Changes in the Transcription Pattern of Flax Cotyledons after Inoculation with Flax Rust

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The rate of ^{32}P incorporation into RNA fractions of flax cotyledons (*Linum usitatissimum* L. var. Bison) was found to increase two- to three-fold by 48 h after inoculation with flax rust [*Melampsora lini* (Pers.) Lev., race no. 3]. This was accompanied by a change in the nucleotide composition of the newly transcribed sodium chloride-soluble RNA fraction. A comparison of the nucleotide composition of the RNA synthesized in the host-parasite complex at different stages of development indicated the preferential synthesis of one or more molecular species of RNA with a high A+U/G+C ratio at a relatively early stage of infection. Treatment of healthy plants with indol-3-ylacetic acid also resulted in a substantial stimulation in the rate of ^{32}P incorporation into RNA but this was not accompanied by a detectable change in the nucleotide ratios of the newly synthesized RNA. These results suggest that the synthesis of one or more additional RNA species or the augmented synthesis of certain species of RNA may be a specific phenomenon elicited by host-pathogen interaction.

It has been demonstrated that the development of obligate fungal parasites on their hosts is controlled by genes for resistance in the hosts and genes for virulence in the pathogens (Flor, 1956; Person, 1967). Host-parasite interactions that lead to susceptibility or resistance of a host plant to a given pathogen are therefore likely to take place at the level of transcription and translation at a very early stage of the infection process (Heitefuss, 1968).

The effects of rust infection on nucleic acid content and metabolism have been reviewed by Heitefuss (1966). Two- to three-fold increases in RNA content accompany the increases in respiration rate that occur in host-parasite combinations leading to susceptible host reactions. These increases are not usually recorded until 4-6 days after inoculation and their relevance to the host-pathogen interactions that trigger disease development is therefore uncertain. On the other hand, cytological and cytochemical observations (Person, 1960; Rohringer & Heitefuss, 1961; Whitney, Shaw & Naylor, 1962; Bhattacharya, Naylor & Shaw, 1965) have shown that the nuclei and nucleoli in rust-infected leaves increase in size and RNA and protein content by 48 h after inoculation. It is therefore possible that nucleic acid metabolism in the host is affected at a very early stage of host-parasite interaction. There is as yet, however, no published evidence for a qualitative change in the species of RNA synthesized in the host after infection.

We have undertaken a study of RNA synthesis in cotyledons of a susceptible variety of flax after inoculation with a virulent race of flax rust to try to elaborate the genetic control mechanism that underlies disease development. Reported below are experiments on the transcription pattern of healthy and inoculated flax cotyledons. These include (a) the rate of RNA synthesis as determined by the rate of ^{32}P incorporation into RNA and (b) qualitative changes in the RNA as judged by the nucleotide composition of the newly synthesized RNA.

Particular aspects of this work have been reported (Chakravorty & Shaw, 1970).

MATERIALS AND METHODS

Plant material. Flax plants were grown in growth chambers with an illumination of 800-1000 ft-candles for 16-18 h daily at 25°C. One week after the seeds were sown, plants were sprayed with a dilute suspension of uredospores in water (approx. 50 mg of spores/100 ml). The pots containing the inoculated plants were placed in a refrigerator at 4°C in a water-saturated atmosphere and the electricity was disconnected for 18 h, thus bringing the interior of the refrigerator up to room temperature. The pots were then returned to the growth chamber. The control plants were sprayed with water alone, but were otherwise treated identically.

Growth of flax rust. Infected cotyledons were sectioned and observed under the microscope. Growth of the rust on flax cotyledons during the first 48 h after inoculation was similar to that described by Littlefield & Aronson (1969).

Incubation procedure. At various periods after inoculation bundles of 100–150 plants were placed upright on Petri dishes so that the basal 0.5–1.0 cm of the hypocotyls was submerged and the cotyledons were 5–7 cm above the surface of the incubation medium. The medium contained 100–150 μCi of $\text{NaH}^{32}\text{PO}_4/\text{ml}$ (carrier-free; Atomic Energy of Canada Ltd., Ottawa, Ont., Canada) and 6 μg of gramicidin D/ml (Sigma Chemical Co., St Louis, Mo., U.S.A.) in 15 ml of water. During incubation the radioisotope reached the cotyledons by translocation through the hypocotyls. This procedure and the addition of the broad-spectrum antibiotic gramicidin D, were adopted as a necessary precaution against bacterial contamination (Tester & Dure, 1967; Chakravorty, 1969b). At the end of the incubation period (6 h at 25°C), the cotyledons were detached, quickly frozen and stored at –20°C until required. The hypocotyls were discarded.

Freshly collected flax-rust uredospores (about 150 mg fresh wt.) were hydrated by stirring in glass-distilled water for 15 min at 0°C and washed with approx. 200 ml of glass-distilled water by centrifugation at 10000g for 15 min in a Sorvall RC2-B refrigerated centrifuge. The spores were then resuspended in 10 ml of glass-distilled water. [^{32}P]Phosphate and gramicidin D were added to the suspension to give a final concentration of 25–30 $\mu\text{Ci}/\text{ml}$ and 6 $\mu\text{g}/\text{ml}$ respectively and the mixture was incubated for 6 h at 25°C. The spores were collected by centrifugation at 600g for 10 min and washed twice with 100 ml of buffered sucrose (0.25M-sucrose in 5 mM-tris-succinate buffer, pH 7.2) by centrifugation as described above. They were then frozen and stored. RNA was extracted from these spores with 10g of flax cotyledons as non-radioactive carrier.

Under the above experimental conditions less than 5% of the uredospores actually germinated and produced only short germ tubes (5–10 μm). Nevertheless, these hydrated spores were found to synthesize a variety of RNA molecules, including ribosomal RNA.

Isolation of ribosomes. Ribosomes were isolated from the frozen tissue as described by Waters & Dure (1966). The ribosomal pellets were resuspended in 0.1M-tris-succinate buffer, pH 7.8. An equal volume of 1% sodium dodecyl sulphate was added to this and the mixture was incubated for 5 min at 37°C. RNA was isolated from this as described below.

Extraction of RNA. All operations were performed at 0–4°C unless otherwise stated. Extraction and purification of RNA from flax cotyledons, rust uredospores and resuspended ribosomal preparations were carried out essentially as described by Chakravorty (1969a,b). About 10g of frozen cotyledons was ground in a mortar with 5g of pre-chilled purified sea sand (Merck and Co. Inc., Rahway, N.J., U.S.A.) and extracted with 100 ml of a solution containing 0.1M-tris-succinate buffer, pH 7.8, 0.6% diethylpyrocarbonate (Eastman Kodak, Rochester, N.Y., U.S.A.; see Solymosy, Fedorcsak, Gulyas, Farkas & Ehrenberg, 1968) and 1% sodium dodecyl sulphate. The homogenate was filtered through six layers of gauze and the filtrate was incubated at 37°C for 5 min. An equal volume of redistilled phenol, which had been previously equilibrated with 0.05M-tris-succinate buffer, pH 7.8, and containing 0.1% of 8-hydroxyquinoline, was added to the filtrate. The mixture was vigorously shaken at room temperature for 30 min, and the aqueous layer

was separated from the phenol layer and the interphase by centrifugation for 1 h at 10000g. The phenol layer and the interphase were treated with an equal volume of 0.05M-tris-succinate buffer, pH 7.8, containing 1% sodium dodecyl sulphate, shaken and centrifuged as described above. RNA was precipitated from the combined aqueous phases with 2 vol. of ethanol at –18°C overnight.

RNA preparations were purified by precipitation with cetyltrimethylammonium bromide (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.) by the method of Ralph & Bellamy (1964).

Fractionation of RNA. (a) Differential salt precipitation. Purified RNA preparations were dissolved in 0.01M-tris-succinate buffer, pH 7.8, containing 5 mM-EDTA and 4 μg of polyvinyl sulphate (Seiko-Kagaku Yakuhi, Osaka, Japan)/ml. Any insoluble material was removed by centrifugation. The clear solution was made to 1.2M with NaCl. This was frozen and stored overnight at –20°C, thawed at approx. 5°C and the flocculent precipitate containing high-molecular-weight RNA was collected by centrifugation at 10000g for 20 min. The precipitate was washed once with 1 ml of 1M-NaCl and the supernatant fractions were combined. The pellet was dissolved in 0.05M-tris-succinate buffer, pH 7.8, containing 5 mM-EDTA. Both the NaCl-soluble and NaCl-precipitated RNA fractions thus obtained were dialysed overnight against 3 litres of 0.025M-tris-succinate buffer, pH 7.8, containing 2 mM-EDTA. The buffer solution was changed three times during the dialysis.

(b) Sucrose-density-gradient centrifugation. RNA preparations (NaCl-precipitated fractions) were sedimented on 6–22% (w/v) linear sucrose gradients. Besides sucrose (Ultra-pure, ribonuclease-free density-gradient grade; Schwarz BioResearch, Orangeburg, N.Y., U.S.A.), the gradient solutions also contained 0.05M-tris-succinate buffer, pH 7.8, 1 mM-EDTA and 1 mM-NaCl. The gradients were centrifuged at 26000 rev./min in a Spinco SW 27 rotor for 17 h at 4°C. The gradients were fractionated and the samples for the measurement of radioactivity and RNA content were prepared exactly as described by Chakravorty (1969a).

Nucleotide-composition analysis. RNA fractions after dialysis were precipitated with an equal volume of 10% (w/v) trichloroacetic acid and washed three or four times with 5% (w/v) trichloroacetic acid by centrifugation at 0°C. The precipitate was dissolved in 0.5M-KOH and hydrolysed for 16 h at 37°C. The solution was then neutralized in the cold by adding 2.5M-HClO₄ and the insoluble KClO₄ was centrifuged off. With the NaCl-soluble RNA fraction, the hydrolysate was first acidified to pH 2.5, any insoluble material was centrifuged off and the solution was then neutralized as described above. The nucleoside monophosphates were separated by step-wise elution with formic acid and ammonium formate from columns of Dowex 1 (formate form), essentially by the method of Hurlbert, Schmitz, Brumm & Potter (1954). Nucleotide composition was determined from the relative amounts of radioactivity in the nucleotide peaks.

RESULTS

Effect of inoculation on the rate of [^{32}P]P_i incorporation into RNA. Batches of inoculated and uninoculated (i.e. healthy) plants (1, 2, 4 and 7 days after

Fig. 1. Effect of inoculation on the specific radioactivity of RNA isolated from flax cotyledons. Healthy and inoculated plants at the specified periods after inoculation were incubated with $100 \mu\text{Ci}$ of $\text{H}_3^{32}\text{PO}_4/\text{ml}$ for 6 h before the isolation of RNA. (a) NaCl-precipitated RNA; (b) NaCl-soluble RNA. ●, RNA from healthy cotyledons; ○, RNA from inoculated cotyledons.

inoculation) were incubated with $[\text{P}^{32}]\text{P}_i$ for 6 h. The RNA isolated from these was fractionated into sodium chloride-precipitated and -soluble fractions and the specific radioactivities (c.p.m./ E_{260} unit) of these were determined. The results are presented in Fig. 1. The results for both the salt-precipitated RNA fraction (Fig. 1a) and for the salt-soluble RNA fraction (Fig. 1b) show that the specific radioactivities of RNA isolated from cotyledons 2, 4 and 7 days after inoculation are higher than those of the RNA fractions from the corresponding healthy control. The increase in the specific radioactivity reaches its maximum at 2 days after inoculation and is then followed by an apparent decline. It has been demonstrated that the RNA content of inoculated cotyledons (mg of RNA/g fresh wt.) remains unchanged during the initial stages of infection (0–4 days after inoculation), after which there is a substantial increase, so that 8 days after inoculation the infected/healthy ratio of RNA content rises to 2.6 (Hamilton, 1969). In healthy cotyledons of comparable age there is no significant change in the RNA content. When the increased RNA content of infected cotyledons is taken into consideration, it appears that the actual rate of $[\text{P}^{32}]\text{P}_i$ incorporation into RNA at 7 days after inoculation may be as high as it is at 2 days. The appreciable decrease in

the rate of ^{32}P incorporation into both salt-precipitated and -soluble RNA fractions at 4 days after inoculation (Fig. 1) cannot be readily explained.

The sucrose-density-gradient profiles of the salt-precipitated RNA fraction from (a) healthy and inoculated cotyledons harvested at (b) 2, (c) 4 and (d) 7 days after inoculation are presented in Fig. 2. A comparison of the E_{260} and radioactivity profiles in Figs. 2(a) and 2(b) reveals that the specific radioactivities of both 28S and 18S RNA from inoculated cotyledons at 2 days after inoculation are significantly higher than those of RNA from the healthy controls. The specific radioactivities of 28S and 18S RNA in Figs. 2(c) and 2(d) are also higher than those of the control (Fig. 2a). In these experiments, RNA was isolated from the cotyledons directly, without prior cell fractionation. In other experiments ribosomes were isolated from healthy and inoculated cotyledons. The labelling patterns of the 28S and 18S RNA isolated from the ribosomes were identical with those obtained from the unfractionated tissue.

Effect of indol-3-ylacetic acid on the rate of ^{32}P incorporation into RNA. It has been reported that rust-infected plant tissues generally have a higher auxin content than the corresponding healthy tissue (Daly & Inman, 1958; Pilet, 1960; Sequeira, 1963). Since auxin has been shown to cause a substantial stimulation in the rate of RNA synthesis (Key & Shannon, 1964; Roychoudhury, Dutta & Sen, 1965) the following experiment was designed to examine the possibility that the observed increase in the rate of ^{32}P incorporation into the inoculated plants (Fig. 1) may be an auxin-mediated phenomenon.

Healthy and inoculated plants (48 h after inoculation) were pre-incubated with $10 \mu\text{g}$ of indolylacetic acid/ml and $6 \mu\text{g}$ of gramicidin D/ml for 2 h, followed by incubation with $[\text{P}^{32}]\text{P}_i$ for 5 h in the continued presence of indolylacetic acid. The control plants were pre-incubated and incubated under identical conditions but without indolylacetic acid. The specific radioactivities of salt-precipitated and salt-soluble RNA fractions are presented in Table 1. The results clearly indicate that there is a considerable increase in the specific radioactivities of both these RNA fractions due to treatment with indolylacetic acid. It should also be noted that the specific radioactivities of RNA from indolylacetic acid-treated cotyledons are as high as those of inoculated cotyledons. However, when inoculated cotyledons were treated with indolylacetic acid before incubation with $[\text{P}^{32}]\text{P}_i$, no further increase in specific radioactivity occurred.

The total uptake of the isotope by healthy and inoculated plants (total c.p.m. in the 17000g-supernatant fraction) was not significantly different. In the indolylacetic acid-treated cotyledons, the

Fig. 2. Sucrose-density-gradient profiles of the NaCl-precipitated RNA fraction from healthy and inoculated cotyledons. Experimental details are described in the Materials and Methods section. (a) RNA from healthy cotyledons; (b), (c) and (d) RNA from cotyledons 2, 4 and 7 days after inoculation. ●, E_{260} ; ○, radioactivity (c.p.m.). Fraction size is 1.2 ml. The direction of sedimentation is from right to left.

total uptake was consistently found to be 30–40% higher than in the untreated control. However, the increase in the specific radioactivity of RNA in the indolylacetic acid-treated cotyledons is much higher than the increase in total uptake.

Nucleotide composition of the newly transcribed RNA. The possibility of a qualitative change in the newly synthesized RNA was explored by determining the nucleotide composition of RNA based on the radioactivity of the fractionated nucleotides. Both salt-precipitated and salt-soluble RNA fractions from healthy, inoculated and indolylacetic acid-treated cotyledons were analysed. The RNA

from rust uredospores which had been hydrated in water was also included in these experiments for the sake of comparison. The results are presented in Table 2. There is no significant change in the nucleotide composition of the salt-precipitated RNA (containing 28S and 18S RNA: Fig. 2) after either inoculation or treatment with indolylacetic acid. It is also noteworthy that the A+U/G+C ratio of this RNA fraction is, within experimental error, the same in the inoculated cotyledons at 7 days after inoculation as in the healthy controls, and is different from that of the corresponding RNA fraction from germinating rust spores.

Table 1. *Effect of treatment with indolylacetic acid on the incorporation of RNA into [³²P]P_i by healthy and inoculated cotyledons*

Healthy and infected plants (48 h after inoculation) were treated with 10 µg of indolylacetic acid/ml for 2 h followed by incubation with [³²P]P_i for 5 h in the continued presence of indolylacetic acid. For determination of the total uptake of ³²P, 10 g of cotyledons was homogenized as described in the Materials and Methods section and the homogenate was centrifuged at 17 000g for 30 min. A sample of the supernatant fraction was used for the measurement of radioactivity.

	Sp. radioactivity (c.p.m./E ₂₆₀ unit)	10 ⁻⁷ × Total uptake (c.p.m./10 g of cotyledons)
NaCl-precipitated RNA		
Healthy	6400	5.0
Inoculated	15200	5.6
Healthy, +indolylacetic acid	17800	7.2
Inoculated, +indolylacetic acid	16000	6.9
NaCl-soluble RNA		
Healthy	4200	—
Inoculated	10300	—
Healthy, +indolylacetic acid	12000	—
Inoculated, +indolylacetic acid	11000	—

Table 2. *Comparison of the nucleotide composition of newly synthesized RNA from healthy, inoculated and indolylacetic acid-treated flax cotyledons and germinating flax rust uredospores*

Nucleotide ratios were estimated from the distribution of radioactivity among the nucleoside (2'- and 3'-) monophosphates as described in the Materials and Methods section. Each value represents the mean of three separate analyses, ±S.D.

	Percentage of total radioactivity in				A+U G+C ratio
	CMP	AMP	UMP	GMP	
NaCl-precipitated RNA					
Healthy	26.9 ± 0.3	21.7 ± 0.5	21.9 ± 0.6	29.5 ± 0.8	0.77
Inoculated (2 days)	27.3 ± 0.5	21.6 ± 0.5	22.8 ± 0.7	28.3 ± 1.7	0.78
Inoculated (7 days)	26.9 ± 0.7	21.5 ± 0.8	22.3 ± 0.4	29.3 ± 1.3	0.78
Healthy, +indolylacetic acid	27.8 ± 0.9	21.3 ± 0.6	21.8 ± 0.5	29.1 ± 0.3	0.75
Inoculated (2 days), +indolylacetic acid	26.8 ± 1.0	22.1 ± 0.6	22.4 ± 0.6	28.7 ± 0.7	0.80
Rust spores	23.7 ± 1.0	23.4 ± 0.5	25.0 ± 0.5	27.9 ± 0.9	0.93
NaCl-soluble RNA					
Healthy	30.1 ± 0.5	18.9 ± 0.8	19.6 ± 0.7	31.4 ± 1.7	0.62
Inoculated (2 days)	27.9 ± 0.3	22.6 ± 0.7	24.1 ± 0.4	25.4 ± 1.1	0.87
Inoculated (7 days)	28.3 ± 1.1	21.6 ± 0.6	23.2 ± 0.5	26.9 ± 1.5	0.79
Healthy, +indolylacetic acid	30.7 ± 1.0	18.9 ± 0.8	18.5 ± 0.5	31.9 ± 0.7	0.60
Inoculated (2 days), +indolylacetic acid	27.4 ± 0.7	22.4 ± 0.7	23.1 ± 0.4	27.1 ± 0.7	0.83
Rust spores	31.4 ± 0.7	18.1 ± 0.4	22.3 ± 0.7	28.2 ± 1.3	0.68

A comparison of the nucleotide composition of the salt-soluble RNA fractions in Table 2 reveals a significant change in the A+U/G+C ratio in the RNA at 2 days after inoculation. This change is due to an increase in the content of AMP and UMP and a corresponding decrease in CMP and GMP in the RNA of the inoculated cotyledons. The A+U/G+C ratio of this RNA fraction from cotyledons 2 days after inoculation is higher than the corresponding ratio for cotyledons treated with indolylacetic acid and also for germinating rust spores. This change in the nucleotide composition was not detected in cotyledons 1 day after inoculation (results not shown in Table 2). The above results suggest that AMP- and UMP-rich RNA molecules

are synthesized in the cotyledons 2 days after inoculation. Similar RNA molecules are either not synthesized in the healthy and indolylacetic acid-treated cotyledons, or are synthesized at considerably lower rates.

DISCUSSION

The experiments described above have revealed two distinct changes in the transcription pattern of flax cotyledons 2 days after inoculation with flax rust uredospores. These are (a) an increase in the rate of ³²P incorporation into RNA and (b) a measurable change in the nucleotide composition of a fraction of newly synthesized RNA.

The results presented in Fig. 1 have shown that, at 2 days after inoculation, there are substantial increases in the specific radioactivities of both sodium chloride-precipitated RNA, which contains high-molecular-weight (28S and 18S) RNA, and the sodium chloride-soluble RNA, which presumably contains low-molecular-weight RNA (tRNA, 5S rRNA and heterogeneous DNA-like RNA). When the salt-precipitated RNA was resolved into 28S and 18S RNA by sucrose-density-gradient centrifugation, the specific radioactivities of both these RNA fractions were found to have increased proportionally at 2 days after inoculation (Figs. 2*a* and 2*b*). It therefore appears that one of the earliest observable changes in the transcription pattern after inoculation is a stimulation in the synthesis of most, if not all, classes of RNA molecules.

The total RNA content of rust-infected plant tissues has been shown to be significantly higher than that of the healthy tissues. This quantitative difference has been demonstrated at 8–10 days after inoculation (Quick & Shaw, 1964; Hamilton, 1969), and is due largely to the direct contribution of fungus RNA, but partly to an increase in host RNA. The remarkable increase in the rate of rRNA synthesis as early as 2 days after inoculation (Fig. 2*b*) partly accounts for the greater accumulation of RNA in the diseased tissue in the later stages of the disease. Heitefuss (1968) reported that in a wheat variety exhibiting temperature-sensitive resistance to stem rust, increased susceptibility induced by higher temperatures is correlated with a large increase in RNA content. This phenomenon does not occur at lower temperatures, at which the plants show resistance. Increases in RNA content in infected tissue thus appear to be a general feature of host-parasite combinations giving susceptible reactions. Although the significance of this phenomenon in disease development is not clear, the following observations indicate that acceleration in the rate of RNA synthesis in inoculated flax cotyledons is initially a parasitically induced activity of the host cells and not merely the result of a substantial direct contribution by the growing fungus. First, at 48 h after inoculation the growth of the rust fungus on the host is extremely limited (Littlefield & Aronson, 1969). In addition it has been observed that the increase in the rate of RNA synthesis is not proportional to the intensity of infection. Secondly, the remarkable similarity in the nucleotide compositions of the salt-precipitated RNA (comprising 75–80% of the total extractable RNA) from healthy and inoculated cotyledons (2 days after inoculation) suggests that the direct contribution of the fungus to the total newly synthesized RNA is small in the early stages of infection. Thirdly, chemical determinations of RNA concentrations in

infected leaves do not reveal increases in total RNA until 4–6 days after inoculation (Hamilton, 1969).

The accelerated synthesis in the spectrum of RNA molecules in the inoculated cotyledons may be a result of (a) an increase in the RNA polymerase activity, (b) derepression of novel genes or (c) both increased RNA polymerase and the derepression of a part of the genome. Table 2 shows that the A+U/G+C ratio of the salt-soluble RNA fraction from cotyledons 2 days after inoculation is higher than the corresponding RNA fractions from both healthy cotyledons and germinating rust spores. This result suggests production of new species of RNA molecules or preferential synthesis of those RNA species with high AMP and UMP contents in response to inoculation. It has not been possible to associate these RNA molecules with any new cellular functions. It is conceivable, however, that many major changes occur during disease development for which RNA synthesis is necessary (Heitefuss, 1966). Consistent with this contention is the appearance of new proteins and enzymes in the inoculated plants (Staples & Stahmann, 1963, 1964) and also the finding that although a low concentration of actinomycin D (2.5 µg/ml) does not inhibit uredospore germination, it does inhibit the development of the rust in flax cotyledons, but only if the plants are treated with the drug within the first 5 or 6 h after inoculation (Shaw, 1967). Treatment of the plants with actinomycin D later than 6 h after inoculation has no effect on the growth and development of the rust, indicating that DNA-dependent RNA synthesis in the host at a very early stage is a critical and obligatory step in the growth and development of the rust fungus on its host.

The results presented in Table 1 indicate that inoculation with rust spores and treatment with indolylacetic acid result in a similar stimulation in the rate of RNA synthesis in flax cotyledons. Several workers have reported an increase in the auxin content of plant tissues infected by a variety of micro-organisms, including the rusts (Daly & Inman, 1958; Pilet, 1960; Sequeira, 1963). Antonelli & Daly (1966) observed a striking increase in the rate of decarboxylation of indolylacetic acid in a resistant host-parasite combination. It has been speculated that resistance may be due to the prevention of indolylacetic acid-induced RNA synthesis (Heitefuss, 1968). Although it is possible that an increased content of indolylacetic acid may result in a higher rate of RNA synthesis, it is open to question whether the observed stimulation in RNA synthesis in the cotyledons 2 days after inoculation (Fig. 1) is mediated by indolylacetic acid or not. In rust-infected wheat an increase in indolylacetic acid content is detectable only 5 days after inoculation (Shaw, 1967). No comparable results are available for rust-infected flax.

The results presented in Table 2 reveal that acceleration of RNA synthesis caused by indolyl-acetic acid is not accompanied by a demonstrable change in the A+U/G+C ratio of the newly transcribed RNA. The observed change in the A+U/G+C ratio of RNA at 2 days after inoculation thus appears to be a specific result of host-pathogen interaction. These results suggest, but do not conclusively prove, that the initiation of disease development may be triggered by the synthesis of new types of RNA molecules. It must also be pointed out that the changes in the transcription pattern described here may only reflect a general effect of rust infection that may have little or no relevance to the primary host-pathogen interactions that determine resistance or susceptibility. The synthesis of only a few mRNA molecules may be all that is necessary to initiate a chain of events that ultimately determines resistance or susceptibility (Heitefuss, 1968). The appearance of a relatively small number of molecules of RNA, however different, could not possibly be detected by the techniques used in this work. Further studies using a more sensitive RNA-DNA hybridization technique should ascertain which of these alternative possibilities is correct.

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