Neth. J. Plant Path. 70: 37-52

THE INTERACTION OF SOME PHYTOPATHOGENIC FUNGI WITH PLANT TISSUE[1]

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The phytoalexin test of MÜLLER was applied to various host/parasite combinations. The highest production was obtained when spores of *Sclerotinia fructicola* were on slices of potato tuber, and spores of *Colletotrichum lindemuthianum* in contact with bean pod tissue. During the period of incubation of the fungus on the host plant tissue substances were formed or excreted by the plant tissue. Several amino acids and carbohydrates (saccharose, glucose and fructose) could be demonstrated by paper chromatography. Most of these substances were found at much higher concentrations in the controls (i.e. drops of double distilled water incubated upon host tissue), than in the supernatant of the spore suspension after the incubation period. Drops of spore suspension and drops of double distilled water on glass cavity slides never contained these substances, or they were in such low concentrations as to be undetectable by paper chromatography. Inhibition of spore germination by a phenolic compound was not confirmed in the used test objects and host/parasite combinations.

INTRODUCTION

The production of inhibiting substances induced in a resistant host plant by a pathogen is one aspect of the defense mechanism of the plant (reviews by GÄUMANN, 1951; ALLEN, 1959; MÜLLER, 1959). BERNARD (1909, 1911), NOBÉCOURT (1925) and CHESTER (1933) have emphasized the dynamic character of this process, without establishing proof of it. MÜLLER, who did much work on resistance of potatoes to late blight caused by *Phytophthora infestans*, stressed this point when he became interested in the physiological basis of the differences in response between resistant and susceptible hosts. The course of reaction differs particularly in speed of response (MÜLLER *et al.*, 1939; MÜLLER & BEHR, 1949; MÜLLER, 1953).

In resistant plants a rapid response occurs and the hypersensitive reactions in the host cells take place before the fungus has a chance to become established; the slower response of susceptible plants allows time for growth and reproduction (MÜLLER & BÖRGER, 1940). From observations on the course of cytological changes (MEYER, 1940) and experimental results (MÜLLER & BÖRGER, 1940), MÜLLER postulated the formation of fungitoxic substances, which he called "phytoalexins".

Later MÜLLER (1956) transferred drops of spore suspension of *Phytophthora infestans* and *Sclerotinia fructicola* on to the aseptically exposed sterile inner epidermis of young bean pods and tested these drops after various times of incubation for antibiotic activities. These experiments showed that drops incubated in contact with bean and fungus gave rise to antibiotic substances which were lacking in drops without fungus spores, or were not present at concentrations inhibitory to the fungi tested.

The possibility that the active substances came from the spores was not

[1] Accepted for publication 13 January, 1964.

systematically excluded by MÜLLER, as it could have been by incubating another series of drops with spores on an inert substratum. However, he found that apricot leaves, a susceptible host, did not produce phytoalexin after inoculation with *Sclerotinia*. Therefore, it appeared to him unnecessary to test this possibility (personal communication). Australian co-workers of MÜLLER (CRUICKSHANK & PERRIN, 1960, 1961; PERRIN & BOTTOMLEY, 1962) were successful in identifying one of the phytoalexins which he had previously demonstrated. They found that the phytoalexin, obtained from pea pod which had been inoculated with *Sclerotinia*, is a chromano-coumarane.

Experiments reported here extend the phytoalexin concept also to other host parasite combinations than those reported by MÜLLER and colleagues. Moreover, efforts were made to determine what substances are formed in the diffusates during contact of parasite with the plant tissue.

MATERIALS AND METHOD

The fungi under investigation were *Sclerotinia fructicola* (Wint.) Rehm, strain 'Rader' (syn. *Monilinia fructicola* (Wint.) Honey) and *Colletotrichum lindemuthianum* (Sacc. et Magn.) Bri. et Cav. The former was received from the Central Bureau of Fungi Cultures (C.B.S.) at Baarn and the latter from the Institute of Phytopathological Research (I.P.O.) at Wageningen.

C. lindemuthianum was cultivated during the experiments on a synthetic medium, composed as follows: 2.8 g glucose, 1.23 g $MgSO_4$. $7H_2O$, 2.72 g KH_2PO_4, 2.0 g neopepton, 20 g agar and 2000 ml distilled water. *S. fructicola* was cultivated on cherry agar. The plant tissues used were leaves and pods of *Phaseolus vulgaris* 'Vroege Wagenaar'. This variety of *P. vulgaris* is moderately susceptible to *C. lindemuthianum*. Inoculation experiments with *Colletotrichum* used here, only produced necrotic spots on the leaves and spots with acervuli on the bean pods. Leaves and pods were inoculated in petri dishes lined with wet filterpaper.

Pods and leaves were always used as fresh material directly from the plant. The bean pods were disinfected by 70% ethanol, 7 minutes followed by washing in running tapwater for half an hour. The method used here was described by MÜLLER (1956) for studying diffusable substances, under aseptic conditions, without interference from wound substances. The bean leaves were washed with running tapwater for two hours, dried between sterile filterpaper and brought in sterile petri dishes lined with wet filterpaper. Eight or ten drops of spore suspension or double distilled water were equally divided over the whole leaf surface.

Tubers of *Solanum tuberosum* 'Furore' were also used. A stock of potatoes were stored at 5° C. After cleaning the tubers with a brush and soap, the potatoes were disinfected for ten minutes with 4% formol. The tubers were then cut under aseptic conditions in slices; these were laid in sterile petri dishes lined on the inner side with wet filterpaper. After incubation for 18 hours at 23° C drops of spore suspension were placed aseptically at three or four different places of one slice.

The possibility that the active substances came either from the spores or from the plant tissue alone was tested by incubating three other series of drops: one series with spores on sterile glass cavity slides and two series without spores,

one on plant tissue and the other on sterile glass cavity slides. They all were placed in petri dishes lined with wet filterpaper. After the incubation period at 23° C the drops were collected and the spores were centrifuged out. The supernatant was then tested for the appearance of substances affecting germination or germ tube growth of test spores. This was also done in petri dishes lined with wet filterpaper on glass cavity slides. The germination and the length of the germ tubes were counted and measured after an incubation period of 24 hours at 23° C.

Paper chromatography was used to determine substances formed on plant/fungus contact. The drops of spore suspension and double distilled water were gathered from the bean pods after 24 hours, from the bean leaves and the potato slices after 48 hours; 0.5 ml of the liquid from bean pod and potato slices was adequate for one chromatogram. Compounds in the liquid collected from bean leaves were undetected in 0.5 ml; therefore 6 ml was used for each chromatogram.

After centrifuging the supernatant was dried in a vacuum container and all were redissolved in equal amounts of water. One hundred µl of the redissolved substances were brought on Whatman no. 1 paper. Solvents were butanol: acetic acid: water (4:1:5) and water saturated phenol for amino acids, amides and carbohydrates; CO_2 in water (pH = 4.8), 2% acetic acid, and chloroform: acetic acid: water (2:1:1) for phenolic compounds.

The detecting reagents used were: benzidine-trichloro acetic acid for carbohydrates; ninhydrin for amino acids and amides; Folin Denis plus NH_3 vapour, or $FeCl_3$ as indicators for phenolic compounds. These reagents are described by LINSKENS (1959), except Folin Denis. This reagens was composed according to HAIS & MACEK (1958); it is a solution of Na_2WO_4, phosphor molybdenic acid and phosphoric acid (H_3PO_4). Folin Denis is, according to PROCHÁZKA (1958), selective for phenolic compounds and gives in a NH_3 vapour blue spots with phenolic compounds.

INHIBITION OF SPORE GERMINATION

The interaction of the fungi with plant tissue was proved by determining the spore germination in the supernatants of drops, in which spores were in contact with plant tissue.

Sclerotinia fructicola

S. fructicola – *pods of* Phaseolus vulgaris

The experiments were running between 8.4.1960 and 18.1.1962 (see Fig. 1). In two experiments there is a convincing difference between the germination of spores in drops of spore suspensions collected from bean pods and in drops of double distilled water gathered from bean pods after an incubation of 24 hours.

In six experiments there was only a retardation in the germination of the spores and the development of the germ tubes of *S. fructicola*. In the experiments II and V of 21.4.1960 and 20.9.1961 (not inserted in Fig. 1) there was no difference at all between the supernatants of the drops of spore suspension and of double distilled water, which were in contact with bean pod tissue. The spore germination was nearly 100%.

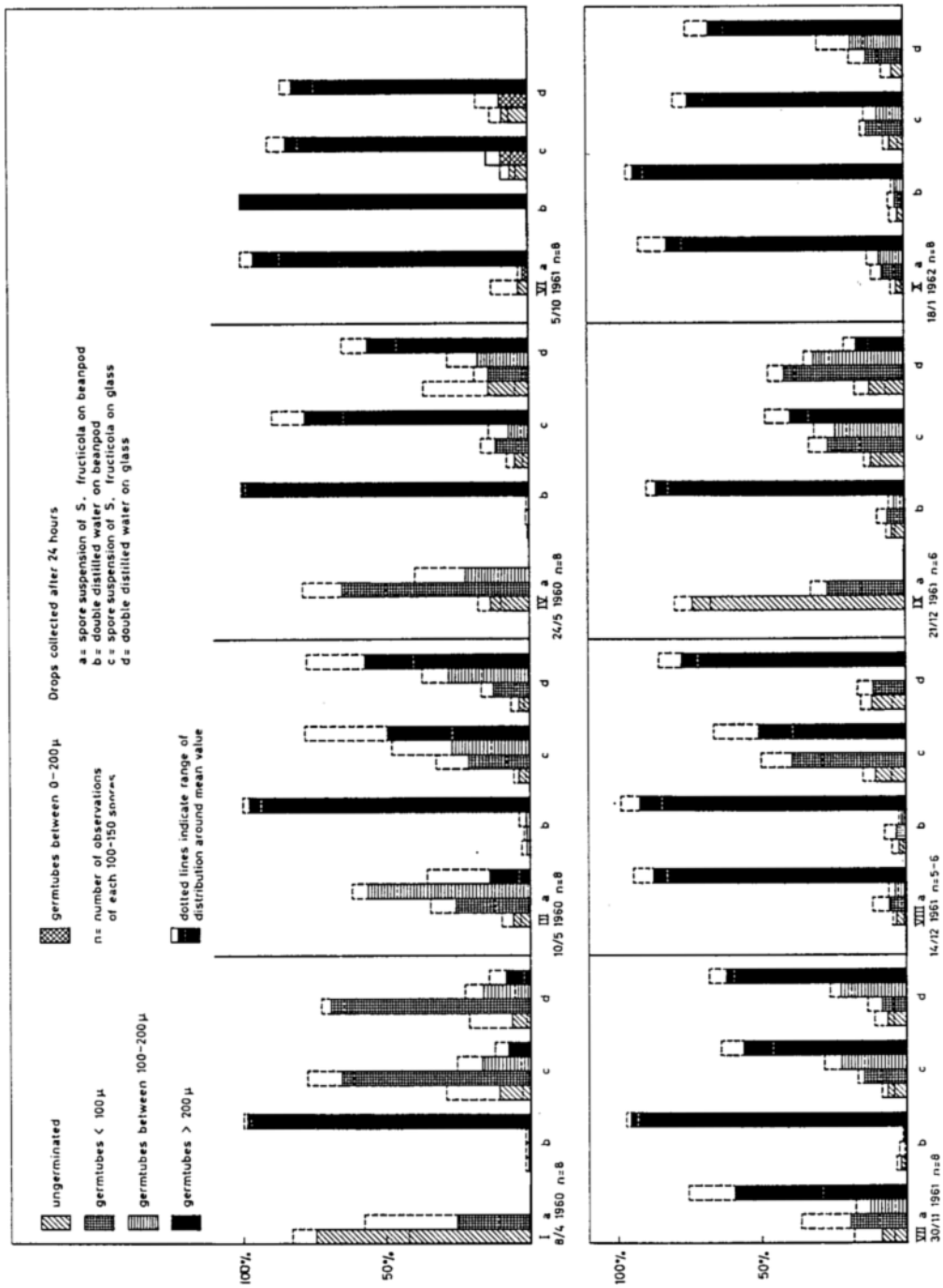

FIG. 1. Inhibition of spore germination and growth of germ tubes of *Sclerotinia fructicola* in diffusates collected from bean pods.

In general one can say that diffusates in which spores of *S. fructicola* had grown, give a retarded germination compared with the germination of test spores in drops of double distilled water or in drops of spore suspension of *S. fructicola* collected from glass after an incubation period of 24 hours, whereas drops of double distilled water collected from bean pods promote the spore germination of *S. fructicola*. It is evident there is an interaction between *S. fructicola* and bean tissue, but this interaction is not constant.

S. fructicola – *slices of tuber of* Solanum tuberosum

The spores were in contact with the potato tissue for 24, 48 and 72 hours (Fig. 2). By this contact a spore germination inhibiting principle is formed or excreted by the potato tissue. The percentage of the spore germination has its lowest point in drops in which the spores were in contact with potato tissue for 48 hours. By repeating this experiment we got the same results.

There was no promoting effect of the spore germination in the drops of double distilled water collected from the potato tissue as we found in the experiments with the bean pods (Figs. 1 and 3). It is possible that there is no excretion at all of substances by the potato tissue into the drops of double distilled water. Also, these experiments do not exclude the possibility that there is a compound under the excreted substances with an inhibiting effect on germination of the spores.

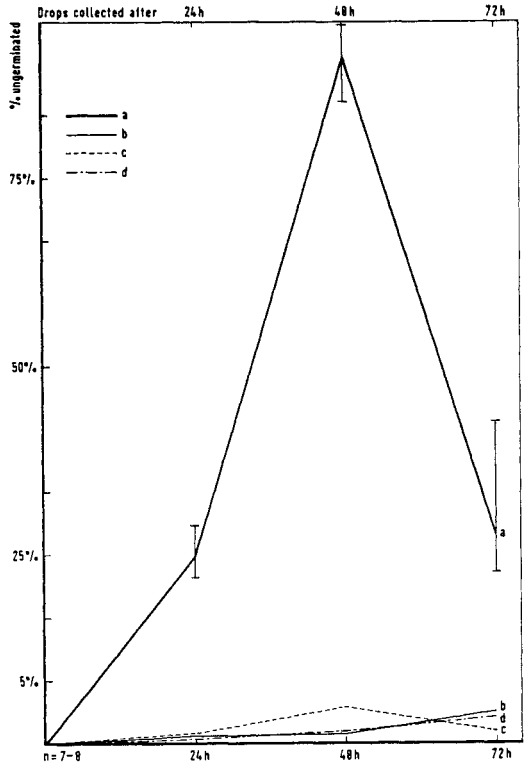

FIG. 2.
Inhibition of spore germination of *Sclerotinia fructicola* in diffusates collected from potato tuber tissue after different incubation periods.
a: spore suspension and b: double distilled water in contact with potato tissue, c: spore suspension and d: double distilled water on glass cavity slides.

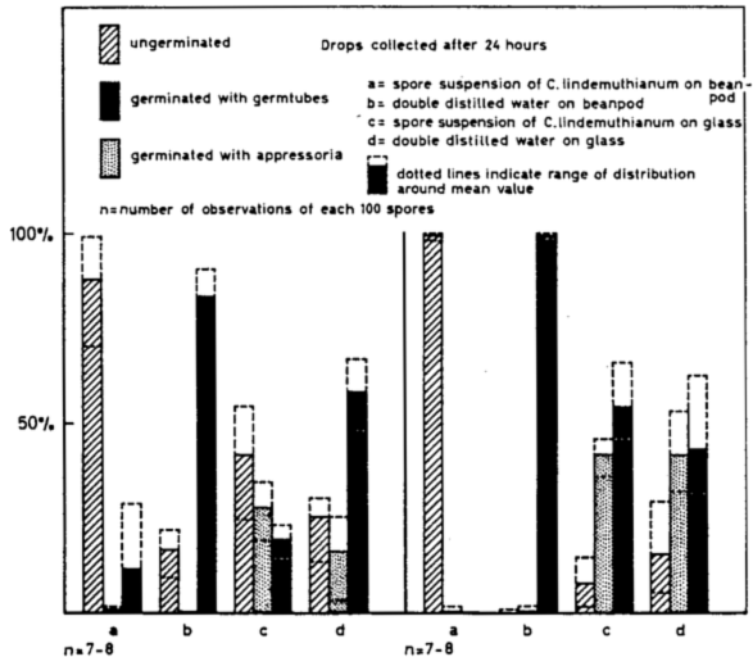

FIG. 3. Inhibition of spore germination and growth of appressoria and germ tubes of *Colletotrichum lindemuthianum* in diffusates collected from bean pods.

Colletotrichum lindemuthianum

C. lindemuthianum – *pods of* Phaseolus vulgaris

The experiments were carried out on 23.11.1961 and 27.11.1961. In replicate experiments a strong inhibition of spore germination in the diffusates of the spore suspensions was observed (Fig. 3). The drops of double distilled water incubated 24 hours on bean pods had a stimulating effect on spore germination of *C. lindemuthianum*. The pH of both diffusates was 5.4.

C. lindemuthianum – *leaves of* Phaseolus vulgaris[2]

Fig. 4 summarizes the results of six experiments in which drops of spore suspension and drops of double distilled water were placed either on bean or on glass slides. The drops were collected after an incubation of 48 hours and tested for substances affecting germination, appressoria formation, or germ tube growth of test spores. Each figure is a mean of ten observations; each observation is based on one hundred spores. The deviation indicated by dotted lines is the mean error in per cent of the ten observations. In experiments I and IV a significant difference is observed between germination of spores in spore suspensions collected from bean leaves and in double distilled water collected from bean leaves and also in the drops gathered from glass.

In experiments II and III we see a retardation in the germination of the test

[2] These experiments were carried out by Drs. RIETY VAN OORSCHOT as a part of her practical work on this laboratory.

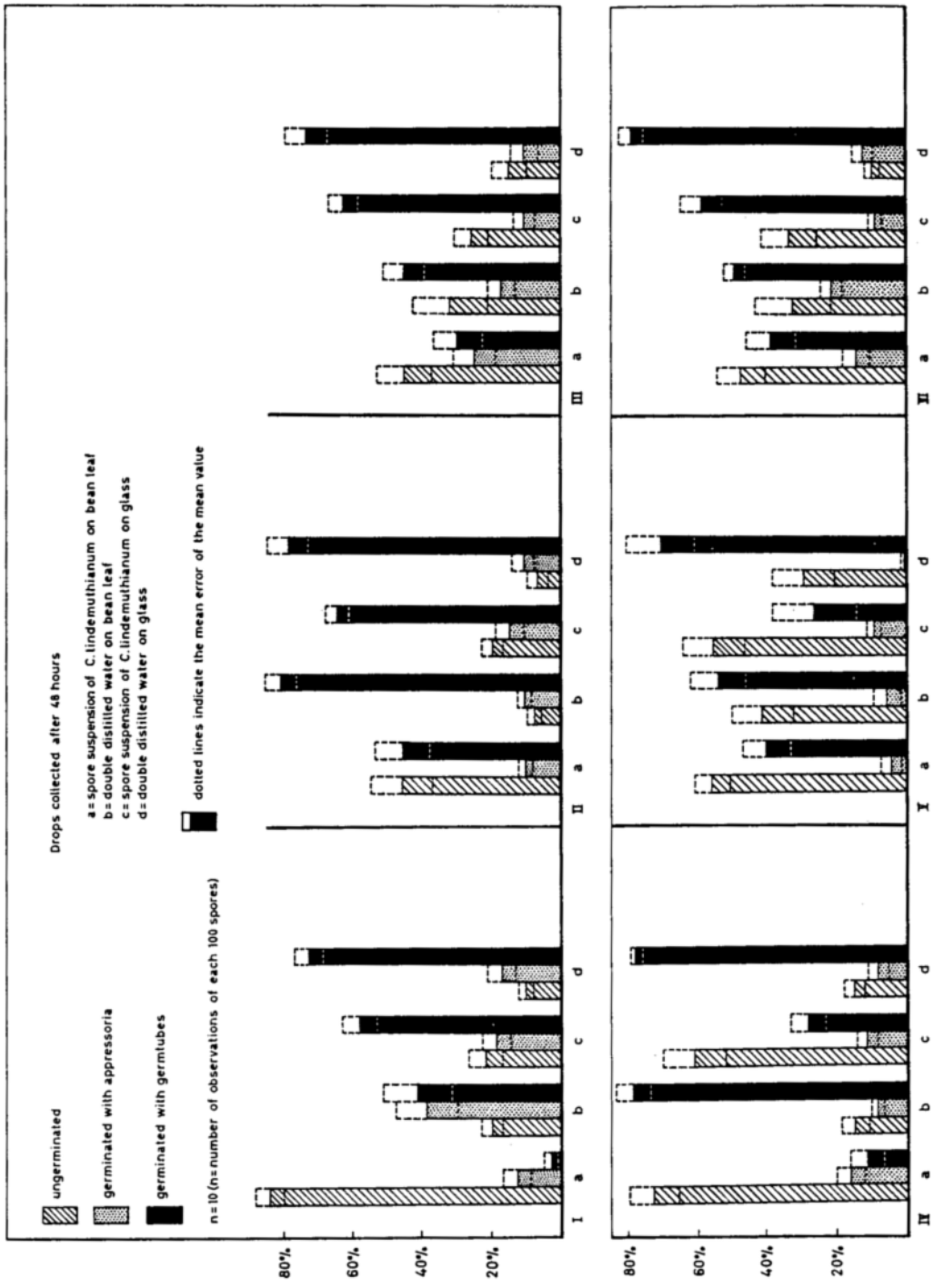

FIG. 4. Inhibition of spore germination and growth of appressoria and germ tubes of *Colletotrichum lindemuthianum* in diffusates collected from bean leaves.

spores, while in experiments V and VI we can hardly speak of a retardation in the germination of the test spores in the drops collected from bean leaves with and without spores and in the spore suspension drops collected from glass cavity slides.

The results were the same as by the interaction between spores of *S. fructicola* and pods of *P. vulgaris*. The supernatants of drops in which spores of *C. lindemuthianum* had germinated give a retardation in germination of test spores in comparison with the supernatants of drops of the three running check experiments.

Substances formed by contact between fungus and plant tissue

By means of paper chromatography we tried to determine the substances formed by interaction of spores and the plant. In the developed chromatograms was reacted on carbo-hydrates, amino acids, amides and phenolic compounds.

Carbohydrates

S. fructicola – *pods of* Phaseolus vulgaris

With benzidine-trichloro acetic acid three spots were observed in the chromatograms of the supernatants of drops of spore suspension and double distilled water, which were in contact with bean pod tissue. They were identified as saccharose, fructose and glucose. The spots of saccharose, found in the chromatograms of the drops of double distilled water, were much greater and deeper stained than from the drops of spore suspension gathered from bean pods. The reverse can be said of glucose but not of fructose (Table 1). Incubating drops with and without spores on glass never produced any of the above mentioned sugar spots. We conclude that the saccharose excreted by the bean pod is partly used by the fungus and the fructose component is used more effectively than glucose. Mobilization and metabolism of carbohydrates were also found in pollinated styles (Linskens, 1955; Tupý, 1961).

Table 1. The extent of the spots in cm^2 of saccharose, glucose and fructose, found in the diffusates from bean pods in contact with spores of *Sclerotinia fructicola* and double distilled water respectively.

Diffusate:	Saccharose		Glucose		Fructose	
	Spore suspension	Double distilled water	Spore suspension	Double distilled water	Spore suspension	Double distilled water
Experiment 1	5.0	18.2	5.0	3.2	5.5	3.2
2	3.4	8.9	3.5	2.5	5.1	5.0
3	3.9	8.2	4.1	3.4	5.5	5.0
4	2.5	9.2	4.0	3.2	5.8	6.3
5	2.3	10.0	4.4	1.9	5.8	5.9
m =	3.4	10.9	4.2	2.84	5.54	5.08

In their respiratory processes pollen tubes consume mainly the fructofuranose component of sucrose. Similarly, in this present study a relatively

higher glucose level was found in diffusates of the spore suspensions of *S. fructicola* than in diffusates of double distilled water in contact with the bean pod tissue. Also, a difference in the proportion glucose/fructose, i.e. an average of 0.76 and 0.56 respectively was observed.

C. lindemuthianum – *pods of* Phaseolus vulgaris

Here we also identified saccharose, fructose and glucose. The spot of saccharose found in the chromatograms of the diffusate of double distilled water was much greater (12.5 cm^2) and deeper stained than in the diffusate of spore suspension (5 cm^2). The reverse can be said of fructose but not of glucose (see Table 2).

TABLE 2. The extent of the spots in cm^2 of saccharose, glucose and fructose found in the diffusates from bean pods in contact with spores of *Colletotrichum lindemuthianum* and double distilled water respectively.

Diffusate:		Saccharose		Glucose		Fructose	
		Spore suspension	Double distilled water	Spore suspension	Double distilled water	Spore suspension	Double distilled water
Experiment	1	5.0	12.5	6.1	7.8	6.7	3.8
	2	11.5	20.0	8.5	14.2	8.6	0

Conclusion: the saccharose excreted by the bean pod is partly used by *C. lindemuthianum* and here the component glucose is more effectively used than fructose. This will appear as a relatively higher fructose level in the diffusates of the spore suspensions of *C. lindemuthianum* than in the diffusates of double distilled water in contact with bean pod tissue. The spores of *C. lindemuthianum* should then consume, following the above mentioned theory of TUPÝ, mainly glucose in their respiratory processes instead of fructo-furanose or the glucose is used in higher quantities for other processes than in the above mentioned case.

C. lindemuthianum – *leaves of* Phaseolus vulgaris

The quantity of carbohydrates, if present, was very small and highly variable.

SUBSTANCES REACTING WITH NINHYDRIN

S. fructicola – *pods of* Phaseolus vulgaris

The number of substances reacting with ninhydrin was variable (8–13); the following amino acids and amides were determined: aspartic acid, lysine, valine, serine, alanine, tyrosine, glutamic acid, arginine, phenyl-alanine, glycine, asparagine and leucine (iso-).

In drops of double distilled water there was always a higher concentration than in the diffusates of the spore suspension (Fig. 5).

S. fructicola – *potato tissue*

It was striking that, in drops of double distilled water incubated 48 hours on slices of potato, the amino acids were always in higher concentration than

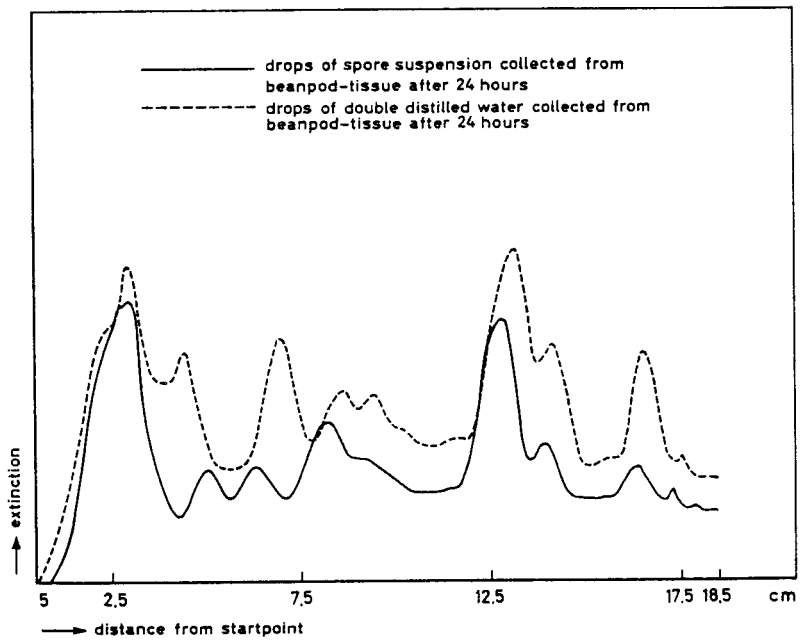

FIG. 5. Quantities of substances reacting with ninhydrin found in the supernatants of drops of double distilled water and in those of a spore suspension of *Sclerotinia fructicola* after an incubation period of these drops of 24 hours on bean pods measured by an extinction writer (Zeiss Extinctionsschreiber II).

in the drops of spore suspension collected from the potato tissue. The number of amino acids ranged from 8 to 12, and their quantities were variable. The following eight were found regularly: aspartic acid, arginine, leucine (iso-), glutamic acid, alanine, phenyl-alanine, serine and valine.

Usually no valine was present in the diffusates of spore suspension. When drops with and without spores were incubated on glass cavity slides, ninhydrin reacting substances were never found.

C. lindemuthianum – *pods of* Phaseolus vulgaris

The number of substances in both diffusates reacting with ninhydrin was about 12. In the diffusates of double distilled water was a higher concentration of the different amino acids than in the diffusates of spore suspensions. The amino acids determined were the same as found by the interaction between spores of *S. fructicola* and pods of *P. vulgaris*. Drops incubated on glass contained no ninhydrin reacting substances.

C. lindemuthianum – *leaves of* Phaseolus vulgaris

The quantity of substances reacting with ninhydrin was also very small and quite variable.

SUBSTANCES REACTING WITH FOLIN DENIS OR $FeCl_3$, AS AN INDICATOR FOR PHENOLIC COMPOUNDS

S. fructicola – *pods of* Phaseolus vulgaris

The chromatograms were sprayed with Folin Denis and exposed to NH_3 vapour. In the chromatograms of four early experiments a blue spot was found with an $R_F = 0.86$–0.98 in drops of spore suspensions and not in drops of double distilled water. But after 2.9.1960 this spot was not detected again. In drops incubated on glass we never found any substance reacting with Folin Denis.

S. fructicola – *potato tissue*

With the reagents of Folin Denis a spot was sometimes found with R_F=0.80 in drops of the spore suspension and not in drops of double distilled water. However, this spot was usually not present in both diffusates.

S. fructicola – *leaves of* Phaseolus vulgaris

After development and spraying the chromatograms with Folin Denis, exposing to a NH_3 vapour, some blue spots were found (Table 3), indicating phenolic compounds. By testing the eluates of these spots for their fungitoxic capacity, we could not find a spore germination inhibiting effect. The spot with the highest R_F value (0.83–0.87) gave a slight stimulation to spore germination of *S. fructicola.*

C. lindemuthianum – *pods of* Phaseolus vulgaris

Phenolic compounds were not found either with Folin Denis or with $FeCl_3$.

C. lindemuthianum – *leaves of* Phaseolus vulgaris

The same method was used as mentioned under "Interaction between spores of *C. lindemuthianum* and pods of *Phaseolus vulgaris.*" Here too some phenolic compounds were found (Table 3), but tests of eluates of one dimensional chromatograms, did not show any spore germination inhibiting effect.

TABLE 3. R_F-values of phenolic compounds found in diffusates of *Sclerotinia fructicola* and *Colletotrichum lindemuthianum* collected from bean leaves.

Reagens	Solvent	Diffusates of	
		Leaf + *C. lindemuthianum*	Leaf + *S. fructicola*
1% $FeCl_3$	CO_2 in H_2O	$R_F = 0.88$ $R_F = 0.92$	$R_F = 0.88$ $R_F = 0.92$
Folin Denis	2% acetic acid	$R_F = 0.86$	nihil
Folin Denis	chloroform: acetic acid: water = 2:1:1	$R_F = 0.51$ $R_F = 0.69$ $R_F = 0.83$	$R_F = 0.52$ $R_F = 0.87$

The conclusions from these experiments are as follows: Interaction between *S. fructicola* and bean pods showed a change in the carbohydrate levels and in the concentration of the ninhydrin reacting substances.

The supernatants of drops of double distilled water contained sucrose and amino acids at much higher concentrations than the supernatants of drops of spore suspension. In the latter the spores were growing during 24 hours and they used substances excreted by the bean pod tissue. In the former the substances excreted by the bean pod tissue were not metabolized. The presence of phenolic compounds in the supernatant of drops of the spore suspension is doubtful.

In both types of drops, collected from potato slices, with and without spores of *S. fructicola*, substances are excreted by the potato tissue in the same manner as by the bean pods. The lack of a promoting effect in the drops of double distilled water on the spore germination is probably due to the question of an inhibiting substance excreted by the tissue of the potato.

During the interaction between *C. lindemuthianum* and bean pod tissue, carbohydrates and amino acids are formed in the same way as by *S. fructicola*. The interaction between *C. lindemuthianum* and leaf tissue of *Phaseolus vulgaris*, however, is not constant. Carbohydrates and amino acids, if present, were found in very small quantities. The phenolic compounds found in the diffusates were, at least in the concentrations used in our experiments, not responsible for the effect on spore germination.

THE INFLUENCE OF SOME AMINO ACIDS ON SPORE GERMINATION OF *SCLEROTINIA FRUCTICOLA*

In Table 4 we see that there is a positive effect of aspartic acid and glutamic acid on the spore germination and the growth of *S. fructicola*. Both amino acids were always present on the chromatograms. Thus, we would expect a promoting effect of the drops of double distilled water collected from the potato tissue, as we found in the drops of double distilled water collected from bean pods. Another reason for this behaviour is perhaps a changing of the H^+-ion concentration.

TABLE 4. The influence of aspartic acid and glutamic acid on the germination of the spores of *Sclerotinia fructicola*.

Solution	Ungerminated %	Length germination tubes[1]		
		< 100 μ	100–200 μ	> 200 μ
Aspartic acid	9.4 (5.6–15.6)	18.1 (13.3–23.4)	13.3 (9.5–19.2)	59.1 (49.6–67.3)
Glutamic acid	5.0 (0.9–6.8)	8.5 (2.8–16.5)	4.8 (2.8–7.7)	81.7 (71.6–88.4)
Double distilled water	3.3 (0.9–7.6)	47.9 (44.7–53.8)	25.3 (21.7–28.6)	23.5 (16.9–27.6)

[1] Expressed in %; every figure is a mean of six counts, each of minimum hundred spores.

THE INFLUENCE OF THE pH-VALUES OF THE DIFFUSATES

There is a striking difference in the pH of drops of spore suspension and drops of double distilled water collected from potato tissue after an incubation period of 48 hours (Table 5). This in contrast with the pH of the diffusates

collected from bean pods. The pH of the diffusates of double distilled water, however, are the same as those of the drops of spore suspension and the drops of double distilled water, incubated on glass cavity slides.

TABLE 5. pH-Values of the diffusates, collected from bean pods and potato tissue, with or without spores of *Sclerotinia fructicola*.

Nature of the liquid	*Solanum tuberosum*				*Phaseolus vulgaris*		
	48h		72h		24h		
Diffusate of spore suspension	8.4	8.5	7.2	7.2	5.8	5.8	5.8
Diffusate of double distilled water	5.6	5.6	5.6	5.6	5.4	5.6	5.4
Spore suspension on glass	5.6	5.6	5.6	5.4	5.6	5.6	5.4
Double distilled water on glass	5.4	5.6	5.6	5.4	5.6	5.6	5.2

Possibly there is an effect of pH on the germination of spores of *S. fructicola* in diffusates of the spore suspension. Therefore we have made spore suspensions of *S. fructicola* in phosphate buffers at pH 8.0, 7.0 and 5.5. As a control double distilled water pH = 5.2, was used. It proves (Fig. 6) that high pH-values affect the spore germination adversely and that near pH = 8.0 the spore germination is reduced to 5–16%. With a pH-value of 5.5 we found a spore germination of about 100%. This experiment was done with two replications with the same result. The observed pH of the drops of double distilled water give, however, no explanation for the absence of a promoting effect of the excretions from the potato tissue. Therefore we suppose, that the spore germination inhibiting principle is a substance formed and excreted by the potato tissue in small amounts in drops of double distilled water. When, on the other hand, drops of spore suspension are in contact with the potato tissue, then the formation and excretion of this substance is strongly stimulated.

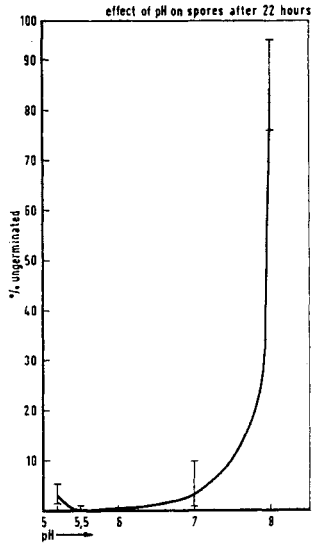

FIG. 6.
The influence of the pH on the inhibition of spore germination of *Sclerotinia fructicola* in phosphate buffers with increasing pH-values and in a check liquid with a pH = 5.2 (double distilled water).

General discussion and conclusion

From experiments described here, it appears that there is an interaction between the fungus and the attacked plant tissue. This interaction is most evident between *S. fructicola* and the tuber tissue of the potato. Here we see also a strong alteration in the pH of the drops of spore suspension in contact with the potato tissue. This alteration was very slight or absent by the interaction of *S. fructicola* and *C. lindemuthianum* with the tissue of the bean pod. There was also a strong inhibition of the germination of spores tested in the supernatants of centrifuged drops of *Colletotrichum* spores, which were in contact with the bean pods 24 hours, although there was no alteration in the pH of the diffusates.

The reaction of the leaf tissue under influence of *C. lindemuthianum* was strongly variable and generally slight, either as regards inhibition of spore germination or the formation of amino acids and carbohydrates. The results of our experiments with *S. fructicola* and the pods of bean are not in complete agreement with those of experiments of MÜLLER (1956). He always found an obvious inhibiting effect upon the spore germination of *S. fructicola* as a result of the interaction between the parasite and the been pod tissue. The temperature experiments of JEROME & MÜLLER (1958) proved, however, that there is an influence of the environmental conditions upon this interaction (see also CRUICKSHANK & PERRIN, 1963). JEROME & MÜLLER stated that the treatment of the plants before inoculation is important.

The reaction rate of the cells is probably the factor that turns the scale (MÜLLER & BÖRGER, 1940). With a high reaction rate of the cells we get a hyperergical reaction and a discordancy between the fungus and the plant (parabiose). A low reaction rate gives rise to a concordancy between host plant and parasite and a congenial relation. Between both extremes, discordancy and concordancy, lays a scale of reaction-possibilities for the cells of the host. It is not astonishing that in one case the reactions of the cells of a distinct cultivar of the bean with a distinct strain of the fungus and/or under typical environmental circumstances run slower than in another case. In the former case the formation of spore germination inhibiting substance(s) during the period of the experiment does not occur or they are formed in much smaller quantities.

The type of the tissue also influences the reaction. This is clearly indicated in experiments done with the pods and the leaves of beans. Interaction of the fungus with the former tissue is much greater than with the latter. Moreover, the excretion of amino acids and carbohydrates in drops is much higher.

The fact that MÜLLER (1956) did not find an inhibiting effect on spores germinated in drops of double distilled water collected from the bean pods, does not prove that the spore germination inhibiting substance is not formed or present in these drops. It only demonstrates that there is a quantitative difference between both diffusates. The influence of the spore germination inhibiting principle is affected by substances with a germination promoting effect, which are also present in the diffusates. The concentration of these substances is always higher in drops of double distilled water than in drops of spore suspension, because the spores, when present, also use substances excreted by the plant tissue. An indication for the presence of a spore germination

inhibiting substance was found in drops of double distilled water collected from the potato tissue (see also GÄUMAN, 1951: 427). CRUICKSHANK & PERRIN (1963) could not prove, however, by spectroscopical analysis, that pisatin was present in healthy tissue of pea pods.

That the effect of the inhibition of the spore germination is caused by a phenolic compound (JOHNSON & SCHAAL, 1952; WALKER & STAHMANN, 1955; MENON & SCHACHINGER, 1957; GÄUMANN & KERN, 1959) was not confirmed in our test objects and host/parasite combinations. This is in agreement with the experiments of CRUICKSHANK & PERRIN (1960, 1961), in which they found pisatin ($C_{17}H_{14}O_6$) as a result of the interaction between *S. fructicola* and pea pods, a substance which has no phenolic character.

From work in medical science BUCHNER (KOLLE & HETSCH, 1938, cited by MÜLLER & BÖRGER, 1940) intended by the term alexin, bactericidal substances on which the bacterial-averting reaction of body-liquids is based. These substances have obviously partly the property to kill definite parasites. It is not proven that the specific substances which cause in vitro precipitation, agglutination or lysis-reactions have a fungicidal or bactericidal effect. From the experiments of CRUICKSHANK (1962) it is evident at present, that the substance pisatin formed by the interaction of *S. fructicola* and pea pod tissue, is not specific and really has an antifungal activity. This substance found by CRUICKSHANK & PERRIN is a chromano-coumarane and it is evident, that the substances formed by the interaction of different parasite/host-plant relations do not necessarily belong to the same group of compounds.[3]

ACKNOWLEDGEMENTS

The author is greatly indebted to Professor Dr. H. F. LINSKENS and Professor Dr. K. O. MÜLLER for valuable discussions and suggestions and to Miss A. BOUWHUIS for technical assistance. Special gratitude is due to Dr. R. G. STANLEY of the Pacific S. W. Forest and Range Experimental Station, Berkeley, California, U.S.A., and to Mr. D. H. PHILLIPS of the Forest Research Station Alice Holt Lodge, Wrecclesham, England, for revising the English text.

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[3] Recently CRUICKSHAW & PERRIN (Life Sci. 2 (1963): 680–682) published the skeletal structure of phaseolin ($C_{20}H_{18}O_4$). This is a heterocyclic compound, also a chomanocoumarine and not a phenol, as indicated in their text. Phaseolin is a compound formed during the interaction between *S. fructicola* and the endocarp of *Phaseolus vulgaris*.

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