Neth. J. Pl. Path. 93 (1987) 135-146

Substances in dead plant tissue that stimulate infection of French bean leaves by Botrytis cinerea

J. VAN DEN HEUVEL

Willie Commelin Scholten Phytopathological Laboratory, Javalaan 20, 3742 CP Baarn, the Netherlands

Accepted 26 March 1987

Abstract

Spreading lesions were found in primary leaves of French bean after inoculation with pieces of dead bean tissue on which a droplet of a suspension of *Botrytis cinerea* conidia was placed, and after inoculation with conidia suspended in a diffusate from dead tissue. After fractionation of aqueous extracts from dead bean leaves, the infection-stimulating activity was confined to the fractions containing sugars and phosphate esters.

The infection-stimulating activity and the concentrations of carbohydrates and orthophosphate in extracts of liquid N_2-killed bean leaves varied slightly with the manner and degree of senescence. No consistent correlation, however, was observed between infection-stimulating activity and concentration of particular components. This was also true for extracts from other dead bean tissues. The stimulation of infection exhibited by extracts from dead flowers of different plant species, however, was closely correlated with the orthophosphate concentration.

Inocula containing only orthophosphate and sugars in the same concentrations as found in the extracts, did not stimulate the infection to the same extent as the extracts did. It is concluded that, in addition to simple carbohydrates and orthophosphate, one or more other compounds in the extracts are involved in the stimulation of infection.

Additional keywords: glucose, sugars, orthophosphate.

Introduction

Infection of undamaged plant tissue by *Botrytis cinerea* Pers.: Fries is stimulated by the presence of nutrients, derived either from the host or from an exogenous source, in the inoculum (Jarvis, 1977).

A wide range of nutrient substances can be leached from living plant tissue, or may diffuse into water droplets lying on such tissue, and accumulate with time. Although their concentrations may vary considerably depending on various factors, e.g. type, age and nutrient status of the tissue, and presence of epiphytic microorganisms, they often remain relatively low (Fokkema, 1981; Rossall and Mansfield, 1981; Morris and Rouse, 1985). In general, the concentrations of nutrients diffusing into inoculation droplets with *B. cinerea* conidia suspended in water are too low to stimulate the formation of spreading lesions (Blakeman, 1980). Therefore, exogenous nutrient substances, such as present in plant extracts, fruit juices, and pollen, are used frequently to

establish a succesful infection by *B. cinerea* (Watanabe et al., 1978; Verhoeff, 1980; Harper et al., 1981; Van den Heuvel, 1981; Kō et al., 1981; Macfoy and Smith, 1986).

Infection of living plant tissue by *B. cinerea* starting from adhering senescent or dead plant material is a well known phenomenon (e.g. Jarvis, 1977; Verhoeff, 1980; Bulit and Dubos, 1982; Humphreys-Jones, 1982). Although it is suggested that such material may serve as a nutritional base for the pathogen, enhancing its pathogenicity (Bulit and Dubos, 1982), experimental evidence for this hypothesis has not been presented so far.

The aim of the present study was to examine whether dead plant tissues contain infection-stimulating substances that are readily available for *B. cinerea* and, if so, to characterize some of the active compounds.

Materials and methods

Growth of bean plants and B. cinerea. French bean plants (*Phaseolus vulgaris* cv. Dubbele Witte zonder draad) and sporulating cultures of isolate BCl of *B. cinerea* were grown as described previously (Van den Heuvel and Waterreus, 1983). Primary leaves from 11-15-day-old plants were used for inoculation.

Preparation of extracts from dead plant tissue. Aqueous extracts were prepared from dead plant tissue that had senesced or died naturally or that had been killed by soaking in liquid N_2. In most experiments, healthy-looking primary leaves from 13-16-day-old French bean plants were used; leaf blades without the petiole were killed and extracted. Also, other parts of French bean plants were extracted: hypocotyls from 2-week-old plants; young (1-2-week-old) green trifoliate leaves; old (3-4-week-old) yellowing or shriveled trifoliate leaves, and flowers (petals plus stamens) that had dried on the plant.

Senescent, shriveling flowers from other plants species growing in the laboratory garden were also taken for extraction: petals plus stamens from *Berberis* spec., *Ranunculus repens*, *Viola tricolor maxima* and *Wisteria sinensis*; and petals only from *Cheiranthus cheiri*, *Lunaria annua*, *Pelargonium* × *hortorum* and *Rhododendron* spec. Pollen was collected from *Typha latifolia*.

All plant tissues were soaked in liquid N_2 for c. 3 minutes and then freeze-dried. The freeze-dried tissues except the pollen material were ground dry to fine fragments in an Ika Culatti micromill fitted with a 1-mm pore sieve. Unless stated otherwise, 0.5 g portions of this material were added to 15 ml distilled water and gently shaken at 20 °C for 15 min (the pollen material was shaken for 1 h). After shaking, the suspensions were filtered through cheesecloth. The residues were rinsed with 5 ml distilled water and squeezed, and the eluate was filtered and combined with the 15-ml filtrate. The extract was centrifuged (1500 *g*, 10 min). The pH of the supernatant was adjusted to pH 5.0 with 1 M HCl or KOH, which resulted in the appearance of a slight precipitation. This precipitate was centrifuged off (1500 *g*, 10 min) immediately, if the extract was to be used on the same day. Otherwise, the extract was frozen and kept at −20 °C until use and centrifuged after thawing. The clear supernatants were used for preparing inocula (to assess their infection-stimulating activity) and for determining concentrations of carbohydrates and orthophosphate.

Inoculations. Unless stated otherwise, the preparation of suspensions of conidia in solutions to be tested, the inoculation of detached leaves with 5-μl droplets of suspension (2×10^6 conidia ml^{-1}) and the conditions of incubation of inoculated leaves were as described earlier (Van den Heuvel, 1981). Five leaves were inoculated (10 droplets per leaf) per replicate treatment. After incubation for 3 to 4 days, the percentage of inoculation droplets that had given rise to a spreading lesion, was determined.

Assays. Concentrations of reducing sugars and total carbohydrates were determined with the methods of Nelson (1944), as modified by Somogyi (1952), and Hewitt (1958), respectively, with D-glucose as the standard. Concentrations of orthophosphate were determined according to the method of Golterman (1970, section 5.6.2, modification a), with KH_2PO_4 as the standard, except that the absorbance of reaction mixtures was measured at 690 mm.

Fractionation of extracts from dead bean leaves. For fractionation experiments extracts were used that had been prepared by shaking diminuted liquid N_2-killed and freeze-dried primary leaves of French bean at 20 °C for 3 h in distilled water (1 g per 15 ml).

Ten ml of such an extract was partitioned twice with 20 ml ethyl acetate. The combined ethyl acetate extracts were evaporated under reduced pressure to dryness, and the residue was dissolved in warm distilled water and tested for infection-stimulating activity.

Traces of ethyl acetate were removed from the water fraction and this fraction was further fractionated according to the method of Redgwell (1980). Ten ml of sample was loaded on to a 6×2.8 cm column of SP-Sephadex C-25 (Pharmacia) mounted in series above a similar column of QAE-Sephadex A-25 (Pharmacia), and then eluted with distilled water. Elution was continued until 200 ml had been collected from the second column to obtain the sugar fraction. The SP column was then eluted with 300 ml 0.2 M NH_4OH to yield the amino acid fraction. The QAE column was eluted with 300 ml 1 M formic acid to yield the organic acid fraction and thereafter with 150 ml of a pyridine/formate buffer to yield the fraction containing phosphate esters and other material. All fractions were evaporated to dryness under reduced pressure and the residues were dissolved in 10 ml distilled water. These fractions were used for assessing infection-stimulating activity and for determining concentrations of carbohydrates and orthophosphate.

Capillary gas chromatography. Extracts to be analyzed by capillary gas chromatography were freed from high molecular weight compounds (including oligo- and polysaccharides) by addition of 40 ml 100% ethanol to 10 ml extract, removal of the precipitate by centrifugation (1300 *g*, 10 min), evaporation of the supernatant to a small volume, addition of 25 ml 100% ethanol and removal of the precipitate by centrifugation (1300 *g*, 10 min). The supernatant was evaporated to dryness under a stream of N_2.

Sugars present in the extracts were silylated with N-(trimethylsilyl) imidazole (TSIM), following the procedure recommended by the manufacturer (Chrompack). One ml TSIM was added to each extract or to 4.5-7.5 mg of a reference sugar. D-xylose was used as an internal standard.

Separation and quantitation of the trimethylsilyl derivatives of the sugars were performed by means of capillary gas chromatography. The instrument was a Packard 430 fitted with a flame ionization detector and a 25 m × 0.25 mm WCOT fused silica capillary column coated with CP Sil 5 CB (Chrompack). The injection port temperature was 250 °C and the detector temperature was 280 °C. The column temperature was raised from 150 to 275 °C at 5 °C min^{-1} immediately after injection. N_2 was used as the carrier gas at 0.7 ml min^{-1}. Volumes of 0.25 μl were injected.

Sugars were analyzed qualitatively by comparison of retention times of compounds in the extract with those of authentic reference sugars. Quantitative determinations based on peak areas were done by the computing integrator of the gas chromatograph.

Results

Inoculations with conidia of B. cinerea on dead plant tissue. Flowers of French bean cv. Monarch which had dried and shriveled naturally were wetted with distilled water, soaked in a suspension of *B. cinerea* conidia (c. 10^4 ml^{-1}) in distilled water and placed on the adaxial side of detached fresh primary bean leaves. After incubation of the leaves for 1 day a water-soaked lesion was visible on the abaxial side of the leaves below each flower. All lesions started to spread rapidly within the next 1-2 days.

Other primary bean leaves were inoculated by (a) placing two 5-μl droplets of a suspension of *B. cinerea* conidia on the adaxial side of the leaves and a 9-mm disk from a herbarium-dried bean leaf on top of each droplet, or (b) placing two 5-μl droplets of distilled water on the leaves, a 9-mm disk from a dried bean leaf on top of each droplet, and a 5-μl droplet of a suspension of *B. cinerea* conidia on each disk. After 3 days of incubation spreading leasions had developed which continued to spread during the next 4 days.

Similar inoculations in which disks from bean leaves were used that had been killed with liquid N_2 and subsequently freeze-dried, also yielded spreading lesions.

Controls in which dead plant tissue without conidia was placed on leaves, or in which conidial suspensions in distilled water without dead plant tissue were inoculated, did never give rise to lesions.

Diffusates from leaves were prepared by incubating herbarium-dried bean leaves on a thin film of water (8 μl cm^{-2} leaf area) at room temperature for 3 to 5 hours. Droplets (5 μl) of the diffusates were placed on fresh bean leaves and mixed with 5-μl droplets of a conidial suspension in distilled water (4×10^6 conidia ml^{-1}). After incubation for 5 days spreading lesions developed from 19 of 20 inoculation droplets. This indicated that the factor(s) responsible for infection stimulation was (were) of an easily diffusable, hydrophilic nature.

Fractionation of extracts from dead bean leaves. Aqueous extracts prepared from diminuted liquid N_2-killed and freeze-dried bean leaves, and containing infection-stimulating activity, were fractionated in order to get more information on the identity of the infection-stimulating factor(s).

The ethyl acetate extract of an active aqueous extract did not show infection-stimulating activity (Table 1), while overnight dialysis of an active extract against running tap water resulted in an almost total loss of activity. This indicates that one or more hydrophilic, low molecular weight compounds are responsible for the infection

Table 1. Infection stimulation by and concentration of reducing sugars and orthophosphate in different fractions of an extract from diminuted liquid N_2-killed and freeze-dried primary leaves of French bean.

Fraction	Number of spreading lesions induced[1]	Reducing sugars (mg ml^{-1})	Orthophosphate (mM)
Whole extract	30	>1.2	18.1
Ethyl acetate extract	0	ND[2]	ND
Sugar fraction	29	>1.2	ND
Amino acid fraction	0	0.135	ND
Organic acid fraction	0	0.034	ND
Phosphate ester fraction	28	0.042	15.2

[1] Thirty inoculation sites per fraction.
[2] ND: not determined.

stimulation.

Further fractionation of the aqueous fraction left after partitioning with ethyl acetate yielded four fractions (Table 1). Of these, the sugar and phosphate ester fractions had the highest infection-stimulating activity, and retained most of the reducing sugar and orthophosphate, respectively, present in the unfractionated extract. The results suggested that sugars or orthophosphate or both were involved in the stimulation of infection.

Infection-stimulating activity of extracts from differently aged bean leaves. The infection stimulation exerted by extracts made from N_2-killed and freeze-dried primary leaves taken from 2-6-week-old bean plants and the concentrations of reducing and total sugars and orthophosphate in these extracts are presented in Fig. 1. Extracts from leaves of 2-week-old plants had always a slightly higher infection-stimulating activity and a distinctly higher concentration of sugars and orthophosphate than extracts from older leaves. The same was true for extracts from leaves (from 2-week-old plants) that had been killed immediately after detachment compared to extracts from similar leaves that had first been stored under dry (r.h. c. 70%; results not shown) or moist (r.h. 100%; Fig. 2) conditions at 20 °C for 2-7 days, except for the orthophosphate concentrations, which did not change much. No explanation is available for the relatively low infection-stimulating activity which was consistently observed in extracts from leaves that had been stored moist for 2 days. It was, however, associated with a fall in the orthophosphate concentration of these extracts.

Infection stimulation by extracts from different plant parts and plant species. Extracts prepared from different liquid N_2-killed and freeze-dried organs of French bean plants did not show much difference in infection-stimulating activity (Fig. 3); extracts from primary leaves displayed a slightly higher activity than those from other tissues. No clear correlation with concentrations of sugars and/or orthophosphate in the extracts was observed.

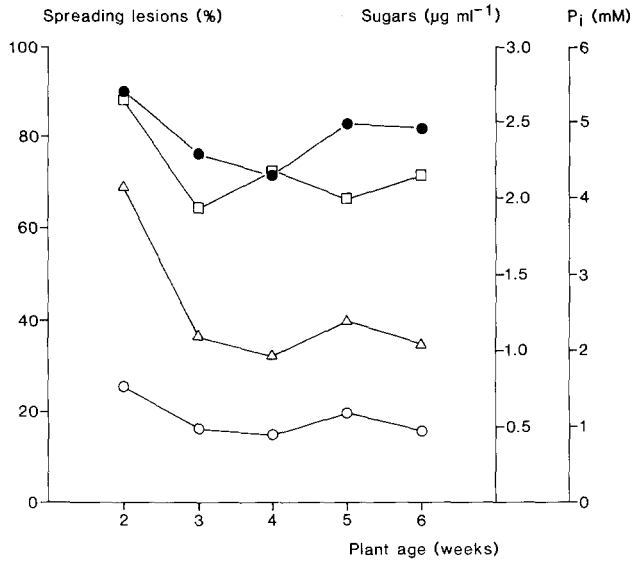

Fig. 1. Infection-stimulating activity (•) and concentrations of reducing (○) and total (□) sugars and of orthophosphate (△) in extracts from liquid N_2-killed primary leaves of French bean plants at different plant ages.

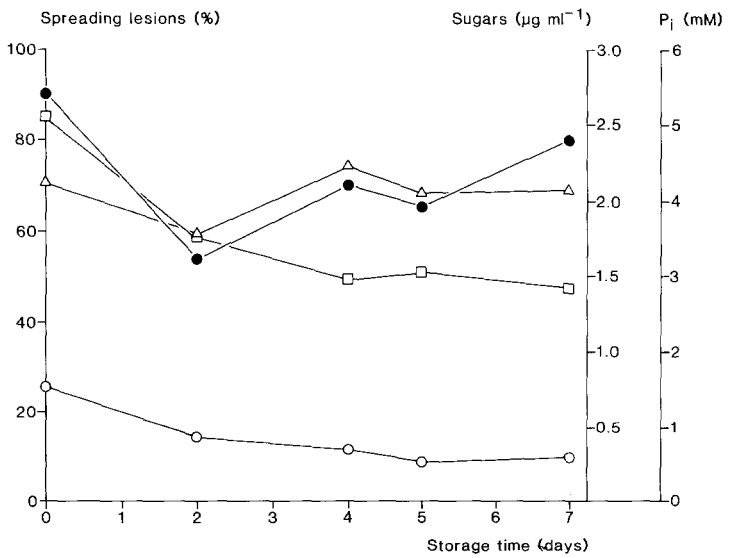

Fig. 2. Infection-stimulating activity (•) and concentrations of reducing (○) and total (□) sugars and of orthophosphate (△) in extracts from liquid N_2-killed primary leaves of 2-week-old French bean plants. The detached leaves had been stored moist (r.h. 100%) at 20 °C for 0-7 days.

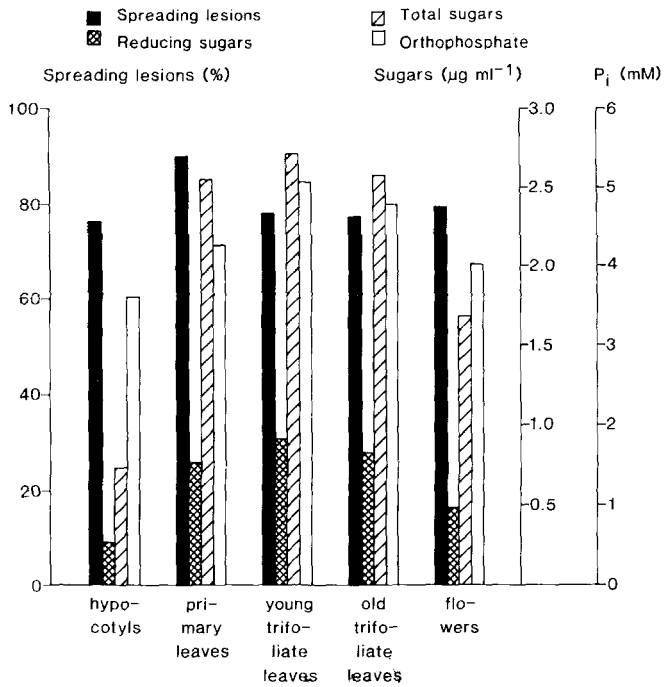

Fig. 3. Infection-stimulating activity and concentrations of reducing and total sugars and of orthophosphate in extracts from different liquid N_2-killed organs of French bean plants.

Clear differences were found between the infection-stimulating activity of extracts from flowers of nine different plant species (Fig. 4). Their infection-stimulating activity appeared to be closely correlated with the concentration of orthophosphate but not of sugars (Figs. 4 and 5).

Extracts from N_2-killed and freeze-dried pollen of *Typha latifolia* stimulated infection very well and contained a relatively high concentration of reducing and total sugars (Table 2).

Analysis of simple carbohydrates in an extract from dead primary bean leaves. A qualitative and quantitative analysis was made of the simple carbohydrates present in an extract from liquid N_2-killed and freeze-dried primary bean leaves. The principal mono- and disaccharides found were D-glucose, D-fructose, sucrose, inositol and an unknown sugar (possibly a disaccharide) (Table 3).

Assessment of infection stimulation by sugars present in extracts from dead bean leaves. Mixtures of the known sugars from Table 3 at 0.5, 1 and 2× the concentrations shown in the table were supplemented with KH_2PO_4 (6.7 or 67 mM), adjusted to pH 5.0 and tested for infection-stimulating activity. If supplemented with 67 mM KH_2PO_4 the mixtures had an infection-stimulating acticity of 66 to 78%, but if supplemented with 6.7 mM KH_2PO_4 infection was not stimulated.

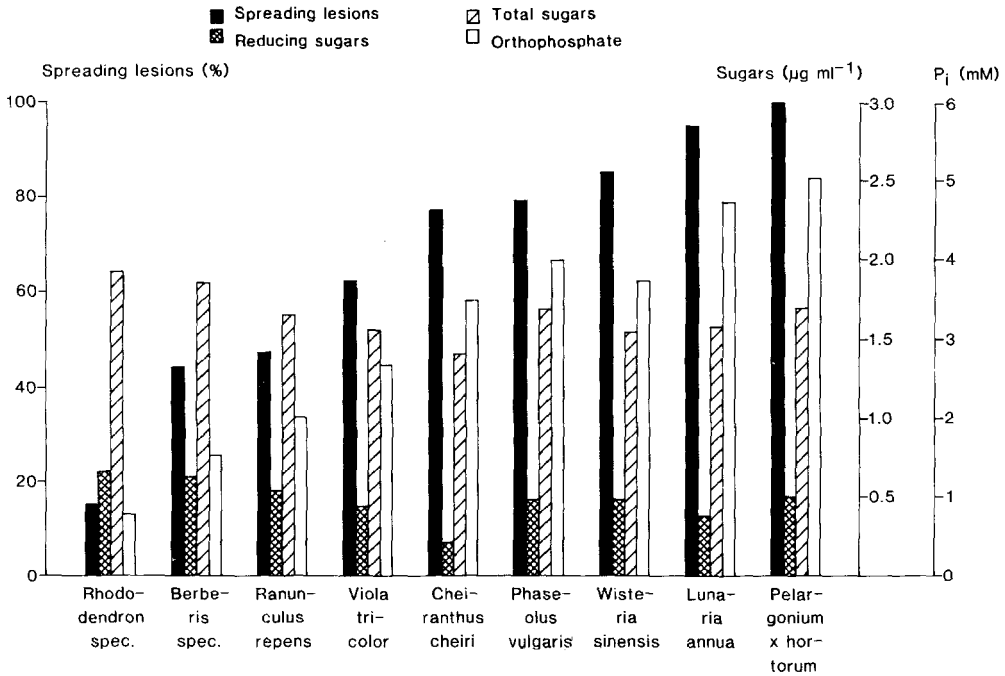

Fig. 4. Infection-stimulating activity and concentrations of reducing and total sugars and of orthophosphate in extracts from liquid N_2-killed flowers of nine different plant species.

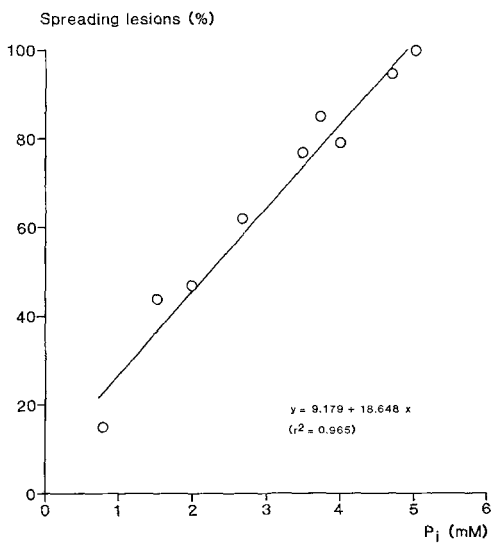

Fig. 5. Correlation between infection-stimulating activity and orthophosphate concentration in extracts from liquid N_2-killed flowers of nine different plant species.

Table 2. Infection stimulation by and concentration of reducing sugars and orthophosphate in extracts from liquid N_2-killed and freeze-dried pollen of *Typha latifolia*.

Spreading lesions (%)[1]	Reducing sugars (mg ml^{-1})	Total sugars (mg ml^{-1})	Orthophosphate (mM)
93[2]	1.92	6.32	2.54

[1] Based on 50 inoculation sites per experiment.
[2] Values are averages from two experiments.

Table 3. Simple carbohydrates detected by capillary gas-liquid chromatography in an extract from liquid N_2-killed and freeze-dried primary bean leaves.

Compound	Concentration (μg ml^{-1})	Compound	Concentration (μg ml^{-1})
D-arabinose	0[1]	L-rhamnose	0
D-ribose	0	Sucrose	66
D-xylose	0	D-galacturonic acid	0
D-fructose	560	myo-Inositol	153
D-galactose	18	Unknown sugar[2]	133
D-glucose	561		

[1] Values are based on D-xylose (internal standard) equivalents. 0: below detection limit.
[2] Other unidentified sugars were present in trace concentrations.

Discussion

Infection of primary French bean leaves by *B. cinerea* was stimulated by plant tissue that had died naturally as well as by tissue that had been killed intentionally. The same applies to diffusates or aqueous extracts prepared from dead plant tissues. Results of fractionation experiments suggested that hydrophilic, low molecular weight compounds, primarily simple carbohydrates and orthophosphate, were responsible for the stimulation of infection. The retention of orthophosphate in the phosphate ester fractions was, though not clearly indicated, inferred indirectly by Redwell (1980).

The combined results of the experiments with leaf extracts show relatively few differences in infection stimulation and reveal only a weak correlation between infection stimulation and concentration of (reducing or total) sugars (r^2 = 0.409 and 0.380, respectively) and no correlation at all between infection stimulation and concentration of orthophosphate (r^2 = 0.000). They do show, however, that the infection-stimulating ability of extracts prepared from liquid N_2-killed and freeze-dried fresh leaves is very similar to that of extracts prepared from leaves that had senesced or died naturally. Therefore, results obtained with the former extracts may be considered to be comparable to those obtained with the latter extracts. Also, extracts from different organs of French bean plants differed only slightly in their infection-stimulating activity and concentration of sugars or orthophosphate (Fig. 3).

The infection stimulation obtained with extracts from dead flowers of different plant species, however, was closely correlated ($r^2 = 0.965$) with the orthophosphate concentration in these extracts (Figs 4 and 5). Three of the four plant species whose flowers had the highest infection-stimulating activity, viz. *Pelargonium* × *hortorum*, *Lunaria annua* and *Phaseolus vulgaris*, are frequently observed, in nature, to have leaves and other plant parts infected by *B. cinerea* via dead flowers.

Earlier experiments, in which the effect of combinations of orthophosphate and glucose on infection of primary French bean leaves was assessed, revealed that relatively high orthophosphate concentrations (> 20 mM) were needed to give a good stimulation of infection, whereas the concentration of glucose seemed less important (Van den Heuvel, 1981). The highest orthophosphate concentrations in the extracts from dead plant tissue were almost always below 10 mM. This indicates that the orthophosphate concentrations found in these extracts, together with the available sugars, are unable to cause the observed successful infection stimulations. Apparently, in addition to orthophosphate and sugars, one or more other compounds in the extracts are involved in the infection stimulation.

Although certain phosphate esters, viz. purine-related compounds and D-glucose 6-phosphate, have been shown to stimulate infection (Kō et al., 1981; Van den Heuvel, unpublished results), they are unlikely candidates, for the following reasons: (a) total phosphate concentrations in the extracts were, in general, only slightly higher than those of orthophosphate; (b) purine-related compounds such as ATP, although stimulating at the 1 mM level, are probably not readily extractable under the conditions employed (e.g. Thore, 1979); (c) D-glucose 6-phosphate was active only at concentrations above 20 mM, and (d) Redgwell (1980) reported that his phosphate ester fractions contained several other substances, such as phenolics and strong anions.

Infection of broad bean leaves by *B. cinerea* was stimulated by orthophosphate, ammonium ions and glutamine, in particular in combination with glucose, fructose, raffinose, maltose and sucrose (Harper et al., 1981; Rossall and Mansfield, 1981) and by the iron-chelating agents ethylenediaminetetraacetic acid (EDTA) and 2,3-dihydroxybenzoic acid (Brown and Swinburne, 1982). Further research on the identity of infection-stimulating compounds in extracts from dead plant tissue should take into consideration the possible involvement of the compounds mentioned above.

Acknowledgements

The author is indebted to Ms M.A. van der Harst, Ms M. Torenbeek, J.A.M. Schram, W. de Jong and Ms J.C. Voorn who participated in some of the experiments.

Samenvatting

Stoffen in dood planteweefsel die de infectie van bonebladeren door Botrytis cinerea stimuleren

In primaire bonebladeren onstonden zich uitbreidende lesies na inoculatie met stukjes dood boneweefsel waarop een druppeltje van een suspensie van conidiën van *Botrytis cinerea* was aangebracht, maar ook na inoculatie met condiën in een diffusaat van dood weefsel. Na fractionering van waterige extracten van dode bonebladeren bleek

de infectiestimulerende activiteit gebonden te zijn aan de suiker- en fosfaatesterfracties.

De infectiestimulerende activiteit en de concentraties van koolhydraten en orthofosfaat in extracten van met vloeibare N_2 gedode bonebladeren varieerden enigszins met wijze en mate van veroudering. Er werd echter geen vaste correlatie waargenomen tussen infectiestimulerende activiteit en concentratie van bepaalde stoffen. Dit gold ook voor extracten van andere dode boneweefsels. De infectiestimulatie teweeggebracht door extracten van dode bloemen van verschillende plantesoorten was evenwel nauw gecorreleerd met de orthofosfaatconcentratie.

Inoculatie met sporensuspensies waaraan orthofosfaat en suikers waren toegevoegd tot de concentraties gelijk waren aan die in de extracten, gaf veel minder infectie dan die met sporen gesuspendeerd in de extracten zelf. Daarom wordt geconcludeerd dat naast eenvoudige koolhydraten en orthofosfaat een of meer andere stoffen in de extracten betrokken zijn bij de stimulatie van de infectie.

References

Blakeman, J.P., 1980. Behaviour of conidia on aerial plant surfaces. In: Coley-Smith, J.R., Verhoeff, K. & Jarvis, W.R. (Eds), The biology of *Botrytis cinerea*. Academic Press, London, New York, Toronto, Sydney, San Francisco, p. 115-151.

Brown, A.E. & Swinburne, T.R., 1982. Iron-chelating agents and lesion development by *Botrytis cinerea* on leaves of *Vicia faba*. Physiological Plant Pathology 21: 13-21.

Bulit, J. & Dubos, B., 1982. Epidémiologie de la pourriture grise. EPPO Bulletin 12 (2): 37-48.

Fokkema, N.J., 1981. Fungal leaf saprophytes, beneficial or detrimental? In: Blakeman, J.P. (Ed.), Microbial ecology of the phylloplane. Academic Press, London, New York, Toronto, Sydney, San Francisco, p. 433-454.

Golterman, H.L. (Ed.), 1970. Methods for chemical analysis of fresh waters. IBP Handbook No. 8. Blackwell Scientific Publ., Oxford, Edinburgh.

Harper, A.M., Strange, R.N. & Langcake, P., 1981. Characterization of the nutrients required by *Botrytis cinerea* to infect broad leaves. Physiological Plant Pathology 19: 153-167.

Heuvel, J. van den, 1981. Effect of inoculum composition on infection of French bean leaves by conidia of *Botrytis cinerea*. Netherlands Journal of Plant Pathology 87: 55-64.

Heuvel, J. van den & Waterreus, L.P., 1983. Conidial concentration as an important factor determining the type of prepenetration structures formed by *Botrytis cinerea* on leaves of French bean (*Phaseolus vulgaris*). Plant Pathology 32: 263-272.

Hewitt, B.R., 1958. Spectrophotometric determination of total carbohydrate. Nature (London) 182: 246-247.

Humphreys-Jones, D.R., 1982. Leaf blotch of potato caused by *Botrytis cinerea*. Plant Pathology 31: 273-275.

Jarvis, W.R., 1977. *Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. Research Branch Canada Department of Agriculture, Monograph No. 15.

Kō, K., Akutsu, K., Kobayashi, Y., Om, Y., Watanabe, T. & Misato, T., 1981. Stimulative effects of purines and their relative compounds on lesion formation of gray mold on cucumber plant. Annals of the Phytopathological Society of Japan 47: 228-233.

Macfoy, C.A. & Smith, I.M., 1986. Interrelationship between nutrients, pathogenicity and phytoalexin metabolism of *Botrytis cinerea* on clover leaves. Journal of Phytopathology 116: 193-200.

Morris, C.E. & Rouse, D.I., 1985. Role of nutrients in regulating epiphytic bacterial populations. In: Windels, C.E. & Lindow, S.E. (Eds), Biological control on the phylloplane. The American Phytopathological Society, St. Paul, p. 63-82.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry 153: 375-380.
Redgwell, R.J., 1980. Fractionation of plant extracts using ion-exchange Sephadex. Analytical Biochemistry 107: 44-50.
Rossall, S. & Mansfield, J.W., 1981. Nutrients and lesion formation by *Botrytis cinerea* on leaves of *Vicia faba*. Transactions of the British Mycological Society 76: 172-175.
Somogyi, M., 1952. Notes on sugar determination. Journal of Biological Chemistry 195: 19-23.
Thore, A., 1979. Bioluminescence assay: extraction of ATP from biological specimens. LKB-Wallac Note, 6 pp.
Verhoeff, K., 1980. The infection process and host-pathogen interactions. In: Coley-Smith, J.R., Verhoeff, K. & Jarvis, W.R. (Eds), The biology of *Botrytis*. Academic Press, London, New York, Toronto, Sydney, San Francisco, p. 153-180.
Watanabe, T., Adachi, Y., Matsuzawa, Y., Kō, K. & Misato, T., 1978. Stimulative effects of some fruit-saps on development of gray mold disease on cucumber plant. Annals of the Phytopathological Society of Japan 44: 519-522.