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The TNO Research Unit for Internal Therapy of Plant Diseases

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In 1950 Oort was invited to establish a Research Unit for Internal Therapy of Plant Diseases and to become its Chairman.

The original goal of this Research Unit was to find other ways of fighting fungal plant diseases than by applying fungicides which only act externally. The idea was to develop *systemic* fungicides which would be taken up by the foliage or by the roots and be transported within plants. It was thought that in this way plants could be protected against fungal attack from within.

From the outset Oort realized the necessity of a many-sided approach. This was reflected in the composition of the Research Unit which, till the present day, unites the combined efforts of phytopathologists, plant physiologists, organic chemists and biochemists.

The demands made on systemic fungicides are very high: they should be easily translocatable and highly fungicidal without showing undue phytotoxicity.

The first efforts were directed towards rendering well-known fungicides translocatable by proper structural variation. Of conclusive importance in this stage of the work was the development by van Raalte of the potato petiole test for establishing the translocatability of chemical compounds. On this basis Pluijgers developed the systemically active compound S-carboxymethyl-dimethyldithiocarbamate ("G 33") which was a remarkable compound in more than one respect. In the first place, both on foliage and on root application it did protect cucumber plants against the attack by *Cladosporium cucumerinum*. Secondly, it had very marked plant growth-regulating activity, causing, for instance, strong epinasty when applied to tomato plants. In the third place, and most remarkably, G 33 was not fungitoxic in vitro; neither was it converted by plants or microorganisms into significant amounts of fungitoxic compounds.

From this it became clear very early in the work that whether or not systemic *fungicides* are a real possibility, systemic *protection* with chemical compounds is possible quite apart from their intrinsic fungicidal activity. This gave the approach an extra dimension since now the pursuit of systemic protection could be continued on a much wider basis. It was realized that the relation between plants and their fungal parasites depends on very specific biochemical interactions between two organisms and that these relations may be disturbed in much more specific ways than just by killing off the invading fungus.

The plant growth-regulating effects of G 33 brought to mind that natural (indole acetic acid) and synthetic (2,4-D) plant growth regulators are known to increase the resistance of certain plants towards fungal attack. This is due to the strong influence of plant growth regulators on plant metabolism. It is known, for instance, that such agents affect the sugar level in plants, which in its turn can be an important factor in determining pathogenicity. The observations by van Andel regarding the systemic protection of plants induced by the administration of certain amino acids, mostly "unnatural" ones, have to be explained along a similar line.

On the other hand, Kaars Sijpesteijn studied systemically active, non-fungitoxic compounds with a totally different mode of action. Phenylthiourea completely protected cucumber plants against *Cladosporium cucumerinum*. This compound is a very active inhibitor of polyphenoloxidases and its protectant action may be partly related with this activity.

The continuation of this work led to the first truly *systemic fungicide* emerging from the efforts of the Research Unit for Internal Therapy of Plant Diseases. Upon studying structural variants of phenylthiourea Pluijgers hit upon a very interesting compound, 1-phenylthiosemicarbazide. This compound and some of its ring-substituted derivatives combined high fungicidal activity in vitro with a high capacity for being taken up by and transported within plant tissues. The study of its mode of action revealed that phenylthiosemicarbazide is taken up and transported as such, but that fungitoxicity depends on a very specific oxidation step.

Studying systemic action by bringing organic compounds into plants inevitably led to the necessity to study as well the effects of plant metabolism on such compounds. It was a lucky coincidence that the latter study started with dimethyldithiocarbamic acid and its derivative G 33. Dekhuijzen developed an extremely sensitive bio-chromatographic test to demonstrate the presence of dithiocarbamic acid derivatives in plant saps and extracts. With the aid of this test Kaslander isolated a number of chemical and biochemical transformation products and conjugates and consecutively elucidated their chemical structures. Later on similar work was performed with other types of compounds. This work demonstrated that one has to be very careful in correlating systemic activity with the chemical structure of the compound originally applied to the plant.

An important line of work was opened by Dekker in his studies on the systemic activity of certain fungicidal antibiotics (rimocidin, pimaricin) against seed-borne diseases. Complete protection of pea plants against *Ascochyta pisi* was achieved by the internal disinfection of pea seeds with these antibiotics. Among a large number of synthetic compounds only pyridin-2-thiol-N-oxide had a similar activity. It should be realized that work along these lines is of significance with a view to the increasing opposition against the use of organomercurials as seed-disinfecting agents.

Interesting results were obtained by Dekker in the systemic combat of powdery mildew of cucumber (*Sphaerotheca fuliginea*). Two completely different types of compounds, viz. procaine (2-diethylaminoethyl-p-amino-benzoate) and 6-azauracil, rendered a high degree of protection against powdery mildew when applied to the roots or to the foliage of cucumber plants. Niemann made an extensive study on the mode of action of procaine and on the effects of structural variations on anti-mildew activity. Dekker studied the mode of action of 6-azauracil, which appeared to interfere effectively with RNA synthesis in the fungal cells and thus with fungal growth.

In the course of years the attention was almost inescapably drawn towards the natural mechanisms which in plants determine resistance and susceptibility to fungal attack. One mechanism, which had been recognized elsewhere, is connected with the so-called "phytoalexins", compounds with fungicidal activity which are formed by cer-

tain plants following rather unspecific mechanical and/or biochemical damage. Plants are unsusceptible towards those fungi which are sensitive to the phytoalexin formed in response to the primary fungal attack. The formation and significance for resistance of one type of phytoalexin, pisatin, were studied by de Wit-Elshove. It appeared that not only the sensitivity of the fungus is of importance, but also its capacity for the biochemical breakdown of pisatin. The occurrence of phytoalexins not belonging to the pisatin-type is being studied by van Dijkman. It is the aim of this work to establish how wide the "phytoalexin"-concept is valid in explaining natural resistance. That it is not a general mechanism has been shown recently by Raa in his study of the factors determining resistance and susceptibility of apple plants against the fungus *Venturia inaequalis*. Here, the non-pathogenic fungal strains excrete toxins causing local damage of the plant which results in the *secondary* formation of fungitoxic quinones. As a result, the invading fungus is killed and confined to strictly localized necrotic spots and can not spread. On the other hand, the pathogenic strains are lacking active toxins and can spread uninhibited within the foliage.
It would seem that the further study of natural resistance mechanisms might afford clues to more specific and effective ways of fighting fungal plant diseases.
In addition to the defense mechanisms of plants attention has been given to the offense systems available to fungi. Among these, extracellular pectic enzymes excreted by fungi play an important part. This aspect and the possibilities to interfere with these mechanisms of attack have been studied by Fuchs.
In the foregoing no attempt has been made to give a full account of the many ways in which the TNO Research Unit for Internal Therapy of Plant Diseases has studied its subject. Neither has the historic line been followed strictly. The main purpose of this review was to show the diversity of approaches resulting from a multidisciplinary study of fungal plant dieases.
We honour Oort, the scientist, because of the quality of his many papers on specific subjects. In connection with the TNO Research Unit for Internal Therapy of Plant Diseases we honour, however, in the first place the scholar, who has added a new conceptual approach to his field of science.

Samenvatting

De Werkgroep Inwendige Therapie bij Planten, TNO

In 1950 werd Oort verzocht een Werkgroep Inwendige Therapie bij Planten op te richten en daarvan het voorzitterschap op zich te nemen. Onderzoekers van verschillende disciplines, fytopathologen, plantenfysiologen, organisch chemici en biochemici, werken hierin nauw samen bij de ontwikkeling van systemische fungiciden en bij de bestudering van de relatie tussen waardplant en parasiet. De hoofdlijnen, waarlangs de problemen benaderd werden en de voornaamste objecten van onderzoek worden in het kort beschreven.