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A biochemical mechanism of natural resistance of apple to Venturia inaequalis

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According to Boone and Keitt (1957) susceptibility and resistance of commercial apple varieties to different physiological races of *Venturia inaequalis*, the incitant of apple scab, is governed by genetic properties of the host as well of the parasite. A gene-for-gene relationship (cf. Flor, 1955) in which seven genes of the parasite correspond to seven genes of the host was recognized by these authors. Fincham and Day (1963) further interpreted these results by assuming that alleles for avirulence (A_1–A_7) of each of the seven genes of the parasite correspond to alleles for resistance (R_1–R_7) of the host plant, in so far that a host plant carrying a resistance allele, e.g. R_1, is resistant only to parasites carrying the corresponding avirulence allele (A_1) and not to those having the allele a_1. Resistance being dominant, avirulence may be dominant as well but this has yet to be proved.

In the case of a resistant combination, a hypersensitive reaction resulting in necrotic spots ("fleck" reaction) is given by the host after penetration of the fungus under the cuticle. In the case of a susceptible combination the fungus equally penetrates under the cuticle but no necrosis follows (Nusbaum and Keitt, 1938).

Results of recent investigations (Raa, 1968a, b,) may throw some light on the nature of scab resistance which apparently is brought about by interaction of products from the avirulence allele of the parasite and from the corresponding resistance allele of the host.

From a cross of the resistant hybrid 'Antonovka' 34–20 and the susceptible commercial variety 'Golden Delicious' we used 42 seedlings which proved resistant to a monoconidial isolate P of *V. inaequalis*, and 70 seedlings which were susceptible.

A very simple test pointed to a clear biochemical distinction between the plants that were resistant or susceptible to strain P. Young as well as mature leaves of resistant and susceptible greenhouse grown seedlings were placed with their petioles in a dialyzed culture filtrate of strain P, grown for 20–30 days in shake culture on a glucose-casamino acids –mineral salts medium. Leaves of the resistant plants wilted within even in a 1 : 640 diluted culture filtrate. In contrast, leaves of the susceptible plants kept their turgor for at least 12 h. Further, if culture filtrate was added twice daily to the surface of the youngest leaves of the plants, necrosis resulted in resistant plants after 2–3 days and in susceptible plants after 7–8 days If young leaves were infiltrated in vacuo with undiluted dialyzed culture filtrate the resistant leaves became almost com-

pletely necrotic, whereas the susceptible ones showed only a few necrotic spots after 18 h at 20 °C. Of orchard trees young soft leaves to be used for the test.

These observations show that in vitro strain P excretes a substance into the medium which elicits a toxic reaction in a resistant, but not in a susceptible host plant. Assuming that the same happens in vivo the phytotoxic action in the resistant plant may evoke resistance in the following way. As shown earlier (Noveroske et al. 1964; Raa, 1968a and b), in leaf homogenates of both resistant and susceptible apple plants, the phloridzin present is subject to conversion by a polyphenoloxidase system and a β-glucosidase. As a consequence polymeric quinones are formed (Raa and Overeem, 1968). The intermediate oligomeric quinones were found strongly inhibitory to pectinase and other enzymes and they rapidly killed germinated spores of *V. inaequalis* (Raa, 1968 a and b). We assume that the local damage of leaf tissue brought about in the resistant plants by the toxin of this fungus, will give rise to the same chain of reactions leading to the killing of the mycelium present. In the case of susceptibility there is no cell collapse which could start this process.

Apple fruit does not contain phloridzin. Chlorogenic acid is the main phenol. Its oxidation products may kill the fungus as effectively as those of phloridzin do.

A closer study of the culture filtrate of strain P revealed that its phytotoxic action on the resistant apple plants was lost after boiling for 4 min. Chromatography of the dialyzed culture filtrate on a column of Sephadex G–100 yielded three toxic fractions, the elution volume/void volume ratios (Ve/Vo) being 1.0–1.1, 1.8–2.0 and 2.35–2.55. The former fraction was green-greyish, the latter two were colourless. Paper chromatography of a hydrolysate (6 N HCl, 115°C, 6 h) of each of the toxic fractions yielded 12 ninhydrin positive spots. The toxins may therefore be small proteins or peptides.

The observation that three compounds in the culture filtrate of strain P are toxic to the resistant plants may suggest that this strain possesses three avirulence alleles (A) which are responsible for the formation of these toxins. The resistant apple plants should then at least possess those three R alleles which correspond to the three A alleles. Unfortunately, however, we know neither the genotype of the apple seedlings with regard to R genes nor that of the *Venturia* strain P with regard to A genes.

Yet it is tempting to suggest that in general the avirulence alleles A_1–A_7 are responsible for the production of seven different compounds ("isotoxins") which are toxic only to those plants which possess the corresponding R allele. Experimental confirmation is strongly needed. The way in which in the plant these compounds might interact with products of the R alleles to give a hypersensitivity reaction is entirely unknown.

Gel filtration over Sephadex G–100 of a culture filtrate obtained by incubation (48 h in 0.5 % glucose) of a mixed population of conidia obtained from scab diseased orchard trees did not yield well-separated toxic fractions. All fractions with Ve/Vo of 1.0–2.1 as well as those of 2.4–3.0 were toxic to the resistant seedlings. The former were greyish-green, the latter colourless. This result may mean that more than three toxins are represented in the mixed population.

Pathogenicity to apple may be regarded as a common feature of *V. inaequalis* isolates and susceptibility as a common feature of apple plants. A specific pattern of resistance is then superimposed on this general susceptibility, since resistance is brought about by the possession of one or more specific resistance alleles in the host which must correspond to equally specific avirulence alleles of the invading parasite. Boone and Keitt (1957) assumed that avirulence and virulence were conditioned by two alleles of the

same gene, but in view of the present interpretation of the activity of the presumably dominant avirulence allele, namely the incitement of toxin production, it appears unnecessary to ascribe the function of virulence to the recessive allele of this gene.

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Samenvatting

Een biochemisch mechanisme voor de natuurlijke resistentie van appel tegen Venturia inaequalis

In cultuurfiltraten van *Venturia inaequalis*, de verwekker van appelschurft, werden stoffen gevonden die toxisch zijn voor blad van appelrassen welke tegen dit physio resistent zijn. Blad van vatbare rassen is ongevoelig. De resistentie komt tot stand doordat deze toxische werking in het blad de vorming veroorzaakt van fungicide oxydatie-producten van phloridzine. Op grond van deze waarnemingen kan een mogelijke interpretatie van de gen-om-gen relatie bij deze plant–parasietverhouding worden opgesteld.

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