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EFFECT OF BROWN RUST ON DRY MATTER COMPOSITION AND NUCLEIC ACID CONTENTS OF WHEAT

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Abstract

Changes in dry matter composition and nucleic acid content of wheat leaves after inoculation with leaf rust fungus *Puccinia recondita* were studied. Dry weight per unit fresh weight increased both in rust infected and fungicide-treated leaves. Fungicidal treatment had little effect on dry weight basis. No significant changes occurred in plant moisture content (PMC) whereas a substantial increase in dry matter content was observed in infected and fungicide-treated leaves. Increase in RNA and DNA contents were evident 2 days after inoculation (DAI) and remained high throughout the period of investigation.

Introduction

Changes in dry matter content and nucleic acid metabolism after infection with black rust of wheat have been reported (Lanetskii, 1982; Bhattacharya & Shaw, 1968; Heitefuss, 1968; Bhattacharya *et al.*, 1965). Increase in RNA level of the infected host tissue have been observed in many host: parasite systems with a little or no change in DNA contents. However, these changes in most of the cases were observed for a maximum of 15-20 days (Heitefuss, 1968; Bhattacharya *et al.*, 1965; Rohringer & Heitefuss, 1961), and changes upto maturity or ear formation were not studied. The present investigation deals with the changes in dry weight and nucleic acid contents in a susceptible variety of wheat after inoculation with brown rust fungus, *Puccinia recondita.*

Materials and Methods

One month old seedlings of wheat cv. Pak-70 were inoculated with a suspension of uredospores of *Puccinia recondita* (250 spores/drop) by hypodermic and foliar inoculations. The inoculum was about a year old and had more than 80% viability when tested in moist chambers at 20°C. The seedlings were grown under field conditons at a temperature ranging between 13° – 27°C and average humidity of 39%. In another set wheat seedlings were sprayed with a systemic fungicide INDAR (BT or 4-n-butyl-1,2,4-triazole) @ 0.5 liter/ha., when traces of rust pustules were observed on leaves followed by another spray after 15 days. Control plants were grown at a distance from the inoculated plants to minimize the dissemination of spores from the infected plants. Leaves from healthy, rust infected and fungicide-treated plants were sampled at 3-7 days interval for analysis.

One cm^2 leaf area was removed from the base of the leaf tissue, and inter-relationship between fresh and dry weight of healthy, infected and fungicide-treated plants were calculated by the method used by Shaw & Colotelo (1961). Plant moisture content (PMC) was determined by the relative difference in fresh and dry weight of samples and is expressed as percentage of fresh weight. RNA and DNA were estimated by the methods proposed by Schmidt & Thannhauser (1945) and Schneider (1945).

Results

Dry weight and moisture content changes

A paired 't' test of the data showed that the percentage of rust in rust infected plants was significant ($p < 0.001$) and 60% of the total increase in dry weight in infected

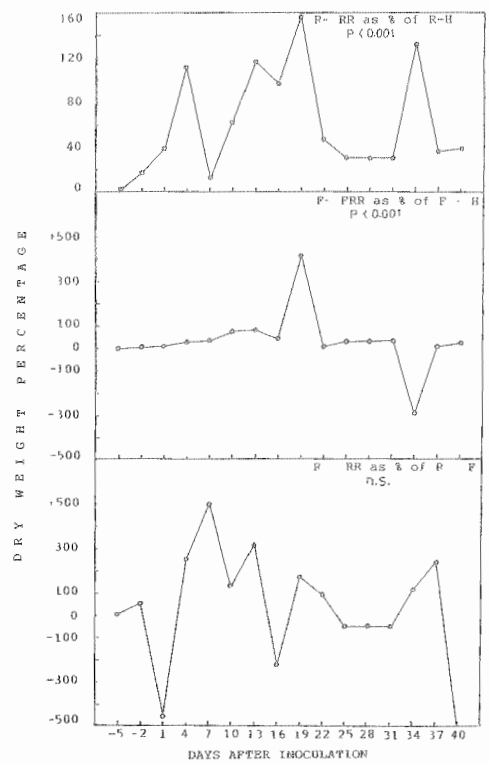

Fig.1. Comparison of dry weight of healthy (H), rust infected (R) and fungicide-treated (F) leaves of wheat.
RR – Rust removed from infected plants.
FRR – Rust removed from fungicide-treated plants.

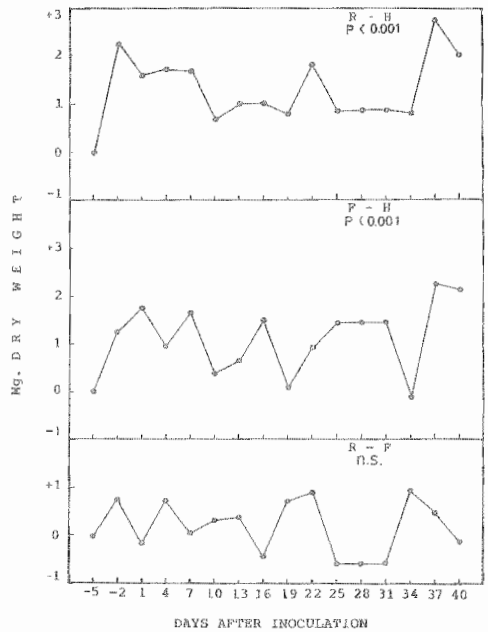

Fig.2. Dry weight differences between healthy (H), rust infected (R) and fungicide-treated (F) leaves.

plants could be attributed to the weight of the fungus itself. The application of the fungicide showed little effect on the rust development and only 32% of the dry weight increase could be attributed to the weight of the fungus (Fig. 1). Another paired 't' test showed that there was no significant difference in the dry weight between rust infected and fungicide-treated leaves since only a difference of 0.181 mg. cm^{-2} in dry weight was observed between rust infected and fungicide-treated samples, whereas a difference of 1.307 mg. cm^{-2} was observed between rust infected and healthy leaves and 1.126 mg. cm^{-2} between fungicide-treated and healthy leaf samples. The pattern of difference in dry weight between rust infected and healthy samples and between fungicide-treated and healthy samples was more or less similar except that there was a slight increase in dry weight in infected plants as compared to fungicide-treated ones (Fig. 2).

Moisture content in healthy, rust infected and fungicide-treated leaf samples was 72.05, 71.59 and 69.72%, however, on different days after inoculation, the PMC was lower in infected and fungicide-treated samples than that of healthy ones. The dry matter composition remained high in rust infected and fungicide-treated tissues over healthy ones throughout the investigation period.

Dry weight per unit fresh weight gradually increased and from 0 days after inoculation upto the stage of maturity (31DAI) it increased by 44% in healthy, 122% in rust

infected and 105% in fungicide-treated leaves. However, a gradual decrease was observed in all the three treatments after the plant attained maturity. This decrease was 54% in rust infected and 42% in fungicide-treated samples over control 40DAI (Fig. 3).

Nucleic acid changes

Increase in the RNA content of rust infected leaves was evident 2 DAI and was higher than that of healthy leaves upto 30 DAI (Fig. 4). The RNA content increased with an increase in rust infection and 30 DAI it was three times higher than that of uninoculated control with sharp decline at subsequent interval (Table 1.). The application of fungicide had no effect on the RNA content and followed that same pattern as that of healthy

Table 1. Changes in RNA content of wheat leaves after infection with *Puccinia recondita* and treatment with fungicide.

Days after Inoculation	Total RNA (μg. / mg. fresh weight)				
	Healthy	Infected	Fungicide Treated	% increase over healthy control	
	(H)	(R)	(FT)	Infected	Fungicide treated
2	0.5712 ± 0.0064	0.6264 ± 0.183	0.5850 ± 0.0325	109.66	102.41
9	0.4317 ± 0.0098	0.5440 ± 0.0470	0.5080 ± 0.0000	126.01	117.67
16	0.5424 ± 0.0151	0.5936 ± 0.0000	0.4949 ± 0.0130	109.44	91.24
23	0.5576 ± 0.0834	0.5973 ± 0.0074	0.5670 ± 0.0477	107.12	101.68
30	0.5626 ± 0.0026	1.7040 ± 0.0000	0.6586 ± 0.0607	302.88	117.06
37	0.5653 ± 0.0053	0.7346 ± 0.1406	0.6280 ± 0.0700	129.95	111.09

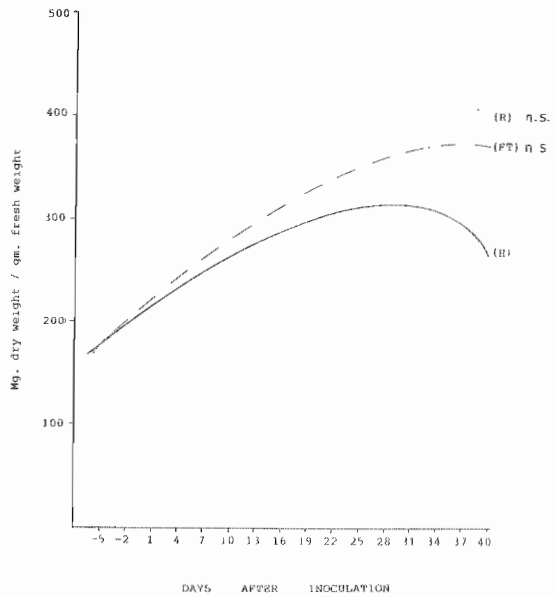

Fig.3. Dry weight per unit fresh weight in healthy (H), rust infected (R) and fungicide-treated (FT) wheat leaves.

samples. DNA content was more in rust infected than healthy plants throughout the period of investigation (Fig. 5) and a similar increase in DNA content of fungicide-treated plant was observed (Table 2).

Discussion

Dry weight of rust infected and fungicide-treated leaf samples was highly significant, but the net difference in dry weight cannot be attributed to rust infection alone, since rubbing the leaves will only remove the rust pustules and not the haustoria from within the cells of the host (Shaw & Colotelo, 1961). However, a part of the difference in dry weight between different samples can be attributed to the increase in dry matter content in the host tissue. This increase may vary from 25-50% of the total gain in weight against 25% or less as reported by Shaw & Colotelo (1961) and Shaw & Samborski (1956).

The increase in dry matter content in infected plant may be related to the induced synthesis of metabolites. There are reports of the increase in starch (Mirocha & Zaki, 1966), soluble carbohydrates (Goodman *et al.*, 1965), nitrogenous fraction (Jensen, 1969), minerals (Sasikumaran *et al.*, 1979) and phenolic compounds (Jung Yie & Ju Ying, 1981; Galil *et al.*, 1981) in plants infected by various pathogens. Increase in dry weight per unit fresh weight in the rust infected plants was however not as high as repor-

Table 2. Changes in DNA content of wheat leaves after infection with *Puccinia recondita* and treatment with fungicide.

Days after Inoculation	Total DNA (μg. / mg. fresh weight)				
	Healthy	Infected	Fungicide Treated	% increase over healthy control	
	(H)	(R)	(FT)	Infected	Fungicide treated
2	0.4549 ± 0.1523	0.9104 ± 0.1463	0.2322 ± 0.0569	200.13	51.04
9	0.3181 ± 0.0149	0.6568 ± 0.0096	0.5240 ± 0.0191	206.47	164.73
16	0.5315 ± 0.0058	0.6021 ± 0.0305	0.6706 ± 0.1148	113.28	126.17
23	0.7445 ± 0.0461	0.8336 ± 0.0056	1.0984 ± 0.0000	111.96	147.53
30	0.6320 ± 0.0159	1.0275 ± 0.0348	0.9546 ± 0.0373	162.58	151.04
37	1.1453 ± 0.0226	1.2960 ± 0.0402	0.8653 ± 0.0526	113.16	75.55

ted in virus infected plants (Jensen, 1968, 1969) where the dry weight doubled in the infected leaves.

Increase in RNA content following brown rust infection is similar to TMV infected tobacco leaves (Heitefuss & Wolf, 1977; Rohringer & Heitefuss, 1966) and in black rust infected wheat leaves (Bhattacharya *et al.*, 1965). A 3-fold increase in RNA level was observed with the onset of heavy rust infection. Fungal mycelium of stem rust of wheat is known to contain high concentration of RNA (Lanetskii, 1982; Heitefuss, 1968). The contribution of the host and parasite towards this increased level of RNA is difficult to evaluate and presumably both are involved in the system. Alteration in the nucleotide composition of RNA may be the reason but the results of Zscheile *et al.*, (1969) appears inconsistent with this hypothesis.

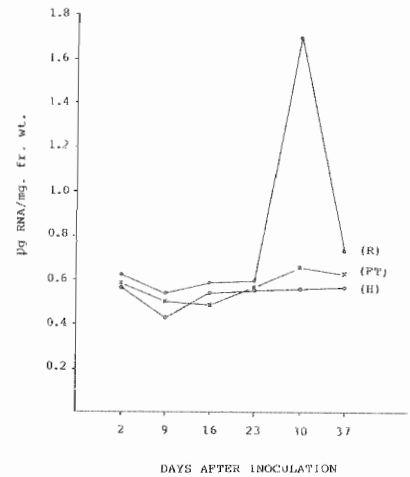

Fig.4. RNA content of healthy (H), rust infected (R) and fungicide-treated (FT) leaves. Standard errors (SE) are small and are covered by the points.

With the onset of heavy rust infection several changes occur which include the collapse of the nuclei of the epidermal and sub-epidermal host cells due to proteolysis, and only the deep seated cells remain unaffected by the fungal mycelium. This could be one of the reasons for the decrease in RNA contents of the host cell. Histological studies of the host tissue may confirm this hypothesis.

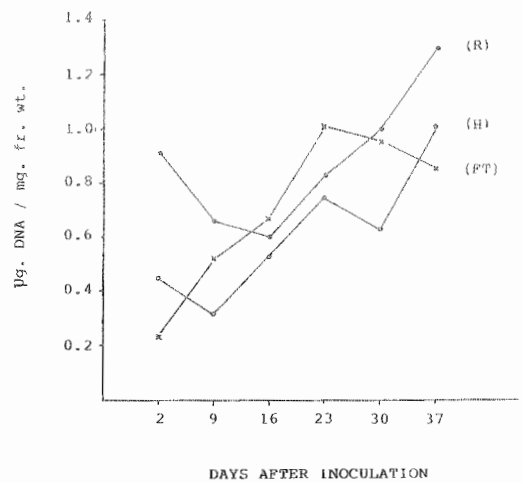

Fig.5. DNA content of healthy (H), rust infected (R) and fungicide-treated (FT) leaves. Standard errors (SE) are small and are covered by the points.

The level of DNA in infected tissue increased 2 DAI, when sporulation began and was twice the amount of healthy leaves. This is similar to the observations of Heitefuss (1968), Staples & Ledbetter (1960) and unlike the report of Bhattacharya *et al.*, (1965). After an initial increase, a decrease in DNA was observed till 16 DAI perhaps due to the senescence and death of the invaded cells. Similar decrease from 4-12 DAI has been observed in black rust infected wheat leaves (Bhattacharya & Shaw, 1968). In the later stages of rust development (23 DAI) the increase in DNA content may not be due to the DNA synthesis by the fungus since a similar increase was observed in healthy plants also.

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