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ABSTRACTS OF COMMUNICATIONS PRESENTED AT THE JOINT MEETING OF THE "NEDERLANDSE PLANTEZIEKTENKUNDIGE VERENIGING" AND THE "NEDERLANDSE KRING VOOR PLANTEVIROLOGIE" ON 24 MAY 1962, WAGENINGEN

L. M. BLACK[1] and ROY MARKHAM[2]: – Base-pairing in the ribonucleic acid of wound-tumor virus[3, 4]

Wound-tumor virus (WTV) was purified from root tumors of sweet clover (*Melilotus officinalis* (L.) Lam.) by a series of steps including both rate and quasi-equilibrium zonal density-gradient centrifugations. Sucrose or glycerol was used to provide the gradients of density in the centrifuge tubes. Destruction of virus during purification was reduced by operation at about 4 °C and maintenance of the virus in a glycine-$MgCl_2$ solution wherever possible. Nevertheless, the yield of virus from a 500 g sample of root tumors was only about 0.75 mg.

The RNA content of the virus is about 20% as indicated by determinations of the nucleic acid and protein content, ultraviolet absorption curve and specific volume of the virus. The virus is about 60 mμ across and has a sedimentation coefficient of about 500 S. If the RNA content of a virus particle is considered as a single molecule, its molecular weight would be about 1.5×10^7. At the time the preliminary results on WTV RNA were reported at the Netherlands Phytopathological Meeting in May 1962 an analysis of an inadequate amount of RNA failed to reveal base pairing. Later, two completely independent experiments each showed base pairing. In these experiments the analysis was performed by dispersing the purified WTV pellet, freed of sucrose or glycerol, in 1 N HCl. After standing overnight the protein was centrifuged out and the RNA in the supernatant hydrolized by heating to 100 °C for 1 hour. The cooled hydrolysate was evaporated to dryness at room temperature, dissolved in a small amount of 1 N HCl and spotted on paper. A solution of tertiary butanol, HCl and H_2O was used to develop the chromatogram and the four bases were eventually eluted from the spots in 0.1 N HCl and measured in the Cary Model 14 spectrophotometer.

In the first of the two later experiments, the ratios: adenine/uracil, guanine/cytosine and adenine + uracil/guanine + cytosine, were 0.96, 0.99 and 1.60 respectively; the corresponding ratios were 0.98, 1.01 and 1.56 in the second experiment. The total RNA represented by the isolated bases was 0.19 mg and 0.16 mg in the first and second experiments respectively.

It may be that the pairing of the WTV RNA bases is due to the nucleic acid forming a double-stranded RNA helix similar to that of the DNA helix. One or a very few such molecules in the WTV virus particle could provide continuous molecular strands within the long filaments observed by BILS & HALL[5] to have been extruded from virus particles in such preparations. The formation of such a complementary structure would also convey a definite protection against chance damage by enzymes and hydrolytic agents, and so might be essential for maintaining the integrity of such a large RNA.

J. H. FREITAG[6] – Cross protection of three strains of the aster yellows virus in the leafhopper and in the plant

Three California strains of aster yellows virus can be differentiated on the basis of symptoms developing on *Nicotiana rustica*, plantain, aster and periwinkle. *Nicotiana rustica* is the best of these differential hosts. Vein clearing, proliferation, virescence and phyllody are common symptoms of all three strains. The Severe strain is characterized by spindly growth, severe chlorosis, etiolation, elongation and proliferation of terminal shoots. The Dwarf strain can be distinguished by stunting and development of numerous small shoots bearing dwarfed

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green leaves resulting in a bunchy top effect. The Tulelake strain is readily differentiated by severe stunting, chlorosis, prominent bright yellow venation and leafrolling. The terminal shoots are severely dwarfed, thickened and develop swollen, wavy irregular veins.

Common plantain inoculated with Dwarf and Severe strains either simultaneously or first with one strain and then another usually developed symptoms of only one strain. However, some plants first showed symptoms of one and then also symptoms of the second strain. The Tulelake strain failed to protect against Dwarf in plantain. Plants inoculated the first week with Tulelake and challenged during a second week by Dwarf developed symptoms of Dwarf.

Nicotiana rustica plants infected with Dwarf were protected from Severe or Tulelake strains. Likewise plants infected with Severe were protected against Dwarf or Tulelake strains. However, the most interesting interaction resulted when plants were first infected with the Tulelake strain and then challenged a week later by either the Dwarf or Severe strain. In both instances the plants after having developed definite and pronounced vein yellowing indicating infection by the Tulelake strain recovered and the new growth was normal with few if any symptoms of either the Tulelake or the challenging strains. The flowers and leaves were normal in appearance suggesting that a mutual antagonism between the strains resulted in the development of apparently normal healthy plants.

When six-spotted leafhoppers, *Macrosteles fascifrons* (Stål), were first fed two weeks on a plantain infected with Dwarf and then two weeks on one infected with Severe strain, the great majority transmitted only Dwarf when they were transferred individually to a fresh plantain test plant daily. Likewise leafhoppers first fed on Severe strain failed to transmit the Dwarf strain and those fed on Dwarf and then Tulelake strain transmitted only Dwarf. However when leafhoppers were first fed two weeks on Tulelake and then two weeks on Dwarf, all of them transmitted Tulelake at first, but 16 of 24 tested later transmitted only Dwarf during the last part of their lives. The Tulelake strain in this test did not protect against the Dwarf strain, although the Dwarf strain gave protection against the Tulelake strain.

The results of experiments in which leafhoppers were fed alternately two days on Dwarf and two days on Severe for a period of 20 days, in an attempt to mix up the strains in the insect, also resulted in the great majority of insects transmitting only one strain. Leafhoppers that had acquired one strain were protected from transmitting the second strain. In other experiments when leafhoppers were first fed two days on one strain and then two weeks on a second strain, in an attempt to give them an unequal charge of the two strains, they again transmitted only one strain. The data obtained indicate that the strains cross protect against each other and that leafhoppers can only transmit one strain even though given an ample opportunity to acquire a second strain. About 95 per cent of the leafhoppers given feeding periods first on one strain and then on a second strain transmitted only one strain. They usually transmitted the first strain, but some individuals transmitted only the second strain. Only 5 per cent of the leafhoppers transmitted two strains. These individuals usually infected a high percentage of plants with one strain while intermittently transmitting the second strain to only a few plants.

Most individual leafhoppers tested proved to be equally efficient vectors of the three strains. A number of insects infected more than 90 per cent of the plants on which they fed. Several individuals transmitted the virus to as many as 50 consecutive test plants during the daily transfers.

LEONARD J. ALEXANDER[1], D. H. M. VAN SLOGTEREN[2], NEELTJE P. DE VOS[2] and D. Z. MAAT[3] – Comparison of certain strains of the tobacco mosaic virus in the Netherlands and the United States, and resistance to them

It was previously shown by MCRITCHIE and MCRITCHIE & ALEXANDER who worked in Ohio, U.S.A. that four pathogenic strains of the tobacco mosaic virus exist. These strains were differentiated by 1. differential hosts, 2. by cross-protection tests and 3. by the gel diffusion reaction. They also presented evidence to show that different strains of the virus adversely affected the yield of different tomato cultivars in different degrees. A high degree of resistance to all four pathogenic strains of the virus was isolated in the accession, P.I. 128650-6Y.IV-12-1-2 of *Lycopersicon peruvianum*. Other accessions of *L. peruvianum* and one breeding line of *L.*

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esculentum were used as the differential hosts. Since major efforts were under way to transfer the high degree of resistance possessed by 128650 to a commercial variety, it appeared desirable to compare strains of TMV in The Netherlands with the four strains found in Ohio.

In a separate paper, ALEXANDER presented evidence, based on host differentials, which indicated that Ohio pathogenic strains I, II, and IV exist in The Netherlands, but that there was little evidence, from the limited number of collections studied, that Ohio strain III existed. However, it was found that the differential hosts did not give as clear cut identification of The Netherlands strains of the virus as they did in Ohio, and that probably The Netherlands strains, while closely related to Ohio strains I, II and IV are not identical. Since MCRITCHIE and ALEXANDER had not performed cross-absorption tests with the four Ohio strains of TMV, it seemed desireable to make this type of study. Accordingly antisera were prepared from the four Ohio strains of the virus. When the titre of the antisera from the four strains was approximately equal the rabbits were bled and the antisera stored frozen.

When the four Ohio antisera and a very high titre Lisse antiserum were absorbed with healthy tobacco sap and cross-absorbed separately with the four Ohio antigens plus healthy sap and reacted with each of the four Ohio antigens it was found, 1. that all strains were related, 2. that Strains I and II were closely related, 3. that Strains III and IV were related but much more distantly, and 4. that Strain IV was more closely related to Strains I and II than was III.

When certain single lesion isolates from five tomato collections of TMV multiplied in Samsoun tobacco were reacted with the five antisera the results indicated 1. a close relationship to Strain IV, 2. less close relationship to Strains I and II and 3. very little relationship to Strain III. No distinction could be made between the isolates which caused a necrotic reaction on Necrotic White Burley and those which caused a systemic reaction. These results appear to be in accord with those secured by ALEXANDER with host differentials.

A comparison was made between TMV isolates secured from commercial brands of tobacco and these were also found to be more closely related to Strain IV, less closely related to Strains I and II and more distantly related to Strain III.

Again with the five antisera no differences could be detected between the collections of TMV which gave necrotic lesions on Necrotic White Burley, necrotic lesions followed by systemic reaction and systemic reaction.