MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY ON APRIL 22, 1977

P. BAAS *(Rijksherbarium, Leiden)*

Leaf Anatomy of some Ilex hybrids

The main differential leaf and nodal anatomical characters of a number of *Ilex* species and their artificial hybrids has been investigated. It appears that the hybrids either share a certain character of one of the parents, completely lack such a character, are intermediate between the two parents for the two alternative character states, or show a completely new character not known to either parent. A certain pair of alternative characters may be inherited differently in different crosses involving the same parent species. Only in the case of marginal sclerenchyma strands, is this character never transferred to hybrid offspring in species combinations for which this character is differential. The lignification of the unspecialized abaxial epidermal cells is dominantly inherited in all but one hybrid involved. The development of a distinctive adaxial hypodermis is usually suppressed in hybrids of which one of the parents is lacking in this character. New characters have been found for hybrids of Ilex *opaca* × *aquifolium* where the vasculation of petiole and midrib, and the presence of periclinal subdivisions in the adaxial epidermis constitute features not known from either parent species.

The possible significance of the four different ways of expression of parental characters in hybrid offspring is in the field of genetic control of anatomical characters and in the systematic and phylogenetic interpretation of present day diversity in *Ilex* leaf anatomy. Some characters are probably controlled by homozygous recessive genes, others by dominant genes, and the majority by multiple genes. If a significant role of hybridization is presumed for the diversification of Ilex leaf anatomy in evolution, the present results aid in our understanding of the reticulate pattern of leaf anatomical variation within the genus, and may also explain the unusual variability of some characters below the species level reported for some taxa (BAAS 1975). Finally the leaf anatomical character complexes may aid the taxonomist and horticulturist in tracing parent species of putative *Ilex* hybrids.

A detailed, illustrated account of the leaf anatomy of *Ilex* hybrids will be published elsewhere (BAAS 1978).

BAAS, P. (1975): Vegetative Anatomy and the affinities of Aquifoliaceae, Sphenostemon, Phelline, and Oncotheca. *Blumea* 22: 311–407.

— (1978): Leaf and nodal anatomy of some Ilex hybrids. *Bot. J. Linn. Soc.* 75, in the press.

A. M. W. MENNEGA and B. J. H. TER WELLE *(Projectgroep Houtanatomie, Instituut voor Systematische Plantkunde, Utrecht)*

Mega-styloids in the secondary xylem of the genus Henriettea (Melastomataceae)

An unusually large type of styloid crystals was recorded in the secondary wood of a number of representatives of the genus *Henriettea*. The Ca-oxalate crystals, for which the name megastyloids is proposed, are 24 to 80 μm wide and 240 to 560 μm long. They are not situated in cells of any of the normal wood tissues, but seem to be distributed at random, their longitudinal axis being at various angles with the axis of the trees. Although the first impression is that the crystals are lying free in the wood, an extremely thin cell wall which envelopes the crystals tightly could be made visible by special treatment of the sections. Large crystals were already found at a distance of 150 μm from the cambium.

Mega-styloids were present in all (8) samples of *Henriettea succosa*, in part of the material of *H. maroniensis* and *H. ramiflora*, but they were absent in our (restricted) material of *H. granulata*, *H. martiusii*, *H. spruceana* and *H. stellaris*. In the genus *Calycogonium*, belonging to the same tribe, styloids of a smaller type occur. Those crystals are found in the elongate cells of the longitudinal parenchyma. Hitherto no crystals had been recorded for the wood of Melastomataceae.

Crystals of the mega-styloid type have been recorded for the leaves and petioles of several Monocotyledons and for a restricted number of Dicotyledons, but as far as we know never for the secondary wood.

A full account of our paper has been published in the IAWA Bulletin, 1977/2: 31–35.

M. T. M. WILLEMSE and M. A. W. FRANSSEN-VERHEIJEN *(Botanisch Laboratorium, Landbouwhogeschool, Wageningen)*

Changes in the cell organell population during megasporo- and gametogenesis in Gasteria verrucosa.

The changes in the cell organell population during the megasporo- and gametogenesis in *Gasteria*, which can be described as a variation of the *Polygonum* type, has been studied submicroscopically.

During the leptotene, the formation of transparent zones surrounded by membrane-like structures can be observed. Blebbing of the outer membrane takes place in the subsequent zygotene. This is probably followed by the separation of vacuoles in the cytoplasm. A decrease in the number of ribosomes, starting in the late pachytene, continues until diakinesis. After the first meiotic division there is an increase in the number of ribosomes.

During diakinesis the cell shows a polarity in its cytoplasm. This is expressed by the preference of location of the dictyosomes at the micropylar end and by the formation of electron transparent zones in the hyaloplasm at the chalazal end of the cell. These electron transparent zones are located where the megaspore cell is to be formed. The young megaspore also shows a characteristic less electron dense hyaloplasm.

In the beginning of megagametogenesis, the formation of starchlike and lipid-like material starts. Subsequently vacuolisation begins and a big central vacuole develops. After the second mitosis, the reserve material disappears and the formation of the egg apparatus, the central cell and the antipodal cells starts. Thereafter the storage of reserve products is again concentrated in the central cell.

On the ultrastructural level the changes and composition of the population of cell organelles until the formation of the megaspore are comparable with those during microsporogenesis (WILLEMSE 1972). The duration of the development to the megaspore on the racemous inflorescence also shows a sequence simultaneous with the development of the microspore. Only some events during megagametogenesis are comparable with those during formation of the microprothallium.

WILLEMSE, M. T. M. (1972): Morphological and quantitative changes in the population of cell organelles during microsporogenesis of Gasteria verrucosa. *Acta Bot. Neerl.* 21: 17–31.

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON MAY 18, 1977

J. G. TH. HERMSEN *(Instituut voor Plantenveredeling, Landbouwhogeschool, Wageningen)*

Questions on plant resistance and breeding for resistance

Research in many disciplines is either directly or indirectly related to resistance. The resistance phenomenon is complicated because of the large differences both among host plants and

parasites. Research on resistance as a means of biological control of diseases and pests is in the field of plant breeding.

Many questions pertinent to resistance are relevant to plant breeders.
– What is meant by horizontal and vertical resistance?
– Are these types of resistance genetically related or independent?
– Is race-specificity restricted to vertical resistance?
– Does erosion of horizontal resistance occur?
– Can vertical resistance become horizontal, if only a sufficient number of (major) genes are involved?
– Is it always possible to separate resistance into components and are these components genetically related? In other words: does it make sense to breed for separate components of resistance?
– What is the effect of environmental conditions on the reliability of assessments of disease attack?
– It is possible to detect resistance in a host without the parasite being involved?
– What is the level of generalisation of the theories and practical methods concerning resistance?

The main task of a breeder in relation to resistance is: breeding varieties with as many and stable resistances as possible. Reserach on genetic and biochemical relations between horizontal and vertical resistance in addition to results from population-genetic studies on resistance deepen the insight in what happens in field and glasshouse. Furthermore it informs us about the ways to influence and eventually control these processes.

J. C. ZADOKS *(Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen)*

Pathosystems; an introduction to concepts and problems

In this introductory paper general concepts were discussed. Resistance and virulence can be studied from three different points of view; epidemiology, physiology, and genetics. The phenomena of physiological specialization and gene-for-gene relation were described. The consequences for practice were discussed briefly, considering three historical periods. In the premendelian period with low N-levels gene mixing and horizontal resistance predominated; the latter still survives in some crops. In the R-gene period with medium N-levels vertical resistance was promoted, with evident successes and equally evident failures (boom-and-bust cycle, *Vertifolia* effect). New possibilities appeared, gene pyramiding, gene mixing (multiline varieties), gene deployment (spatial and temporal), and gene recycling. In the recent period with high N-levels horizontal resistance has been much advocated. There are evident successes, without and with breeder's intervention, but these are still few in number. Where natural pathosystems have been studied, mixed situations of horizontal and vertical resistance were found.

J. E. PARLEVLIET *(Afdeling Plantenveredeling, I.v.P., Landbouwhogeschool, Wageningen)*

Population-genetic aspects of host parasite systems

Resistance can be distinguished into true resistance and escape resistance. In the former, resistance mechanisms operate after intimate contact between the host tissue and the parasite has been established, and resistance is expressed by a reduced growth of the parasite in or on the host tissue. The latter functions before the parasite has made contact with the host tissue and is expressed by a reduced chance of such contacts. True resistance genes are assumed to act in a gene-for-gene way with virulence genes in the parasite. Genes governing escape resistances are supposed to function independently from genes in the parasite; there is no gene-for-gene interaction. Horizontal resistance, characterized by the absence of measurable host-parasite interactions when the resistance level of several host cultivars for a number of

parasite isolates is determined, is expected in case of escape resistances and polygenic true resistances. The latter type, operating on a gene-for-gene basis, gives a horizontal resistance pattern as simulation studies (PARLEVLIET & ZADOKS 1977) showed. These studies also showed that gene-for-gene systems offer advantages for both the host and the parasite compared with independent gene action. For the parasite, especially the obligate ones, the continued presence of sufficient host tissue is essential. Genes governing high levels of virulence are less fit to the parasite population than genes for intermediate levels of virulence, as they may endanger the existence of the host. The rabbit-myxomatosis relation in Australia is a good example. The present host and pathogen populations are moderately resistant and moderately virulent respectively, and co-exist, although a highly virulent strain was introduced in a highly susceptible host population many years ago.

The gene-for-gene system provides the basis for a stable equilibrium between host and parasite provided both populations are highly variable (MODE 1958; PERSON 1966). This stable equilibrium is obtained at a moderate level of the parasite population (PARLEVLIET & ZADOKS 1977). The more resistance and virulence genes participate in the host-parasite system the better this equilibrium is maintained.

In Agro-ecosystems the individual resistance genes are exposed one-by-one in various cultivars to a parasite population not anymore checked by the danger to extinct its host as man maintains the host. Use of polygenic and/or multigenic resistances, preferably incorporated into a system of integrated parasite control, may keep the parasites at bay more permanently.

MODE, C. J. (1958): A mathematical model for the co-evolution of obligate parasites and their hosts. *Evolution* 12: 158–165.

PARLEVLIET, J. E. & J. C. ZADOKS (1977): The integrated concept of disease resistance: A new view including horizontal and vertical resistance. *Euphytica* 26: 5–21.

PERSON, C. (1966): Genetic polymorphism in parasitic systems. *Nature* 212: 266.

A. H. EENINK *(Instituut voor de Veredeling van Tuinbouwgewassen (IVT), Wageningen)*

Crop protection by stable resistances

Stability of monogenic and polygenic resistance. Resistance is the cheapest way of crop protection. However, it is only valuable if its level is high enough, so that the damage threshold is not reached, and if it is stable.

Stability of resistance depends on many factors such as environment, genetic homogeneity of the host population, and parasitic ability (loci for pathogenicity, spore production, mutation frequency etc.). Stability may increase if more pathogenicity genes and/or complex pathogenicity genes are required to overcome the resistance. Both mono/oligogenic resistance and polygenic resistance can be stable or unstable. For instance many long lasting simply inherited resistances occur. If genes of a polygenic resistance have only an additive effect such a resistance may be unstable.

Stable monogenic or polygenic resistances can be obtained from 'old' populations already used for a certain period, and from 'new' populations such as primitive cultivated types and wild species.

Monogenic/oligogenic resistance. In certain cases recombination of 'old', already broken, genes results in stable resistances. For instance it was found that such a resistance in spinach to *Peronospora spinaciae* was governed by two closely linked genes of which one was overcome several years ago. In lettuce, recombination of 'old' genes also results in valuable resistance. From genetic research it appeared that in a limited number of lettuce varieties more than 15 different loci for resistance to *Bremia lactucae* occurred. Resistance genes differed significantly in their action as dominant and recessive genes, and duplicate and dominant and recessive complementary gene action were found.

In the IVT *Lactuca* gene bank various wild species with resistance were present. There is no guarantee at all that R-genes from wild species will give a more stable resistance than R-genes from cultivated types.

Polygenic resistance. Level of polygenic partial resistance may change because the host population is not in equilibrium. Fitness differences may occur between various resistances genotypes if in certain years of absence of the parasite no selection pressure is exerted, also resulting in a change of resistance level.

From our investigations on partial resistance to *Bremia lactucae* and *Myzus persicae* it appeared that both environment and physiological conditions of the host influence resistance level.

Resistance may increase e.g. as a result of transgression after intercrossing progenitors with a certain level of resistance.

Combination of absolute monogenic and partial polygenic resistance. Combination of monogenic and polygenic resistance may result in a valuable durable resistance. Combination of these two types of resistance can easily be carried out with a self-pollinator by backcrossing. With a cross pollinator first the level of partial resistance may be improved by sib-mating and then both types of resistance can be combined by backcrossing. In each backcross population 50% of plants occur without the absolute resistance (R-gene absent) and these plants can serve as a check on the level of partial resistance.

With vegetatively propagated crops about the same procedure can be used. Varieties of self-pollinators and 'clone crops' with both types of resistance can be maintained without any danger of occurrence of the '*Vertifolia* effect'. This effect will only occur with varieties of cross-pollinators with both resistances if partial polygenic resistance is fitness negative in absence of selection pressure by the parasite.

J. C. M O O I *(Instituut voor Plantenziektekundig Onderzoek, Wageningen)*

Race specific resistance and race non-specific resistance in potato to *Phytophthora infestans*

In potato two kinds of resistance to *Phytophthora infestans* are distinguished, race specific resistance and race non-specific resistance. Race specific resistance or vertical resistance is controlled by R genes, each of which protects the host against incompatible races of the fungus, but not against compatible ones. This kind of resistance is unstable, because after introduction of a resistant host compatible races may develop rapidly particularly if resistant hosts are grown on large areas. Even if the host carries more then one R gene resistance is broken down.

Race non-specific resistance or horizontal resistance is much more stable because it is active against all races of *P. infestans*. It is therefore preferred to race specific resistance in breeding for resistance to late blight, though it does not give complete protection against the disease. The inheritance of race non-specific resistance is believed to be polygenic. The degree of resistance can be assessed in laboratory experiments by measuring resistance to infection, resistance to invasion and resistance to sporulation. The possibility of studying the effect of genes, controlling race non-specific resistance, on these components can be discussed, especially in relation to the influence of time and amount of tuber production of the host on expression of resistance. Up till now no genes controlling race non-specific resistance have been identified.

I. W. B O U K E M A *(Instituut voor de Veredeling van Tuinbouwgewassen (IVT), Wageningen)*

Breeding for resistance to *Cladosporium fulvum* in tomato

Cladosporium fulvum causes leaf mould in tomato. More than 10 races of this fungus are known. Resistance is found in wild species, among them *Lycopersicon pimpinellifolium* and *L. peruvianum*. Most resistant varieties were attacked by new races 2 to 4 years after they had been introduced to commerce. The newest race, which can overcome the resistance of the gene Cf5, appeared in Belgium in 1976.

From the history of the development of new races in relation to resistance breeding it

appears that resistance of varieties containing only one resistance gene is apt to be broken soon. Combination of 2 genes of which the resistance of each separate gene has already been broken sometimes still gives resistance for a number of years. How stable a combination of 2 resistance genes which have not yet been broken will be, cannot be predicted from information available at the moment, but it seems that the resistance of this combination will last longer. Therefore it is recommended to incorporate in varieties at least 2 resistance genes, preferably 2 genes of which the resistance has not yet been overcome but in any case besides a 'new' gene an 'old' one.

At the IVT many sources of resistance are present. For about 7 sources it has been demonstrated that the resistance depends on different genes. Many are still under investigation.

It was also investigated whether in tomato horizontal resistance was present. A number of varieties appeared to have partial resistance to a mixture representing all existing races. This resistance was shown to depend on an incompletely dominant major gene, namely on the oldest known resistance gene Cf1. Selection for this resistance is difficult. Further, near-isogenic lines are made, each containing one of the known resistance genes, using Money-maker and Ailsa Craig as recurrent parents. These lines will facilitate the biochemical research on resistance mechanisms.

P. J. G. W. DE WIT *(Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen)*

Biochemical asoects of compatibility and incompatibility in the system *Cladosporium fulvum*-tomato

Infection of tomato plants by *Cladosporium fulvum* Cooke was studied using light and scanning electron microscopy. Races 4 and 5 of *C. fulvum* were used in this study, while near-isogenic lines of tomato carrying the Cf_4 gene (susceptible to race 4 and immune to race 5) and the Cf_5 gene (immune to race 4 and susceptible to race 5) served as differentials.

Fungal growth and degree of penetration were the same for both interactions. After penetration of fungal hyphae in the incompatible combination growth was arrested completely, while the plant reacted by deposition of callose and extracellular material. In the compatible combination fungal growth was abundant without damaging the plant during the first ten days after inoculation.

The following biochemical aspects involved in compatible and incompatible interactions: RNA-synthesis, change in soluble proteins, and production of phytoalexins, were studied.
RNA-synthesis. Three to four days after inoculation an increased incorporation of ^{3}H-orotic acid into m-RNA-like material was observed, especially in the incompatible interaction.
Change in soluble proteins. By using electrophoresis in 7% polyacrylamidegel, two new protein components appeared in the soluble protein fraction of the incompatible combinations four to five days after inoculation. Nine to ten days after inoculation, a third protein component appeared in the incompatible as well as in the compatible combinations.
Phytoalexins. Four to five days after inoculation, just after the resistant reaction was completed, there was a larger accumulation of fungitoxic material in the leaves of incompatible than in those of compatible combinations. The identity of this material is nog yet known, but rishitin is not involved. When sliced tomato fruits were inoculated with spores of *C. fulvum*, rishitin and two other not yet known compounds accumulated, whether or not the interaction was incompatible.