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STUDIES ON *BOTRYTIS CINEREA* IN TOMATOES INFLUENCE OF METHODS OF DELEAFING ON THE OCCURRENCE OF STEM LESIONS[1]

Botrytis cinerea *in tomaten*

De invloed van het op verschillende manieren verwijderen van bladeren op het ontstaan van stengellesies

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Deleafing is a normal procedure in tomato crops and subsequent infection of the leaf scars by *Botrytis cinerea* frequently gives rise to stem lesions. Where deleafing is done by breaking off the petiole close to the stem, a cambium forms and the leaf scar is sealed after about seven days. Two to three days after deleafing, only a thin surface layer of dried cells is visible but at that time the leaf scar is unlikely to become infected by conidia of the fungus. When deleafing is done by cutting the petiole about 5 cm from the stem, abscission of the stumps occurs after about three weeks. When the petiole stump is inoculated with conidia of *B. cinerea*, abscission occurs after about eight days. In neither case is abscission complete. In old, but still green leaves, an abscission layer gradually develops at the petiole base, so deleafing by cutting the petioles of old leaves might be a promising method of avoiding stem infection by *B. cinerea*.

INTRODUCTION

Botrytis cinerea Pers. ex Fr. causes considerable losses in tomato crops by attacking stems, fruit stalks and fruits. Most stem lesions originate from infection of leaf scars. As deleafing is a normal procedure in tomato growing, the question arose as to whether an abscission layer is formed at the petiole base when deleafing is done by cutting the petiole at some distance from the stem, instead of by breaking, when the tissue ruptures at the junction with the stem.

The length of time for which the leaf scars remained open to infection by conidia of *B. cinerea*, after deleafing by breaking off the petiole close to the stem, was also of some interest.

MATERIALS AND METHODS

Young (approx. 35 cm stems) and old (approx. 100 cm stems) tomato plants of the variety 'Moneymaker', grown in pots, were used for the experiments.

Material for microscopical examination was collected and fixed one, two, three, five, seven and nine days after deleafing. The latter operation was carried out either by breaking off the petiole close to the stem or by cutting it about 3 or 5 cm from the stem. The fixative used was formal-acetic acid-alcohol (1 part 6% formaline to 1 part glacial acetic acid to 18 parts 70% ethanol by volume). Microtome sections 8 μ and 16 μ in thickness were stained with safranine and aniline blue or with Heidenhain's haematoxylin (JOHANSEN, 1940).

In a series of five experiments with 35 cm plants, leaf scars or petiole stumps were inoculated with dry conidia obtained from 12 to 16 days old cultures of

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B. cinerea growing on potato dextrose agar (Difco). Inoculated plants were kept under high humidity for 16 to 24 hours before putting them on the glasshouse bench. The number of stem lesions, and petiole stumps which had fallen before the fungus had entered the stem, was determined.

RESULTS

When deleafing was done by breaking off the petiole of the fifth leaf close to the stem of 35 cm plants, an open wound was made. Under glasshouse conditions late in summer, some dried cells were present in the pith parenchyma region after 24 hours. Two or three days after deleafing, a cambial layer of 3 to 5 cells was present in the pith and cortex parenchyma but there was no cambial activity near the epidermis and in the region of the vascular bundles. Seven days after deleafing there was a continuous cambial layer of 5 to 7 cells beneath the wound, this layer being thinner near the epidermis and in the region of the vascular bundles. Dried cells remained on the wound surface and these could be found easily even six weeks after deleafing.

When this treatment was applied to the fifth leaf of 100 cm plants, the same meristematic activity took place but more time was taken to seal off the wound completely. Three days after deleafing, hardly any meristematic activity could be found in these plants, and even after five days a cambial layer of 3 to 5 cells was present in the pith and cortex parenchyma only. After eight to nine days the wound was sealed. There was more cell wall debris on the leaf scars of these older stems.

In 100 cm plants, the beginning of an abscission layer was found at the base of the petioles of old but still green leaves (Fig. 1). When the leaf lamina had begun to turn yellow, cambial activity could be found in the pith and cortex parenchyma. When it was entirely yellow, the abscission layer was nearly complete, being well developed in the pith and cortex parenchyma but relatively thin near the epidermis; there was, however, no cambial activity in and immediately around the vascular tissues.

When deleafing was done by cutting the petioles of 35 cm plants about 3 cm from the stem, the stumps remained green for two or three weeks before turning yellow. By that time an abscission layer had started to develop at the base of the petiole, especially in the pith and cortex parenchyma. Where the petiole stump was entirely yellow, the abscission layer was ten to sixteen cells wide. Near the epidermis this layer was about six cells thick but there was hardly any meristematic activity in the region of the vascular bundles. The yellow petiole stumps fell at the slightest touch.

When petiole stumps of about 3 cm on 35 cm plants were inoculated with dry conidia of *B. cinerea* immediately after removing the leaves, abscission took place in a comparatively short time. The fungus started growing towards the stem but five to eight days after inoculation, depending upon the diameter of the petiole, a white ring appeared round the base of the stump and this was soon followed by abscission (Fig. 2). In some cases, however, the abscission layer proved to be incomplete as the stump remained attached to the stem; this led to invasion of the stem by the fungus. In sections, the abscission layer appeared to have developed in all tissues except in those in and immediately around the vascular bundles.

TABLE 1. Influence of the method of deleafing on the occurrence of stem lesions with 35 cm plants, after inoculating the fresh wounds with dry conidia of *B. cinerea*. The figures represent the percentages of stem lesions after 15 days, while those between brackets represent the percentages of petiole stumps fallen from the plant before the fungus had entered the stem.

Verband tussen de wijze van bladverwijderen en het optreden van de stengellesies na inoculatie van de verse wonden met conidiën van B. cinerea. *De cijfers geven de percentages stengellesies na 15 dagen weer, terwijl die tussen haakjes de percentages bladsteelresten weergeven die afgevallen zijn voordat de schimmel de stengel bereikte.*

Series	Method of deleafing			
	Petiole broken off close to the stem	Petiole cut leaving a stump		
		of 1 cm	of 3 cm	of 5 cm
1	90	100 (0)	10 (80)	0 (90)
2	70	60 (10)	0 (90)	0 (90)
3	80	85 (0)	15 (65)	5 (85)
4	75	85 (5)	10 (60)	0 (80)
5	90	95 (0)	15 (65)	0 (95)

On 35 cm plants, petiole stumps of various lengths and leaf scars, resulting from deleafing by breaking, were inoculated immediately after deleafing. After fifteen days, counts were made of the number of stem lesions and of petiole stumps which had fallen before the fungus had entered the stem. The results of five experiments, in each of which twenty plants per deleafing treatment were used, are given in Table 1. These show that only with longer petiole stumps hardly any stem lesions did develop.

When leaf scars of the fourth and fifth leaf of 35 cm plants were inoculated at one, two, three, four and five days after deleafing by breaking off the petiole close to the stem, stem lesions developed in the first group and also to a lesser extent in the second. Compared with inoculation immediately after deleafing, the average percentage of stem lesions which occurred following inoculation after one day was 75% and after two days 25%. All counts were made fifteen days after inoculation.

DISCUSSION

When young tomato plants were deleafed by breaking off the petiole close to the stem, only a small layer of dried cells could be found on the wound surface, up to two days after deleafing. This layer did not cover the xylem vessels, though as it was found that no infection took place when inoculation was delayed for two days after deleafing, it seems probable that this layer of dried cells prevents mycelial penetration of the wound surface. However, infection can also occur due to uptake of spore-containing droplets through the broken xylem vessels exposed in the scar, as was shown by WILSON (1963, 1966). But this type of infection was not observed in our experiments although the vessels remained open well beyond two days.

Deleafing by cutting the petiole at not too short a distance from the stem may prove of value. When not infected, the stump turns yellow and abscission takes place after about three weeks; when infected it falls within ten days. This period might be shortened if deleafing were restricted to old, but still green

leaves, where an abscission layer is already developing. In combination with a high level of nitrogen nutrition of the host, which is also unfavourable to the development of the fungus (VERHOEFF, 1965), this might be a promising line of investigation in the control of *B. cinerea* in tomatoes.

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SAMENVATTING

Het verwijderen van bladeren bij de teelt van tomaten is een normale cultuurhandeling. *Botrytis cinerea* kan evenwel juist via bladlittekens gemakkelijk de stengel aantasten. Normaliter geschiedt het verwijderen der bladeren door deze nabij de stengel af te breken. Er ontstaat dan een open wond, die pas na ongeveer zeven dagen geheel is afgesloten. Twee dagen na het op deze wijze verwijderen van bladeren is het gehele wondoppervlak, met uitzondering van de houtvaten, met een dun laagje ingedroogde cellen bedekt. Klaarblijkelijk is dit voldoende om infectie door *B. cinerea* tegen te gaan, want twee dagen na het verwijderen der bladen slagen kunstmatige infecties op de bladlittekens nauwelijks meer, althans bij planten met circa 35 cm lange stengels.

Worden de bladeren op ongeveer 3 cm van de stengel afgesneden en treedt geen aantasting van *B. cinerea* op in de bladsteelrest, dan kleurt de stomp geel en wordt na ongeveer drie weken afgestoten of hij droogt geheel in. Wordt de bladsteel wel aangetast, dan vindt afstoting betrekkelijk snel plaats. Na vijf tot acht dagen is een abscissielaag in alle weefsels aanwezig, behalve in en dicht om de vaatbundels. De afstoting is dan ook niet compleet, zodat bladsteelresten aan de stengel kunnen blijven hangen. De schimmel kan dan vanuit deze resten de stengel aantasten. Dit gevaar is veel minder groot als een grotere bladsteelstomp aan de stengel blijft (tabel 1).

Het verwijderen van bladeren door afsnijden van bladstelen zou met oudere, maar nog groene bladeren uitgevoerd kunnen worden, omdat in bladstelen van dergelijke bladeren reeds een begin van een abscissielaag aanwezig is. Is een oud blad geel gekleurd, dan is de abscissielaag vrijwel compleet; alleen in de vaatbundels zijn nauwelijks celdelingen opgetreden.

Gecombineerd met een hogere stikstofbemesting kan het verwijderen van oud blad, door snijden in plaats van door afbreken, een methode zijn om de ontwikkeling van *B. cinerea* in tomatestengels tegen te gaan.

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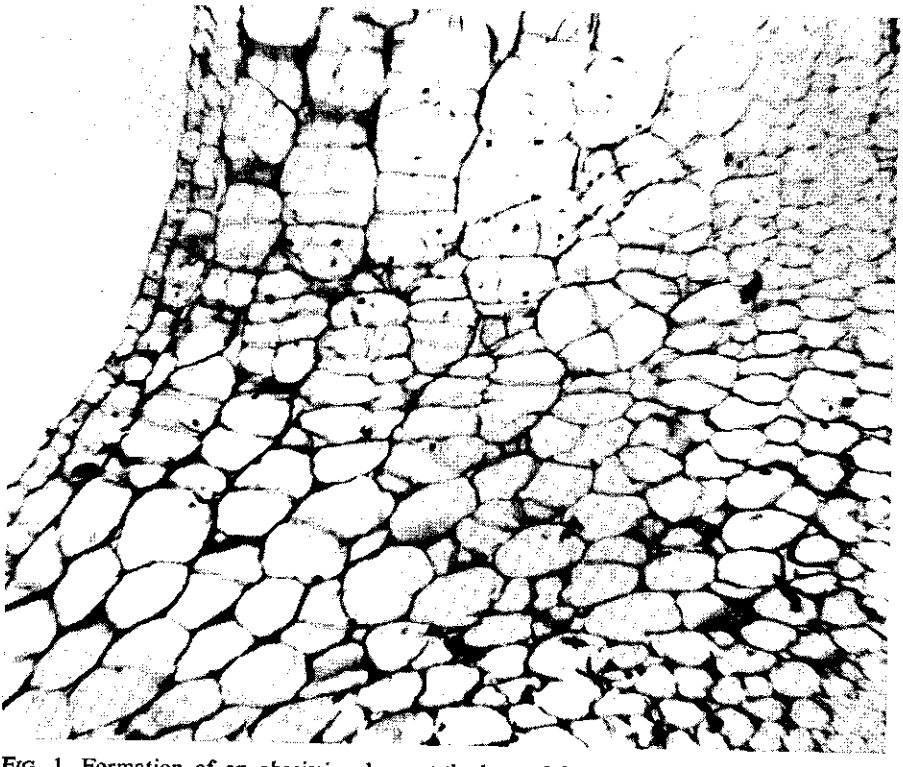

FIG. 1. Formation of an abscission layer at the base of the petiole of an old but still green leaf.

Ontwikkeling van een abscissielaag in de basis van een bladsteel van een oud, maar nog groen gekleurd blad.

FIG. 2. A petiole stump invaded by *B. cinerea*, nearly detached from the stem.

Een bijna van de stengel afgevallen bladsteelrest, aangetast door B. cinerea.