

PRELIMINARY NOTE ON NUCLEIC ACID SYNTHESIS
IN TOBACCO PLANTS INFECTED
WITH TOBACCO MOSAIC VIRUS¹

*Met een samenvatting: Nucleïnezuur-synthese in tabaksplanten,
geïnfecteerd met tabaksmozaïekvirus*

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A number of viruses are difficult to extract from plants. If these viruses cause almost no symptoms on infected plants, it becomes difficult to be sure if a plant is healthy. Since synthesis of a virus means for the plant synthesis of a new substance it might be possible to measure changes in metabolism induced by infection. Most viruses are nucleoproteins and according to GIERER & SCHRAMM (1956) is for tobacco mosaic virus (TMV) the nucleic acid the most important part. Experiments were undertaken to investigate differences in nucleic acid (NA) metabolism between healthy and infected plants. As part of these experiments changes in NA content of homogenates that were kept at 5 °C for different periods of time were measured. Throughout the experiments tobacco plants (White Burley) infected with TMV were used. In all experiments full-grown systematically infected leaves were compared with the same amount of fresh weight of healthy leaves of the same physiological age. The leaves were homogenated with Tris buffer pH 7.0. After homogenating the pH was found to be 6.8 and decreased to 6.6 at the end of the experiment. NA was extracted according to SCHNEIDER (1945) and measured as P by KING's modification of FISKE & SUBBAROW (1932).

Figure 1 gives the account of a typical experiment. In this particular case leaves were harvested 30 days after the plants were infected. The homogenates

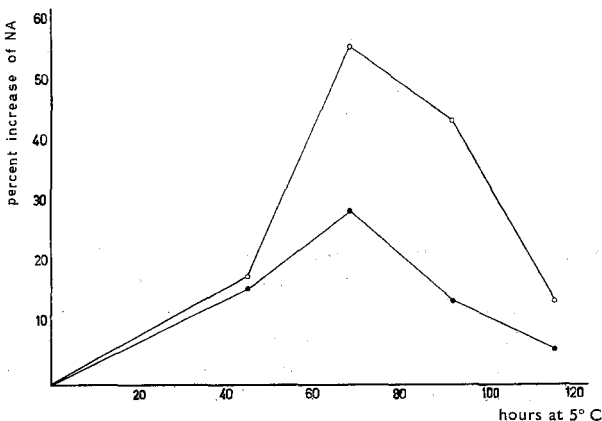


FIG. 1.

Percent increase of NA in homogenates of healthy (•—•) and infected (○—○) plants stored at 5 °C for several hours.

Procentuele toename van nucleïnezuur in homogenaten, die een bepaalde tijd bewaard werden bij 5 °C.

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were kept at 5 °C for several days and samples taken at appropriate intervals of time. During the first few days the NA content of the homogenates increases, the increase being greatest for the homogenates of infected plants. After about 65 hours the NA content decreases in both homogenates, remaining highest for infected plants.

The homogenates were examined more closely. They were divided in two parts. One was kept for 45 hours at 5 °C, the other was washed twice, taken up in buffer and kept for 45 hours at 5 °C. NA content of the different samples was measured. Table 1 gives an account of a typical experiment in which leaves were harvested after 30 days of infection. At the beginning of the experiment NA content of the unwashed homogenates was compared with NA content of the pellets and combined supernatants. The recovery of NA after washing was nearly 50 percent, being about the same for both homogenates. TMV is found in the supernatant. Since 42 percent of the NA of infected plants was TMV, the amount of NA left in the pellet of infected plants was smaller than for healthy plants. If the NA of infected plants was corrected for TMV the amount of NA in the pellet was relatively the same for both homogenates.

TABLE 1. Percent recovery of NA after washing the homogenates twice.
Percentage nucleïnezuur, teruggevonden nadat de homogenaten gewassen waren.

	Healthy / gezond	Infected / geïnfecteerd
Pellet + supernatant / <i>neerslag</i> + <i>bovenst. vloeistof</i>	52	54
Pellet / <i>neerslag</i>	29	20

Table 2 confirms former experiments in that increase of NA of unwashed homogenates stored at 5 °C is greatest for infected plants. NA content of washed homogenates increases as well. However, the increase of healthy plants is about four times that of unwashed homogenates, while the increase of infected plants is relatively the same as that of unwashed homogenates. So, the increase of NA in washed homogenates became greatest for homogenates of healthy plants.

TABLE 2. Percent increase of NA in unwashed and washed homogenates kept for 45 hours at 5 °C.
Procentuele toeneming van nucleïnezuur in gewassen en onbehandelde homogenaten, die 45 uur bij 5 °C bewaard waren.

	Healthy / gezond	Infected / geïnfecteerd
Unwashed / <i>onbehandeld</i>	11	23
Washed / <i>gewassen</i>	42	24

It seems likely that homogenates of healthy plants contain a substance that inhibits synthesis of NA; the substance is removed by washing. Homogenates of infected plants might not contain the inhibitory substance. That makes it unlikely that the substance is a simple ion. However, there are other possible explanations. More experiments are required to elucidate some of the problems. They may also throw light on the question if the enzyme that catalyzes the synthesis of NA is of the nature of the one found by GRUNBERG-MANAGO & OCHOA (1955) in *Azotobacter* that catalyzes synthesis of polynucleotides.

SAMENVATTING

Wanneer gezonde en zieke tabakbladeren, na met Tris buffer van een pH 7,0 te zijn gehomogeniseerd, enige dagen bij 5 °C worden bewaard, neemt de hoeveelheid nucleïnezuur gedurende de eerste dagen toe en vervolgens af. De toeneming is het grootst voor zieke planten. Wanneer de homogenaten gewassen worden met de buffer en vervolgens 45 uur bij 5 °C bewaard, wordt opnieuw een toeneming van nucleïnezuur gevonden, die voor zieke planten gelijk is aan die van het ongewassen homogenaat, maar voor gezonde ongeveer vier keer zo groot. Vermoedelijk is in homogenaten van gezonde planten een remstof aanwezig, die door wassen wordt verwijderd.

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