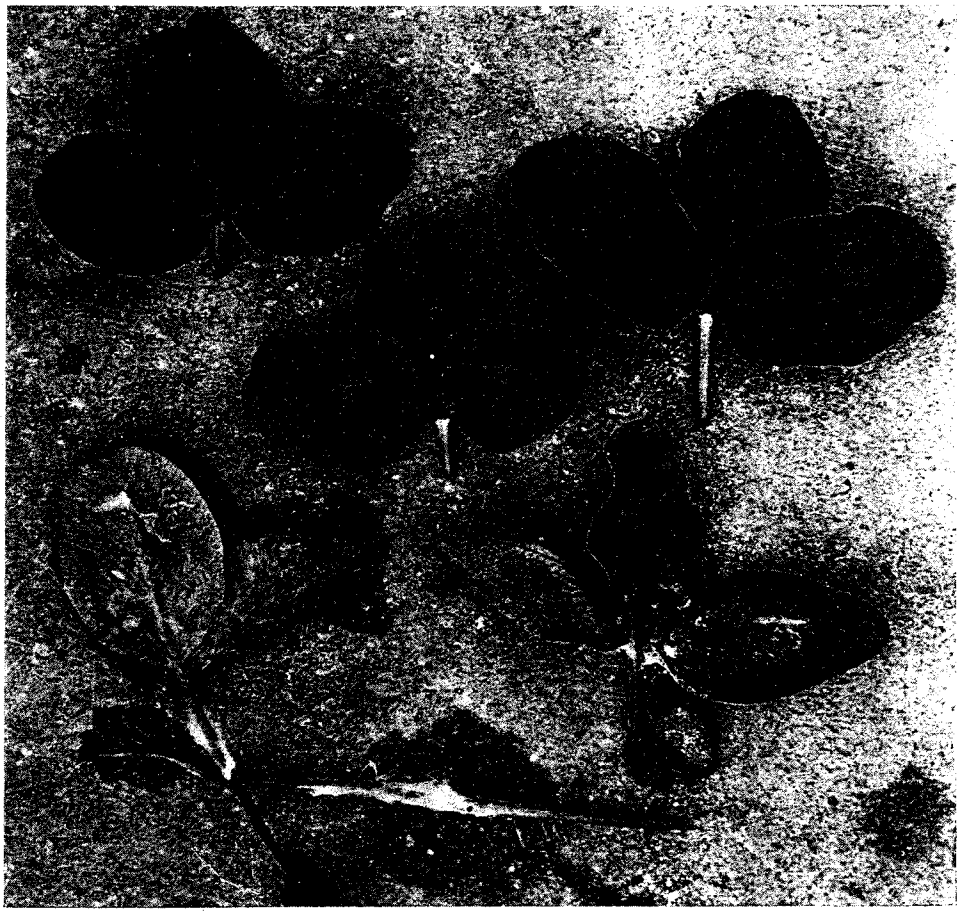

FIG. 1. Infection by ascospores. Dark spots on the leaves. On the lower part of the photo rotting with white mycelium, produced from ascospore infection.

Infectie door ascosporen. Donkere vlekjes op de bladeren. Op het onderste deel van de foto rotting met mycelium, ontstaan uit ascosporeninfectie.

From DIJKSTRA (1964)

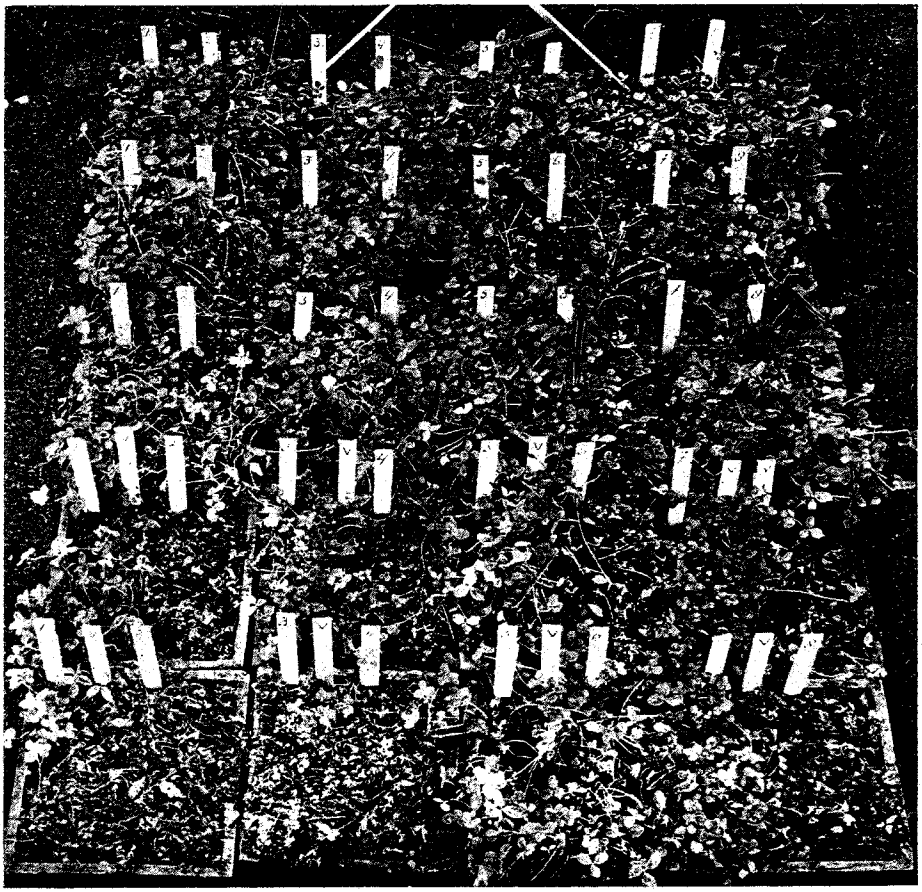

FIG. 2. Rotting caused by *Sclerotinia trifoliorum* after inoculation with ascospores. Upper three rows without freezing, little rot. Lower two rows with freezing at –6 °C, much rot. The boxes are arranged according to variety: 1. 'Early Øtofte Res'. III, 2. 'Italian red clover', 3. 'Medium late Øtofte Res'. III, 4. 'Red Meuse clover', 5. 'Roosendaal red clover', 6. 'Violetta', 7. 'U 036 (Ulva)', 8. 'Groningen red clover'. The photograph was taken about three weeks after having stopped the infection.

Rotting veroorzaakt door Sclerotinia trifoliorum *na inoculatie met ascosporen. Bovenste drie rijen zonder bevriezing, weinig rotting. Onderste twee rijen met bevriezing bij –6 °C, veel rotting. De pannen werden gerangschikt volgens de rassen 1–8.*

From DIJKSTRA (1964)