MEETINGS OF THE BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON NOVEMBER 20TH 1969

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L'analyse de diatomées et de pollen de la crique de Vlaardingen. Une interprétation révisée.

Des analyses quantitatives de diatomées et la représentation des variations dans le pourcentage des différentes sortes par des diagrammes similaires à ceux employés depuis longtemps en palynologie, mais qui n'avaient été que rarement appliqués dans les analyses des diatomées, ont abouti à des résultats surprenants. Il s'agit en l'occurrence de l'examen des diatomées dans les échantillons pris dans des coupes de criques à Vlaardingen (Hollande méridionale), où des fouilles entreprises par l'Institut de Préhistoire et de Protohistoire de l'Université d'Amsterdam ont révélé des habitats des civilisations de Vlaardingen et des Gobelets Campaniformes. On avait admis que dans le lit de la crique le long de laquelle une population Vlaardingen avait habité (vers 2350 av. J.C.) et qui s'était pratiquement comblé, une nouvelle crique s'était formée peu avant l'arrivée de la population des Gobelets Campaniformes vers 1940 av. J.C.: ceci s'est avéré erroné. Il est apparu que la seconde crique s'est formée encore pendant l'époque de l'habitat Vlaardingen, d'abord comme un ruisseau peu profond, qui a rehaussé lentement ses propres rives et qui a rempli ainsi le lit entièrement asséché de la première crique. L'interprétation du diagramme pollinique est fondée en grande partie sur les résultats des analyses des diatomées. Des analyses des diatomées contenus dans des tessons de poterie de Vlaardingen et des Gobelets Campaniformes a prouvé que la poterie est fabriquée sur place en utilisant de l'argile provenant respectivement de la première et de la seconde crique.

Littérature:

Altena, J. F. van Regteren, J. A. Bakker, A. T. Clason, W. Glasbergen, W. Groenman-van Waateringe & L. J. Pons, The Vlaardingen Culture. *Helinium* **2**, 1962, pp. 3–35, 97–103, 215–243; 3, 1963, pp. 39–54, 97–120.

Groenman-van Waateringe, W. & M. J. Jansma, Diatom and pollen analysis of the Vlaardingen creek. A revised interpretation. *Helinium* 9, 1969, pp. 105–117.

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON NOVEMBER 20TH, 1969

INTRODUCTION

The meeting on fundamental research of the Committee of Phytopathology has been the first meeting of this type.

The main purpose is to enhance exchange of methods, results, and ideas of current experimental research in botany and other sciences, that may benefit research in phytopathology. This implies that short papers within the fields of phytopathology, other aspects of botany, and sciences phytopathology is concerned with, are welcome.

It is intended to hold these meetings on fundamental research ("Onderzoekdag") annually.

A. DE WIT-ELSHOVE *(Afd. Fytopathologie van de Landbouwhogeschool, Wageningen)*

Some aspects of the degradation of pisatin by fungi, pathogenic to Pisum sativum L.

In previous communications, it has been demonstrated, that pisatin is broken down by fungi, pathogenic to *Pisum sativum*, in a liquid medium. This degradation now appeared to depend on the glucose concentration in the broth. When the glucose concentration at the beginning of the experiment is higher than 2%, no degradation of pisatin occurred. Catabolite repression might play a role here, as can be inferred from data of experiments with ^{14}C labeled pisatin, showing high amounts of $^{14}CO_2$ liberated during the degradation of pisatin. Detailed results will be published elsewhere.

H. J. TER HAAK and B. SCHIPPERS *(Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn)*

Penetration of Fusarium oxysporum f. pisi race 1 into roots of susceptible and resistant pea cultivars.

Lignituber-like reactions of the epidermis cellwalls are formed where germtubes of the pathogen attempt to penetrate the roots of a susceptible (Rondo) and a resistant (Rovar) pea cultivar grown in soil infested with *F. oxysporum* f. *pisi* race 1 (SCHIPPERS & VOETBERG 1969). The significance of these reactions were studied in relation to resistance and site of infection of the hostplant. The numbers, size and shape of the cellwall-reactions as well as the sites of their formation are equal with both cultivars. With the exception of roottip and roothair zone, cellwall-reactions may occur all over the root surface. They seem to prevent penetration into roots at these sites. The cellwall-reactions aparently are not responsible for the difference in susceptibility between the susceptible and resistant variety. No difference in severity of disease nor in the appearance of first symptoms occurred between seedlings that were placed with their roottips only and those that were placed with their whole roots in infested soil for different periods of time before they were replaced to sterile soil. Symptom-appearance, however, was delayed and less severe with seedlings that had been kept with their roots, except roottip and roothair zone, in infested soil. Laterals started to develop just before the end of the incubation in infested soil. It is supposed that roots only become invaded at roottip and roothair zone. Staining of the cellwall-thickenings with phloroglucine –HCl and with thionin according to Stoughton does indicate, they are cellulosic in nature.

Schippers, B. and Voetberg, Jacoba Sj. (1969). *Neth. J. Pl. Path.* **75**: 241–258.

J. VAN DEN HEUVEL *(Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn)*

Infection of bean leaves by Alternaria zinniae in light and darkness.

Primary leaves of 11 days-old dwarf beans *(Phaseolus vulgaris)* sprayed with spores of *Alternaria zinniae* Pape, showed small purplish brown lesions after incubation in light for 3 days. After incubation in darkness for 1 to 3 days pale brown necrotic spots were formed, the size of which increased with increasing length of the dark period. The reaction of the leaves to infection in light resembles a hypersensitivity reaction; in darkness the leaves are more or less susceptible.

In vitro, spore germination and mycelial growth of *A. zinniae* in light and in darkness did not differ. On the leaf surface spore germination in darkness was better than in light. It must be

assumed that these development stages of *A. zinniae* are not influenced directly by light, but only in an indirect way.

Incubation of leaves sprayed with a culture filtrate of the fungus in light or darkness resulted in small lesions and larger spots, respectively. Injection of small amounts of the culture filtrate into the mesophyll by means of a hypodermic syringe caused necrotic spots, irrespective of the previous light or darkness treatment.

It is not yet clear whether the enhanced susceptibility of bean leaves to this fungus is due to an increased sensitivity to a toxic substance. Research is in progress to examine whether the toxic factor in the culture filtrate is zinniol, a recently isolated and characterized toxin from *A. zinniae*.

J. C. M. BEIJERSBERGEN and C. B. G. LEMMERS (*Laboratorium voor Bloembollen onderzoek, Lisse*)

Enzymatic liberation of α-methylenebutyrolactone from tuliposid A.

The antibiotic substance α-methylenebutyrolactone, isolated from extracts of white skins of young tulip bulbs *(Tulipa gesneriana* L.*)* is liberated in vitro spontaneously from a non-fungitoxic precursor tuliposid A (the glucose-ester of γ-hydroxy-μ-methylenebutyric acid) at pH above 6. Moreover tuliposid A can be split quickly in glucose and α-methylenebutyrolactone at lower pH (5.2) by enzymes present in extracts of bulbscales and pistils. This enzymatic activity could not be found in extracts of the white skins. Yet in extracts of white skins (pH 6.0) tuliposid A and free α-methylenebutyrolactone are found. In extracts made with buffer pH 5.0 the free lactone is absent.

It was demonstrated that factors are present in the extracts (pH 6.0) of white skins, which irreversibly inactivate the bulbscale enzymes which split tuliposid A. These experiments were performed by mixing different extracts before and after dialysis.

The conclusion was drawn that the partial breakdown of tuliposid A in extracts of pH 6.0 of white skins is a result of enzymatic activity. This activity however is, perhaps already during grinding, quickly destroyed.

These experiments indicate that the possibility is still open that during infection by *Fusarium oxysporum* Schlecht. f. *tulipae* Apt in the white skin (in which the fungus can not penetrate) the fungitoxic α-methylenebutyrolactone is liberated by enzymatic activity from a precursor e.g. tuliposid A.

J. C. OVEREEM and D. M. ELGERSMA *(Instituut voor organische chemie, T.N.O., Utrecht and Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn).*

Accumulation of Mansonones E and F in Ulmus hollandica infected with Ceratocystis ulmi.

Thin-layer chromatograms of alcoholic extracts of the xylem of *U. hollandica* 'Belgica' infected with *C. ulmi* showed three spots which were not detected in chromatograms of extracts of healthy wood. On silica gel (solvent chloroform – ethyl acetate 9:1) an orange spot was observed at R_F 0.77, a violet spot at R_F 0.57 and a spot which fluoresced in UV light at R_F 0.24. Both the orange and violet compounds showed fungitoxic activity. Extracts of *U. hollandica* clone 390 (resistant against Dutch elm disease) inoculated with *C. ulmi* contained the same compounds. Again the compounds were not detected in uninoculated wood. From 10 kg of young diseased branches of *U. hollandica* 250 mg of the orange compound and 50 mg of the violet compound were isolated. In 10 kg of healthy wood only traces (fractions of a milligram) of the above-mentioned compounds could be found. By physical methods the orange compound was identified as Mansonone E and the violet compound as Mansonone F. Both compounds

have been isolated earlier from *Mansonia altissima* Chev.[1, 2]. Naphthalene derivatives related to the Mansonones have been found in the heartwood of *U. rubra*, *U. glabra* and *U. carpinifolia*, but not in *U. laevis* and *U. thomassii*[3, 4]. *U. thomassii* contains other naphthalene derivatives[5].

[1] G. B. Marini Bettòlo, C. G. Casinovi and C. Galeffi, *Tetrahedron Letters* **1965**, 4857.
[2] N. Tanaka, M. Yasue and H. Imamura, *Tetrahedron Letters* **1966**, 2767.
[3] M. Fracheboud, J. W. Rowe, R. W. Scott, S. M. Fanega, A. J. Buhl and J. K. Toda, *Forest Prod. J.* **18**, 37 (1968).
[4] B. O. Lindgren and C. M. Svahn, *Phytochemistry* **7**, 1407 (1968)
[5] Chen-Loung Chen and F. D. Hostettler, Tetrahedron **25**, 3223 (1969)

G. A. KAMERBEEK *(Laboratorium voor Bloembollen onderzoek, Lisse)*

Gummosis of tulip bulbs by ethylene.

Traces of ethylene can cause several reactions in flower bulbs, one of these is for instance a retarded development of the growing point in the bulb. Another reaction ethylene can cause, is gummosis of the bulb and the appearance of gum bladders in the bulb scales. The factors which influence the occurrence of gummosis are evaluated.

It is an important fact that only certain tulip cultivars, as for instance "Apeldoorn" and "Mad. Lefeber", show this reaction while others hardly do. Bulbs of cv. "Apeldoorn" give maximal reaction for two to four weeks after lifting. After this period the response slowly decreases in bulbs that were stored at 20 °C. However this does not exclude that after a storage period of two months response can be still considerable. The marginal ethylene concentration that gives a begin of response is of about 0.1 ppm. At higher concentrations the quantity of exudated gum and the number of bladders increase. The time of response can be measured in hours. Time of exposure and concentration of ethylene determine the effect. Higher temperatures stimulate the effect, while at low temperature (5 °C) the effect is negligible.

The endogenous production of ethylene in the bulb is too low to cause an effect. Bulbs that are attacked bij *Fusarium oxysporum* produce ethylene concentrations high enough to cause gummosis. Also *Fusarium* cultured on growth media produces relative high concentrations of ethylene (measured: up to 300 ppm).

Gummosis can also occur after damage of the bulb. Whether this response is caused by an increased ethylene production of the damaged tissue, still needs to be investigated.

H. M. DEKHUIJZEN and J. C. OVEREEM *(Afd. Fytopathologie van de Landbouwhogeschool, Wageningen and Instituut voor Organische Chemie, T.N.O., Utrecht).*

The role of plant hormones on club root formation

The presence of cytokinins in healthy turnips *(Brassica campestris* L. var. *rapa)* and in turnips 4 weeks after infection wich *Plasmodiophora brassicae* has been established. Extracts have been partially purified to remove substances which interfere in the bioassays. The extraction procedure was essentially that described for the isolation of the known purine cytokinins. The extracts were then chromatographed on paper and assayed, either on their ability to stimulate mobilization of ^{14}C labeled α-aminoisobutyric acid from the tip to the base of an oat leaf or on the ability to stimulate cell division of soybean callus. The effect of kinetin in both bioassays can be antagonized by abscisic acid. Extracts of infected turnips were about three times as active in the bioassays as healthy turnips. Infected and healthy turnips contain compounds with the same R_f values, which indicates that the increased cytokinin level originates from the host. One of these fractions cochromatographed with zeatin and zeatin riboside. This result explains the fact that explants of turnips infected with *Plasmodiophora brassicae* are independent of cytokinins for their growth, whereas callus of healthy turnip tissue requires kinetin for continued growth. Growth of infected callus can be antagonized by abscisic acid.

G. T. N. DE LEEUW *(Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn).*

Wilting of leaves induced by systemic invasion of the roots of "Xanthi-nc" tobacco plants with tobacco mosaic virus.

Scions of *Nicotiana tabacum* L. var. "Samsun" grafted on top of *N. tabacum* var. "Xanthi-nc" rootstocks grown on nutrient solution, wilted at about six days after the leaves of the "Samsun" scions were inoculated with tobacco mosaic virus (TMV). To determine whether this wilting was due to a blocking of water transport in the xylem vessels, the wilted "Samsun" scions together with the "Xanthi-nc" stocks were cut off from their root system. Wilting disappeared in about half an hour when the excised parts were kept on water. Necroses were detected in the internal and external phloem tissue of the "Xanthi-nc" stems (DE LEEUW, 1968). Cross sections of the "Xanthi-nc" roots showed necroses in the phloem and necrosis and collapse of endodermal cells. Blocking of the xylem vessels in roots or stems was not observed. Presence of infectious TMV in the roots was demonstrated. Probably the wilting of the leaves had been induced by the inability of water to reach the xylem vessels in the "Xanthi-nc" roots.

DE LEEUW, G. T. N., – 1968. Translocation pathways of tobacco mosaic virus in Nicotiana tabacum L. var. "Xanthi-nc". *Meded. phytopath. Lab. Willie Commelin Scholten* 75, 61 pp.

D. M. ELGERSMA *(Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn).*
Anatomical structure of xylem vessels as a possible factor in resistance against Dutch elm disease.

Conductivity of the vascular system of 2-year-old stem pieces as regards air and water was higher in the susceptible elms, *Ulmus hollandica* "Belgica" and *U. americana* than in the resistant elms *U. hollandica* cl. 296, cl. 390, cl. 405 and cl. 496. The xylem vessels of resistant elms were relatively shorter and the percentage of short vessels was greater than in the susceptible elms. The percentage of vessels with a diameter larger than 65μ was smaller in resistant elms than in susceptible ones. The shorter vessels and the smaller diameter of vessel lumina of resistant elms might be a factor in limiting spreading of the spores of *Ceratocystis ulmi* in the new annual ring.

IDA BLOK *(Instituut voor Phytopathologisch Onderzoek Wageningen).*
The isolation of Pythium species from soil.

Isolation and identification of *Pythium* spp. from soil is part of a larger research project dealing with the relation of the soil-microflora and vegetation. During several years, soil samples from certain experimental fields are being examinated every two months.

Within this framework, we have to consider the following requirements on the isolation technique:

1. the samples have to be worked up quickly,
2. the method has to be quantitatively as reliable as possible, and
3. it must be reproducible.

The use of clear wateragar, with or without specific inhibitors, has an advantage over opaque media, because subculturing the isolates is more easily done. When soil particles are directly spread onto the agar, few *Pythium* isolates and many other fungi are obtained. The use of seeds as baits for *Pythium* is well-known. After some preliminary experimenting corn- and hempseeds were chosen. With hempseed the number of *Pythium* isolates per seed is somewhat smaller than with corn, however, the diversity in species is a little bit higher. The number of *Pythium* isolates obtained depends more on the number of seeds used than on the quantity of soil.

With cornseeds two methods are used: the seeds are either brought into a layer of moist soil, 2 cm deep, or they are brought into a petri-dish with sterile water and some soil. After incubation at room temperature, the seeds are rinsed under tap-water and plated out on water-agar. Both methods give satisfactory results.

The optimum incubation period appeared to be 16 to 24 hours. With hempseeds only the second method is used.

G. J. SAALTINK *(Laboratorium voor Bloembollen onderzoek, Lisse)*

The mode of action of a heat treatment on bulbs attacked by Xanthomonas hyacinthi studied in vitro. (Influence of density of the bacterial suspension and composition of suspension fluid).

In studying the heat treatment of hyacinth bulbs as control measure against *X. hyacinthi*, it is an open question if dying of the bacteria is caused directly or indirectly by the temperature level.

If physiological processes in the bulbs induced by heat treatment are damaging the bacteria, the heat effect is indirect. However, it is possible that the temperature level of the bulb itself is directly harmful to the bacteria.

Introductory experiments have shown that surviving of *X. hyacinthi* in vitro is dependent on the density of the bacterial suspension and the composition of the suspension fluid. For example in pepton the bacteria stayed alive for 190 minutes at 47 °C, in the same experiment in saline for only 10 minutes. The results confirm results of other workers with other bacteria.

A comparison of data about surviving of bacteria in the bulb with surviving in suspensions makes it likely that the cause of the dying process in this case is mainly a direct effect of temperature.

G. J. BOLLEN *(Afd. Fythopathologie van de Landbouwhogeschool Wageningen).*

Effect of pasteurization on spore germination of some saprophytic fungi from soil.

In investigations on pasteurization of greenhouse soils we are confronted with the sudden appearance of some species of fungi in soil which has received heat treatment.

This phenomenon is principally due to a decrease in antagonism between different micro-organisms caused by the death of a large part of the microflora. However, heat treatment also breaks the dormancy of spores of surviving species. *Gilmaniella humicola*, one of the species which shows increased germination after heat treatment of the soil, was chosen for a detailed investigation. This species has only one type of spores (aleuriospores). In addition it has the advantage of being very heat resistant and it can survive heating at 90 °C for 30 minutes.

Soil dilution plates of a pure culture of *G. humicola* in autoclaved compost were made after heating the sample at 80 °C (30 min.) and the number of colonies obtained was compared with that from untreated soil. When the cultures were young many more colonies were counted from untreated soil, but the difference gradually decreased until similar numbers of colonies were found after 13 days. When the colonies became older more colonies (54 ×) were obtained from samples heated at 80 °C increasing until 40 days after inoculation.

The effect of heating at various temperatures on germination of spores from cultures of different ages was determined. The temperature which induced optimal germination varied with the age of the culture (in soil) being 54, 60, 70 and 80 °C at 11, 21, 28 and 82 days respectively. The maximum temperature (thermal death point) proved to be consistent for cultures of various ages. The fungus survived 90 °C (30 min.), but not 92, 5 °C.

When the germination curve (number of colonies versus temperature of preceding heat treatment) of *Gilmaniella* was compared with those of species without heat-activated germination the curve proved to be composed of two parts. Germination initially increased with the temperature of treatment and with the age of the spores ("activation slope"), then – from optimum to maximum – germination decreased with temperature ("death slope").

Optimal germination occurred at a higher temperature if spores came from soil than if they were obtained from potato-dextrose agar, even if the spores from the latter were much drier.