SHORT COMMUNICATIONS

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A new method for inoculation of lettuce with Bremia lactucae

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The cultivation of lettuce (*Lactuca sativa* var. *capitata* L.) has greatly expanded in The Netherlands during the last 10 years because of the development of varieties suitable for growing in greenhouses in autumn and winter and because of the increasing demand for lettuce the year round. However, downy mildew, caused by *Bremia lactucae Regel*, has also increased during this period and losses up to 40% may occur (Verhoeff, 1960). In 1965 two races of the pathogen were known (Rodenburg, 1966). Since then a third race has been found which calls for a continued breeding programme in lettuce (Tjallingii and Rodenburg, 1967).

Methods commonly used for inoculations with *B. lactucae* involve either immersion of the leaves of young plants into a suspension of conidia or a direct spraying of the conidial suspension (Verhoeff, 1960). In this way it usually takes more than 1 week for the disease symptoms to appear in lettuce, i.e. before sporulation starts in the inoculated plants. Under laboratory conditions it was often found difficult to obtain sufficient infection material during winter months to meet the demand of lettuce breeders. Herein a new method of inoculation is described, which minimizes the incubation period as well as ensures heavy infection by *B. lactucae*.

It had been noticed by the senior author on many occasions that sporulation appeared earlier in places where the leaf had been accidently damaged before the conidial suspension was sprayed. In experiments on this phenomenon, it was found that by cutting the tops off the leaves before spraying, sporulation could be markedly increased. It proved to be a further step forward not to spray whole plants or leaves, but discs of 20 mm diameter, punched out of healthy leaves (Yarwood, 1946). The discs were arranged in Petri-dishes on a layer of moist filter paper. By this method sooner and much more spores were produced than on cut leaves (Tjallingii and Rodenburg, 1967). By making small incisions along the edges of the discs the degree of sporulation could still be increased slightly. These experiences led the junior author to the idea that treating the lettuce leaves or the discs with carborundum powder, a method

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known from plant virology, would highly increase the number of minute wounds and therefore the degree of sporulation.

The experiments were carried out with lettuce variety 'Proeftuins Blackpool' and race NL 2 of *B. lactucae*. Before each inoculation, leaves or discs were abraded with carborundum powder with the aid of a dry camel hair brush. Various grades of carborundum powder were tested, viz. 220, 320, 400, 500, 600, 800, 1000 and 1200 mesh particle size. Subsequent to predisposing the leaves by carborundum, they were first rinsed with distilled water and then sprayed with a conidial suspension. The technique was applied on whole plants as well as on leaf discs. Potted plants were incubated in glass chambers lined with sufficient moist peat dust at its bottom and leaf discs in Petri-dishes as described above. After treatment, they were transferred to a greenhouse room with an average temperature of about 18 °C and a day length of about 16 h. All experiments were repeated three times. The results may be summarized as follows.

Whenever carborundum powder was used before inoculation, it was commonly observed that sporulation appeared within 4–5 days, which means a reduction in the incubation period of at least 2–3 days. Moreover, after 7–8 days the final density of *B. lactucae* conidiophores per unit of leaf surface was always much higher than on the objects without carborundum treatment (see Fig. 1).

Of the eight grades of carborundum powder tested, those in the range of 500–800 mesh favoured maximum spore production. With carborundum below 500 and above 800 mesh, sporulation was generally lower. This indicates that sizes of wounds made in the epidermal wall should be within certain limits, if the pathogen were to cause

Fig. 1. Sporulation of *B. lactucae* on lettuce leaf discs, 7 days after inoculation. CH: untreated checks, NCA: inoculated without carborundum treatment, CA: inoculated after carborundum treatment.

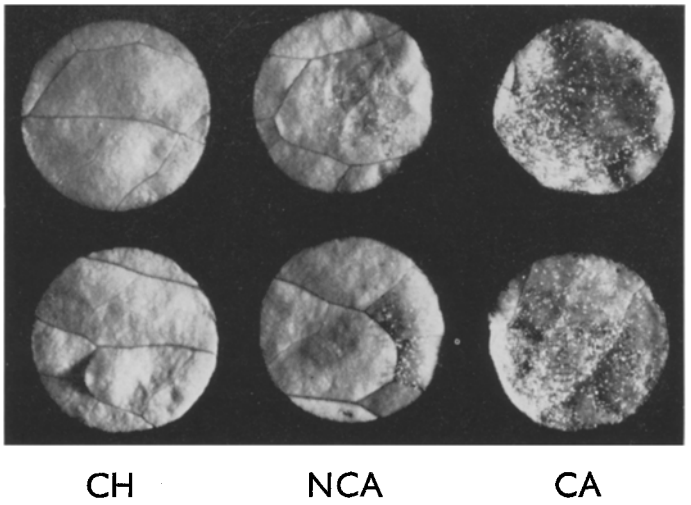

Fig. 1. Sporulatie van B. lactucae op bladschijfjes van sla, 7 dagen na inoculatie. CH: onbehandeld, NCA: met sporen bespoten, zonder carborundum-behandeling, CA: idem, maar van te voren behandeld met carborundumpoeder.

maximum attack and sporulation. In the case of carborundum treatment it is clear that the fungus gains entry through the epidermal walls and that stomatal penetration can only be of minor importance. In the untreated leaf, likewise, penetration through the epidermal walls has been reported to play the greater part by Powlesland (1954) and Verhoeff (1960), whereas Cohen (1952) found a certain percentage of stomatal penetration also and Schultz (1937) could only find stomatal infection.

The described method of inoculation seems to be rewarding because of its efficiency and quickness in producing heavy infections on lettuce with profuse sporulation of *B. lactucae*.

Samenvatting

Een nieuwe methode voor de inoculatie van sla met Bremia lactucae

Het inwrijven van slabladeren en -bladschijfjes met carborundumpoeder vóór de bespuiting met een sporensuspensie van *B. lactucae* heeft een opmerkelijk snellere infectie van de sla en een sterk vergrote sporulatie van de schimmel tot gevolg. De middelfijne poeders, graad 500–800 'mesh', werken effectiever dan de grovere en de fijnere sorteringen. Voor het uitvoeren van proeven met *B. lactucae* en voor het toetsen van slarassen en -lijnen bij de resistentieveredeling tegen valse meeldauw is dit een belangrijke verbetering.

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