Acta Bot. Neerl. 24(2), April 1975, p. 243–255.

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

SYMPOSIUM "PARASITISM IN FLOWERING PLANTS" ON NOVEMBER 16, 1974

J. DAAMS (*Research Laboratories Philips-Duphar B.V., "Boekesteijn", 's-Graveland*)

Parasitic plants as weeds

Only a limited number of the more than 1000 species of parasitic plants have a reputation as weeds in forestry and agriculture, causing unacceptable yield depressions.

In forestry the dwarf mistletoes (*Arceuthobium spp.*) cause, mainly in large area losses of timber of Coniferous trees (Pinaceae). The losses in the forestry regions of the western part of North America are estimated to be of the order of 20 million cubic meters of wood annually. The problems with these species increase as new afforested areas favour the spreading of the parasitic plants. Control is, until now, only possible by expensive cultural methods.

Many species of the Orobanchaceae are known as weeds in a large number of agricultural as well as horticultural crops. In many Mediterranean countries *Orobanche crenata* attacks *Vicia faba* to such an extent that on thousands of hectares this crop cannot be grown anymore. Yield depressions up to 90% have been observed. Besides *Vicia faba* also *Daucus carota* and some other legumes are attacked by this species. Control of this weed is extremely difficult. Handpicking is not feasible. Due to the longevity of the seeds, long term rotation schemes failed. The Orobanche Research team of the University of Malta tries to isolate and to identify the germination stimulator in the roots *Vicia faba*. Other efforts are directed to develop *Orobanche* -resistant varieties of *Vicia faba*. This approach has led to success in control of *O. cumana* in sunflower. Sunflower is an important crop plant in Eastern European countries. *Artemisia maritima* is thought to be the original host of *O. cumana*. Since the beginning of this century this species has spread enormously in Russia and the Balkan countries. In many areas yield depressions reach a level where sunflower growing was not anymore profitable. In this particular case the introduction of new broomrape resistant cultivars, developed in the USSR have, solved the problem. In recent years however, new broomrape strains appear which threaten the crop. In Russia a biological method, making use of midge, *Phytomyza orobanchiae* looks very promising.

O. ramosa, a widely distributed species is well-known as a weed in tobacco, hemp, tomato etc, grown in Europe, Middle East and North Africa. Since a few years this species is rapidly spreading in tomato-growing regions in California. It is considered as a serious threat to this crop. Effective control can only be obtained by soil fumigation.

O. minor, in the past a well-known weed in clover and lucerne also in northern European countries is still a problem in some southern and central European countries, and in Australia. This species disappears probably when better cultural methods are used.

Striga spp. (Witchweeds)

In Asia, Africa and locally in the US a few *Striga* species are important weeds in cereals. *Striga hermotheca* infests large areas of crops such as *Sorghum* spp. in many countries. *Striga asiatica* has been observed in the US in 1956 for the first time. A large effort has been directed to contain further spreading. Use has been made of "trap crops" such as cotton and soybean, species not parasitized by *Striga asiatica* but inducing the seeds to germinate. In this case of a broadleaved weed in a cereal crop also treatments with 2.4-D are effective. A difficulty is that many grasses act as hosts to *Striga* which makes eradication difficult.

Cuscuta spp. (Dodders)
In many countries, all over the world, a large number of *Cuscuta* spp. invade agricultural crops. *C. trifolii* is well-known in clover and lucerne fields, forming often dense nearly impenetrable mats on the vegetation. *C. campestris* is an important weed in crops such as sugar beet, tobacco, potato etc. Low yields of forage crops and seed crops of leguminous plants are mainly due to infestations by *Cuscuta* species. In Yugoslavia 80% of the clover and lucerne fields are infested by dodders of which 20% had to be destroyed. In some cases the sugar content of sugar beets decreased up to 60% by heavy infestation by *C. campestris*. Control methods consist of cultural measures and treatment with herbicides.

General
The importance of parasitic plants as worldwide weeds in crops has come to general attention in the last few years due to the efforts of the Parasitic Weeds Research Group of the European Weed Research Society. The main function of this Group is exchanging and collating information in this field.

C. H. KLAREN (*Biologisch Centrum, Afdeling Plantenfysiologie, Rijksuniversiteit Groningen, Haren*)

On the physiology of the hemiparasite Rhinanthus serotinus

The hemiparasite *Rhinanthus serotinus* (Scrophulariaceae) is capable of autotrophic existence but its growth is stimulated enormously after attachment to the roots of a host plant by means of haustoria (in which a xylem-xylem connection is formed). The question arises what the limiting factors are for the growth of *Rhinanthus* without a host: 1) Is the absorption of water and/or minerals reduced? 2) Is the synthesis of certain organic compounds reduced? To find an answer attached and unattached *Rhinanthus* plants were compared.

Within one day after attachment *Rhinanthus* shows a rapid growth, the contents of water and minerals (nitrate, phosphate, potassium, magnesium, sodium, not calcium), organic phosphorus and nitrogen compounds (phosphate esters, amino acids, nucleic acids, proteins) increase, whereas the contents of carbohydrates, chlorophyll and the enzyme ribulose diphosphate carboxylase decrease. Photosynthesis increases in later stages (HOFSTRA & KLAREN 1973).

Since the transpiration stream does not increase after haustorial contact water absorption can be excluded as limiting factor. The improved water retention may be explained from the great amount of minerals entering the hemiparasite which are absorbed by the cells and enhance their osmotic pressure. This is probably the cause of the observed cell enlargement after attachment.

The hypothesis that the absorption of mineral nutrients is limiting growth could be confirmed by other experiments. The curve giving the relationship between the external phosphate concentration and the rate of phosphate absorption by *Rhinanthus* showed that the affinity for phosphate of the root system is very low.

Tracer studies demonstrated the transfer of organic compounds (e.g. amino acids, sugars) from the host to *Rhinanthus*. There is, however, no evidence that these additional organic compounds are absolutely required by the hemiparasite.

The great growth response of solitarily grown *Rhinanthus* plants to applied xylem sap of the host appeared not to be caused by the hormonal factors present in the host sap. From fractionation experiments it was concluded that the growth stimulating effect of xylem sap for the greater part may be ascribed to the inorganic fraction. The relatively high concentrations of inorganic ions are favourable for *Rhinanthus* with the reduced mineral absorption capacity of its root system.

HOFSTRA, J. J. & C. H. KLAREN (1973): Host-parasite relations in Rhinanthus serotinus. II. Physiological interactions between host and parasite. *Proc. Eur. Weed Res. Counc. Symp. Parasitic Weeds, Malta* 1973. 247–256.

H. CH. WEBER (*Botanisches Institut der Justus Liebig-Universität, Giessen*)

Morphology of haustoria of Rhinanthoideae (Scrophulariaceae)

A comparative study has been made of the morphology of haustoria of 30 Central European Rhinanthoideae. It was shown that all haustoria can be classified more or less in four types. They are named after the most characteristic agent:
The "*Tozzia-alpina*-type" (with a smooth surface but without root hairs), the "*Pedicularis-silvatica*-type" (without a smooth surface and without root hairs), the "*Melampyrum-pratense*-type" (with a smooth surface and root hairs) and the "*Rhinanthus-minor*-type" (without a smooth surface and with root hairs)[1].

Having lost contact with their host roots, haustoria can become very thick, so-called "Speicherhaustorien", but they can also show normal development without any contact with host roots.

Main-roots and secundary roots of many Rhinanthoideae-species have small outgrowths similar to warts, so-called "Warzenhaustorien". These are highly different from known haustoria. Although they are extremely small, they are able to attack thin host roots. Contrary to other haustoria there is no differentiation into vascular-core, haustorial-nucleus and endophyte.

[1] A smooth surface means that the haustorium has no lobes clasping the host root.

P. WOLSWINKEL (*Botanisch Laboratorium, Utrecht*)

Physiological aspects of the parasitism of Cuscuta

The parasitism of *Cuscuta* has been reviewed by SCHMUCKER (1959) and KUYT (1969). More recently new anatomical data, obtained by electron microscopy, have been presented by DÖRR (1972). Our studies on *Cuscuta* are directed to elucidate details of "the process of draining the phloem of the host by the parasite".

Using ^{14}C-labelled metabolites it could be shown that *Cuscuta* is able to withdraw all assimilates which normally move from a photosynthesizing host (*Vicia faba* L.) leaf to growing pods and seeds (WOLSWINKEL 1974a). An important feature of the haustorial organ seems to be the enlargement of the surface area in the haustorial tissues, occurring on three levels (discussed in WOLSWINKEL 1974a). The absorptive cells of *Cuscuta*, showing "transfer cell" characteristics, seem specialised in relation to absorption of solutes from an extracytoplasmic compartment. In addition to a very efficient absorption by the parasite, as suggested by the anatomical details, also an enhanced unloading rate of the host phloem seems to be essential for the transfer of ^{14}C-assimilates from host to parasite (WOLSWINKEL 1974b). The enhanced unloading of the host phloem is apparently under metabolic control since it appeared to be inhibited at 0 °C and after addition of dinitrophenol.

A high invertase acitivity, found in the free space of the contact region of host and parasite, suggests an important role for this enzyme in the parasitic relationship. Invertase was assayed by measuring the glucose formed after incubation of stem segments, with or without haustorial coil, in a buffer solution with sucrose.

Using the graphical method of compartmental analysis, the efflux was studied of potassium, known to be a typically phloem-mobile element, from parasitised broad bean segments in comparison with non-parasitised segments (WOLSWINKEL 1975). A metabolically controlled enhanced release of potassium by the host phloem seems to be an important factor involved in the transfer of potassium from host to parasite.

Having presented data on *Cuscuta*, we can add a remark on the economically important root parasite *Orobanche* (cf. SCHMUCKER 1959 and KUYT 1969). It may be expected that an enhanced rate of unloading of phloem in the host root will be as essential for the transfer of solutes to *Orobanche* as it seems to be for *Cuscuta*.

The data on the enhanced unloading of phloem (found for ^{14}C-assimilates and potassium) may be meaningful for efforts to elucidate the normal movement of solutes in plants between phloem and surrounding tissues. An enhanced unloading of phloem could function as an essential factor determining the sink activity of strong sinks like fruits.

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S. J. TER BORG (*Biologisch Centrum, afdeling Plantenoecologie Rijksuniversiteit Groningen, Haren*)

Ecological similarities and dissimilarities in the hemiparasitic Scrophulariaceae in the Netherlands

In the Netherlands the following hemiparasitic Scrophulariaceae occur (nomenclature follows Heukels-van Ooststroom, 1973): the annuals *Melampyrum pratense*, *M. arvense* (rare), *Parentucellia viscosa* (rare, adventive), *Euphrasia* spp., *Odontites verna*, *O. litoralis*, *Rhinanthus serotinus* and *R. minor*, the three latter with various subspecies; *Pedicularis sylvatica* (biennial, perennial); *P. palustris* (most populations consisting of biennials, probably mixed with winter-annuals; moreover rare annual populations – ssp. *opsiantha*?).

Germination. The seeds of the annual species require a period of chilling. Chemicals tested so far cannot substitute for stratification; gibberellic acid sometimes raises the percentage. They germinate during winter and early spring except for *Melampyrum pratense*, where roots develop in September-October, at temperatures of ca 10 °C; its epicotyl is still dormant then. The cotyledons of *Melampyrum* expand after further stratification, from January onwards. Climatological conditions during seed ripening (temperature, humidity) largely affect the proportion of seeds dormant until next autumn.

In *Pedicularis sylvatica* so far a germination rate of maximally 10% only could be achieved.

In *Pedicularis palustris* (both annuals and biennials) up to 40% of fresh seeds germinate at temperatures of over 20 °C, up to 90% after previous chilling during a month or more. In a supposedly biennial population large numbers of seedlings were found shortly after seed ripening in July 1974, and rosettes in September, suggesting that at least part of the population behave as winterannuals.

Host-parasite relationships. The degree of host dependance is variable: whereas *Parentucellia viscosa*, *Euphrasia* spp., *Odontites verna*, *Rhinanthus serotinus*, and annual *Pedicularis palustris* could be grown without a host, this proved difficult in *Melampyrum pratense*.

These hemiparasites occur in relatively open vegetations. This, however, is not just a consequence of their presence, but also a major primary reason, as indicated by experiments with *Rhinanthus serotinus*. Presence of the parasite depresses total yield (dry weight of shoots) to some extent, most strongly in well-spaced host plants. Moreover the parasite influences the composition of a mixed vegetation, the relative amount of Leguminosae being reduced.

M. M. KWAK (*Biologisch Centrum, afdeling Plantenoecologie Rijksuniversiteit Groningen, Haren*)

Pollination ecology of some hemiparasites with large flowers (Scrophulariaceae)

Operation of the pollination mechanisms of *Rhinanthus serotinus* (Schönh.) Oborny, *R. minor* L., *Pedicularis palustris* L., *P. sylvatica* L., and *Melampyrum pratense* L. by nectar and pollen collecting bumblebees (*Bombus* Latr.) was studied, to collect data on pollination mechanisms of some inland hemiparasites for a comparative study of these processes.

The zygomorphic flowers of members of the Rhinanthoideae exhibit the "classical" type of bumblebee pollination in which the head, in some cases other parts of the dorsum, effects the transfer of pollen.

Each bumblebee species makes its own demands with regard to the following factors:

1. The length of the corolla (taking the nectar-level in the corolla on account).
2. The presence of pollen and nectar.
3. The coincidence of the habitat of the plant and the bumblebee.
4. The interaction between the various bumblebee species.

Insect visitors on the plant species studied were divided in real collectors of pollen and nectar e.g. bumblebees (*Bombus* spp.), and occasional visitors e.g. honeybees (*Apis mellifera*), cuckoo bumblebees (*Psithyrus* spp.), butterflies (Lepidoptera), and syrphids (Syrphidae).

Insect behaviour in relation to foraging and pollination differed with the corolla length and pollinator caste. Three groups could be distinguished: normal collectors (long tongued bumblebees e.g. *Bombus pascuorum* and *B. hortorum*) foraged upright for nectar, while the stigma nototribically contacted residual pollen in the head-thorax crevice; pollen not retained in this crevice is groomed from the body by a forward movement of the middle legs crossed over the dorsum and deposited in the corbiculae. Primary nectar thieves, e.g. *B. terrestris* and *B. lucorum*, obtained nectar by biting a hole into the long corolla tube close to the nectary. Secondary nectar thieves, e.g. *B. jonellus* and *B. pratorum*, secured nectar by using holes bitten by primary thieves.

These short-tongued thieves collected pollen by grasping the edge of the galea with their mandibles, supported their inverted bodies by grasping the galea with their legs and curved their abdomen under the pollenchamber so the venter would receive the load. Pollen was released from the anthers concealed in the galea by wing vibrations. There were three groups of foraging short-tongued bumblebees, depending on their behaviour: nectar collecting individuals, pollen collecting individuals, and individuals collecting both. It may be assumed that cross-pollination was achieved by the latter two groups because those individuals had the best chance of contacting the essential parts of the flower. Their behaviour was similar when visiting either *Rhinanthus*, *Pedicularis* or *Melampyrum*. Although considered in the literature as nectar thieves, my observations show that some individuals in fact brought about cross pollination (particularly, the pollen collectors).

A comparison of the seed-set in cross- and self-pollinated plants was made. The self-pollinated plants were caged in bud. In *P. palustris* 63.6% of the flowers developed capsules with a mean value of 13.0 seeds in pollinated plants. In caged plants 0.4% of the flowers developed capsules with no seeds as a rule. In *P. sylvatica* caged flowers developed a mean of 3.2 seed/flower; cross-pollinated flowers 5.5 seed/flower. In contrast the seed-set in caged plants of *Melampyrum* was not reduced when compared with control plants.

MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY ON OCTOBER 25, 1974

C. VAN DER MEULEN-BRUIJNS
(Biologisch Centrum, Afdeling Plantensystematiek, Rijksuniversiteit Groningen, Haren)

The vascular pattern in the ovary of some Mesembryanthemaceae, compared with that of Melandrium (Caryophyllaceae)

The Mesembryanthemaceae can be divided into two groups according to the placentation:

1. A group with central placentation, which includes three sub-families, of which the Mesembryanthemoideae are assumed to be the most primitive.
2. A group with basal to pseudo-parietal placentation, which includes one sub-family, the Ruschioideae.

Typologically the Ruschioideae are assumed to be derived from the Mesembryanthemoideae, for in some genera of the Ruschioideae the placentation shifts ontogenetically from axile to basal or parietal. This shift is the result of changes in relative growth rates (BUXBAUM 1951).

This statement was confirmed by comparison of the vascular patterns in the ovaries of *Aptenia cordifolia* (L.f.) Schwant. (sub-family Mesembryanthemoideae) and *Dorotheanthus bellidiformis* (Burm.f.) N.E.Br. (sub-family Ruschioideae). Both in *Aptenia* and in *Dorotheanthus* the ventral vascular bundles are located in the central column; in the latter species these bundles are also found in the bottom and the wall of the ovary. The ventral bundles are situated in the septal radii. This last observation is contrary to that of IHLENFELDT (1961), who helds that the ventral vascular bundles are situated in between the septa, both in the Mesembryanthemoideae and in the Ruschioideae.

There is no unanimity in the interpretation of the placental vascularisation in the Caryophyllaceae: are the ovules supplied by one vascular system (ROHWEDER 1967, 1970), or by two separate ones (BOCQUET 1959, MOELIONO 1970)? In accordance with the three above mentioned authors, I *found* two placental vascular systems in *Melandrium rubrum*. Unlike Rohweder, and in accordance with Bocquet and Moeliono, I *interpret* them as two separate vascular systems. These systems can be called the peripheral vascular system, supplying the placentae inferiores, and the axial vascular system, supplying the placentae superiores (MOELIONO 1970).

In comparing the vascular pattern in the ovary of *Aptenia* and *Melandrium*, as a part of that in the flower, it is concluded that the placental vascular bundles in *Aptenia* should be interpreted as homologous with the axial vascular system in *Melandrium*; i.e. the peripheral vascular system is absent in *Aptenia*. As has been elucidated in this study, the vascular pattern in the ovary of *Dorotheanthus* can be infered from that of *Aptenia*, the placental vascular bundles in *Dorotheanthus* also should be interpreted as the axial vascular system.

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MEETING OF THE SECTION FOR PLANT PATHOLOGY ON NOVEMBER 14, 1974

A. DE LANGE and B. DE WIT (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Interactions between saprophytic bacteria and Pseudomonas phaseolicola in vitro and in vivo.

Saprophytic bacteria were isolated from seed and from the primary leaves of Pinto beans. Gram-positive bacteria were predominant on the seed coat whereas most of the phyllosphere bacteria were Gram-negative. Thirty-eight of the 95 isolates from leaves were antagonistic to *Pseudomonas phaseolicola* in vitro; these were mainly non-pigmented Gram-negative bacteria. Each of nine in vitro-antagonistic and of five non-antagonistic isolates was inoculated together with *P. phaseolicola* on primary bean leaves. Most of the saprophytes, and the pathogen, could colonize the leaf surface well in less than seven days. None of the non-antagonistic isolates was antagonistic in vivo. Five of the in-vitro-antagonistic saprophytes could inhibit or suppress growth of the pathogen on the leaf surface. In these instances the reduction in number of *P. phaseolicola* cells seven days after inoculation was established not only when the inoculum contained identical numbers of bacterial cells from saprophyte and pathogen but also when the inoculum contained less saprophytic than pathogenic bacteria. The in-vivo-antagonistic bacteria, with the exception of one, were prototrophic. Among the non-antagonists three isolates were auxotrophic. The antagonistic bacteria mainly belong to the *Enterobacteriaceae* and to the genus *Pseudomonas*.

H. P. MAAS GEESTERANUS (*Instituut voor Planteziektenkundig Onderzoek, Wageningen*)

Hostplant - parasite relationships of the Erwinia carotovora group causing soft rot of potato plants and tubers

Erwinia carotovora var. *carotovora* (Jones) Dye and *Erwinia carotovora* var. *atroseptica* (van Hall) Dye are physiologically very similar but differ widely in pathogenicity. Tubers immersed in a bacterial suspension of the variety *carotovora* may turn into a soft rot, whereas immersed in a suspension of *atroseptica* they do not show any symptom of infection. When sprouts are present one may plant *carotovora* soft rot tubers to find out that they always grow out as healthy plants, while symptomless tubers infected by *atroseptica* may produce plants of which the haulms turn into soft rot. Injecting a *carotovora* suspension into a potato stem causes a local haulm soft rot, whereas, by doing the same with an *atroseptica* suspension, no soft rot appears. It is only possible to induce an *atroseptica* haulm soft rot by infecting the tissue of the mother tuber, when it is still present on the stem base. Probably during the rotting processes of the tuber tissue, toxic products are formed, which are transported with the bacteria into the haulms, making the latter tissue susceptible for the pectinolytic activity of the *atroseptica* bacteria. Reactions of culture filtrates of the *atroseptica* cultures on potato stem cuttings fortify this supposition. Induction of soft rot of latent infected tubers of both micro-organisms under anaerobic conditions indicates the presence of defence mechanisms in the tubers under aerobic conditions. Three types are known: production of phytoalexins, oxidation of polyphenolics and encapsulation by suberin of bacteria present in the intercellular spaces beneath lenticels. The third type may be responsable for the occurrence of the latent lenticel infection. By keeping tubers under water for about 4 days, one may liberate the bacteria from their encapsulation, now permitting these bacteria to induce soft rot. Isolation and identification of the micro-organisms involved, will indicate, whether the tubers were infected by one or both *Erwinia* varieties.

H. VRUGGINK (*Instituut voor Planteziektenkundig Onderzoek, Wageningen*)

Serological recognition of Erwinia carotovora var. atroseptica

The bacteriological procedure for the detection of *Erwinia carotovora* var. *atroseptica*, which causes blackleg, requires much time and effort due to its close similarity to *E. carotovora* var. *carotovora*. Attempts were therefore made to use serological methods as an alternative. Antisera against the bacteria were prepared by conventional methods. In agglutination tests, the antiserum against *E. carotovora* var. *atroseptica* did not react specifically; however, in agar double diffusion tests, a specific reaction could be obtained when the antiserum was first absorbed with an *E. carotovora* var. *carotovora* isolate. The pathogen could be detected directly in sap squeezed from infected stems and rotted tubers. To demonstrate the presence of latent infection the tubers were submerged in water to induce anaerobic rotting during which pathogenic bacteria are increasing. After soft rot had developed, the following two procedures for the identification of the causal organism were compared.

1. A suspension of the rotting tissue of the potatoes was streaked on a pectin-agar medium and incubated. After two days the bacteria were rinsed from the plates and tested serologically with the antiserum against *E. carotovora* var. *atroseptica*.
2. The rotting tissue of the potatoes was rinsed with water, centrifuged and the pellet suspended in a small quantity of water and also tested serologically.

Method 1 appeared to be the more reliable but required more time and work than method 2. A compromise was obtained by testing the rotting tubers first according to method 2 and using method 1 only for those tubers which reacted negatively.

J. C. M. BEIJERSBERGEN and CATHARINA B. G. LAROO-LEMMERS (*Laboratorium voor Bloembollenonderzoek, Lisse*)

In vitro inactivation of tulipalin A by Fusarium oxysporum f. sp. tulipae

Tulipalin (α-methylenebutyrolactone) isolated from tulips (*Tulipa gesneriana* L.) kills *Fusarium oxysporum* Schlecht f. sp. *tulipae* Apt when applied at a level of about 300 mg/litre in potato-dextrose agar. Under certain conditions, when fungus-covered pieces of such agar are placed in liquid media containing tulipalin A, concentration of the tulipalin A diminishes rapidly during incubation. In this process an important role is played by the incubation mixture, the amount and age of the mycelium and the medium on which the fungus has been grown. Tulipalin A was not inactivated by enzymatic or non-enzymatic compounds secreted by the fungus. Inhibition of the growth of the fungal mycelium persisted until the tulipalin A concentration had dropped to a low value and growth resumed. The results suggest that tulipalin A is taken up irreversibly by the mycelium.

ANNIE SWART (*Laboratorium voor Bloembollenonderzoek, Lisse*)

The production of ethylene by Fusarium oxysporum f. sp. tulipae

A culture of *Fusarium oxysporum* Schlecht f. sp. *tulipae* Apt grown on a liquid medium produced at least 2,000 times more ethylene than any of the other *Fusarium* species and formae speciales of *F. oxysporum* tested. The production of ethylene is strongly oxygen dependent. Comparison of several isolates of *Fusarium oxysporum* f. sp. *tulipae* showed that the ethylene production was generally on a high level. The pathogenicity of the isolates seems to be related to the quantity of the ethylene produced.

B. G. VAN 'T LAND, E. D. WIERSMA-VAN DUIN and A. FUCHS
(*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

In vitro and in vivo conversion of pisatin by Ascochita pisi

As has been shown by DE WIT-ELSHOVE & FUCHS (1971) *Ascochyta pisi*, a fungus pathogenic to pea, is able to convert pisatin, the phytoalexin of pea plants, to a product which, contrary to the parent compound, is only sparingly soluble in petroleum ether, but nevertheless shows the UV absorption maxima of pisatin. In view of a chemical characterization of the conversion product these in vitro experiments have been extended, using both unlabelled and ^{14}C-labelled pisatin. Both pisatin and its conversion product were shown to be soluble in ethyl acetate; they could be separated by thin-layer chromatography on silica gel (Merck Kieselgel 60 F 254), their R_f-values being different in various solvent systems ($CHCl_3$: R_f of pisatin 0.30 and R_f of conversion product 0.00; $CHCl_3$/methanol 97 : 3 (v/v): R_f of pisatin 0.75 and R_f of conversion product 0.32).

The rate of conversion of pisatin proved to be dissimilar for different physiological races of *A. pisi* (cf. VAN 'T LAND & FUCHS 1973), being maximal for race B, and minimal for race E; with *A. fabae*, on the other hand, pisatin remained unchanged.

On the basis of the UV and IR spectra of pisatin and its conversion product the inference could be made that the pterocarpan ring skeleton of pisatin did not change upon conversion. However, their mass spectra showed very characteristic differences; whereas the mass spectrum of pisatin had peaks at m/e 314 and 296 (295), that of the conversion product showed peaks at m/e 300 and 282 (281), in both cases the differences of 18 mass units indicating the loss of water. The difference in molecular weight of pisatin and its conversion product equals 14 mass units, which might point to the enzymatic removal of a methyl group upon conversion. Since the NMR spectrum of the conversion product lacked the resonance peak characteristic for the methoxy-aroxy configuration in pisatin, the change can be ascribed to a substitution in pisatin of 3-hydroxyl for 3-methoxyl. Therefore, the conversion product can be supposed to be 3,6a-dihydroxy-8,9-methylenedioxy pterocarpan, or 6a-hydroxyinermin (6a-HI; = 6a-hydroxymaackiain). The fragmentation peaks at m/e 296 and 282 in the mass spectra of pisatin and 6a-HI,respectively, are most probably due to the anhydro-derivatives of these two compounds. These anhydro-derivatives also arise upon acidification of aqueous solutions of pisatin and 6a-HI, respectively.

Since according to CRUICKSHANK et al. (1974) in vitro conversion of phytoalexins does not necessarily reflect changes occurring in vivo, in vivo experiments were also carried out. Formation of 6a-HI was also demonstrated in 5-day old pea seedlings (cv. Gloire de Quimper), the epicotyls of which were inoculated with *A. pisi* (races B and D). After 10 days, lesions were excised and extracted with petroleum ether, followed by ethyl acetate. In the latter extracts a substance was found which in every respect was identical with 6a-HI. Therefore, also in vivo pisatin is converted to 6a-HI. Most probably, this conversion is due to a methyl transferase.

While at concentrations of 100 μg/ml pisatin completely inhibited germination of *A. fabae* and *Monilinia fructicola* spores, 6a-HI hardly showed any inhibition of spore germination, not only of all physiological races, both virulent and non-virulent, of *A. pisi*, but also of *A. fabae* and *M. fructicola*. Therefore, these data seem to emphasize the special significance of pisatin conversion in the susceptibility of pea plants for pathogenic fungi.

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B. G. VAN 'T LAND, E. D. WIERSMA-VAN DUIN and A. FUCHS
(*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

The possible role of ascochitine in the combination Ascochyta pisi-pea

Both *Ascochyta pisi* and *A. fabae* are known to produce a toxic metabolite, ascochitine (BERTINI 1956, OKU & NAKANISHI 1963, 1966), the chemical structure of which has been elucidated by IWAI & MISHIMA (1965). Ascochitine has been shown to interfere with cell membrane functions of higher plants (OKU & NAKANISHI 1966), and might, therefore, play an essential role in the pathogenicity of both *A. pisi* and *A. fabae* towards pea and broad bean plants, respectively.

At concentrations of 6.6 μg/ml ascochitine stimulated pisatin synthesis by pea pods, cvs Cicero and Gloire de Quimper, very markedly; a similar observation has been made recently by OKU et al. (1973).

To study the reverse effect, viz. that of pisatin on ascochitine synthesis, in vitro experiments have been carried out; unfortunately, in vivo experiments failed because of the impossibility to detect any free ascochitine in *A. pisi*-infected pea plants. Erlenmeyer flasks, containing Lilly & Barnett's medium with a series of pisatin concentrations up to 100 μg/ml, were inoculated with *A. pisi* race D. Mycelial weights and also ascochitine concentrations were considerably lower at 60 and 100 μg pisatin/ml culture medium after 72 h of incubation at 23 °C. This resulted in amounts of ascochitine per mg dry weight of mycelium which were only c. 1/5 of those of the controls. Ascochitine could not be isolated from the mycelium as such, but was readily extracted from the acidified culture medium.

The results obtained suggest that ascochitine biosynthesis is controlled by feed inhibition by pisatin. Whereas ascochitine causes a greatly enhanced synthesis of pisatin, pisatin in its turn suppresses ascochitine biosynthesis, and, besides, growth of *A. pisi*. This might mean, that pisatin breakdown by *A. pisi* is a prerequisite for this fungus to be pathogenic to pea. Inability of *A. fabae* to infect pea plants, on the other hand, might be the result of inability to convert pisatin to 6a-hydroxyinermin (VAN 'T LAND et al. 1975).

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

Virus-like particles associated with mycoplasmas in Vinca rosea plants affected by the yellowing disease of Brassica oleracea

Electron microscopy of ultrathin sections of stems of *Vinca rosea* plants affected by the yellowing disease of *Brassica oleracea* revealed virus-like particles associated with mycoplasma-like organisms in the phloem elements. The virus-like particles were about 10 × 50 nm and occurred free in the cell lumen or along the membranes of the sieve tubes. Sometimes the particles surrounded mycoplasmas which appeared to have degenerated. The particles studied

closely resemble the virus-like particles detected by GOURRET et al. (1973) in *Trifolium repens* affected by clover phyllody mycoplasmas. The diseased *Vinca* plants were kindly provided by Dr. G. Marchoux, Montfavet, France.

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J. E. BRÖLMAN-HUPKES (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

A virus-complex in Hippeastrum hybridum

Some of the viruses which occur in *Hippeastrum hybridum* plants produce symptoms in the leaves while others do not. Two of these viruses were examined extensively.

1. The virus that causes lesions on *Hyoscyamus niger* produces mosaic symptoms in the leaves of *H. hybridum* 3–8 weeks after back inoculation. In *Nicotiana clevelandii* this virus becomes systemic after about 19 days. No reactions were obtained on *Gomphrena globosa* or *Chenopodium quinoa*. The length of the virus particles, approximately 750 nm, was determined with the electron microscope utilizing dippreparations from *H.niger*. The purification of this virus by differential centrifugation is presently being attempted.
2. Another virus present in *H. hybridum*, produces lesions on *G. globosa* and *C. quinoa* leaves and became systemic in *N. clevelandii* after about 19 days. *H.niger* did not show symptoms after inoculation. Back inoculation of this virus in *H.hybridum* did not produce symptoms even though the virus was found to be present. Virus-containing-sap, centrifuged on a sucrose gradient, gave practically no band differing from the control. After fractionation however, an infectious zone was found to be present, which contained virus particles 600–650 nm long.

The virus, which produces lesions on *H. niger* should be named the Hippeastrum Mosaic Virus (HMV), while the other virus occurs only latent in *H.hybridum*.

Probably the thrips *Franklienella fusca* Hinds. is a vector of the HMV.

O. KAMOEN and G. JAMART (*Rijksstation voor Planteziekten, Merelbeke (Gent), België*)

Further examination of the strawberry fruit rot control by fungicides

Strawberry fruit rot, caused by *Botrytis cinerea* Pers. ex. Fr., is mainly due to infection of the flowers (POWELSON, 1960, JARVIS and BORECKA, 1968). The standard fungicide treatments applied during flowering are not always succesful. The possible shortcomings of the flowering spray scheme have been examined by analyses of the fungicides used, and by microscopical study of petal infections. Earlier KAMOEN and JAMART (1974) found that immediately after spraying, all flower parts are sufficiently covered with fungicides. After a few days, however, the young opening flowers are insufficiently protected until the next spray. The present experiments have shown that by application of prophylactic sprays during flowering, the fungicide is distributed over flowers and buds. These buds are only covered externally, and open during the following days producing flowers without any fungicide protection on the internal parts until the next spray. Infections can occur on the internal flower parts and we examined if they can be eradicated by the spray the next week. One day after infection of the petals with a spore suspension, germ tubes (50 to 100 μ long) were present only superficially. No penetration had occurred and killing of the germ tubes was possible with a fungicide. The third day after infection the fungus had penetrated and the hyphae were growing internally in the petal tissue. A drop of fungicide was able to stop this internal hyphal growth partially but not completely. Benomyl and dichlofluanide were used for this internal therapy of the petal tissue. The use of

a benzimidazole systemic fungicide has an additional disadvantage in that the pathogen has developed resistance to some of these fungicides. About 40% of the isolates from strawberries sprayed with benomyl in a field experiment were resistant (ED 50 > 10 ppm).

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H. M. DEKHUIJZEN (*Instituut voor Organische Chemie T.N.O., Utrecht*)

A new method for the isolation of plasmodia of Plasmodiophora brassicae

Secondary vegetative plasmodia were isolated by two different methods from *Brassica campestris* callus tissue infected with *Plasmodiophora brassicae*.

The first method, chopped callus tissue produced only small spherical plasmodia (5–30 μ) (KEEN et al. (1969): Phytopathology **59**, 637–644). A new method was developed utilizing enzymes to break down the host cell walls in an hypertonic medium. The plasmodia isolated by this method were of two types, an irregularly-shaped type varying in size from 7–90 μm and a less abundant smaller spherical type of 5–30 μm diameter. Plasmodia isolated by both methods took up oxygen. It has been speculated that the spherical plasmodia originate from cleavage of the large multinucleate plasmodia and are distributed among new host cells during cell division of the proliferating host tissue.

W. H. VAN ECK (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Lytic processes during chlamydospore formation in Fusarium solani f. cucurbitae

Breakdown of cell wall layers of macroconidial cells of *Fusarium solani* f. *cucurbitae* during their transformation in soil into chlamydospores was observed with the electron microscope by OLD & SCHIPPERS (1973). A similar degradation of cell wall material has been demonstrated during formation of chlamydospores from macroconidial cells of the fungus in pure shake culture. This lytic process is therefore likely to be of autolytic origin. Activity of the lytic enzymes chitinase and β-glucosidase was demonstrated colorimetrically both in macroconidia from single spore cultures and from shake cultures. Histochemical analysis at the ultrastructural level appears to be a useful approach to study the origin and distribution of chitinase (N-acetyl glucosaminidase) in chlamydospores from natural soil.

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G. J. BOLLEN and BERNARDA VAN DER POL-LUITEN
(*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Mesophilic heat-resistant soil fungi

The search for heat-resistant micro-organisms forms an integral part of a research program on plant disease control in soils by means of selective heat treatment. The fungi mentioned in this abstract are able to survive a heat treatment at 70 °C for 30 min. in moist soil. Most of

them were Ascomycetes or Deuteromycetes. Among Basidiomycetes only one species, *Tephrocybe carbonaria*, and among Zygomycetes only species belonging to *Mortierella* sect. *Isabellina*, were heat-resistant. Some species form heavy-walled chlamydospores, e.g. *T. carbonaria*, or microsclerotia, e.g. a *Cladosporium* species, but others, like *Sartorya fumigata*, have frail structures.

The occurrence of heat-resistant species was studied in 60 glasshouse, 56 arable, and 127 forest and heather soils. *Gilmaniella humicola* and *Talaromyces flavus* (syn. *T. vermiculatus*) were very common in glasshouse soils (65 and 37%, respectively) and arable soils (45 and 25%, respectively), but rarely encountered in forest and heather soils (4 and 2%, respectively). *G. humicola* was found to be a coprophilous fungus introduced into arable soils with manure.

T. flavus showed a marked antibiotic activity against bacteria and Streptomycetes. To a lesser extent mycelial growth of many fungi was also inhibited.

The distribution patterns of *Trichophaea abundans*, *Mortierella* species belonging to sect. *Isabellina*, *Eleutherascus tuberculatus*, and a *Cladosporium* species differed from that of *G. humicola* and *T. flavus*. These species were often isolated form forest and heather soils, but were rarely found in arable soils and were absent in glasshouse soils. The occurrence of *E. tuberculatus* was restricted to heather soils and reclaimed heather soils.

Three species belonging to the *Eurotiaceae* were very common in all soil types: *Eupenicillium brefeldianum*, *E. lapidosum* and *Sartorya fumigata*. The latter was by far the most dominant species among the pioneers which colonized a burned peat soil after a forest fire.