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SHORT COMMUNICATION

EFFECT OF ROOT PARASITISM BY *OROBANCHE* ON THE RESPIRATION AND CHLOROPHYLL CONTENT OF *PETUNIA*

PRIKHSHAYAT SINGH and P. S. KRISHNAN

Department of Biochemistry, Lucknow University, Lucknow, U.P., India

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Abstract—Infection of *Petunia* by broom rape caused an increase in the respiratory rate of roots but not leaves of the host plant. There was a drop in both the chlorophyll and nitrogen levels of the leaves of infected plants.

INTRODUCTION

ONE OF the most conspicuous effects of infection of higher plants by the microbial pathogens is an increase in respiration.[1,2] Microbial pathogens also induce changes in the rate of photosynthesis in hosts, but the trend of change has not always been consistent;[2] also, the parameter of comparison, whether on the basis of leaf area, fresh weight, or dry weight, influenced the conclusions drawn.[3] An increased photosynthetic rate observed in many cases was correlated with the need for increased catabolism,[4] as evidenced by the respiratory increase. Several lines of investigation in progress in this department and elsewhere[5] suggest that the effects of infection by angiosperm parasites resemble those caused by microbial cellular pathogens. In the present paper we have determined the respiratory activity and the chlorophyll content of *Petunia* plants infected by broom rape.

RESULTS AND DISCUSSION

The analytical data for dry weight and protein content per host plant and the total parasite growth per plant and the data for the respiratory rate of host rootlet and of adjacent parasite tissue and of host leaf and the chlorophyll content of leaf are recorded in Table 1. The values reported are the means of a minimum of six determinations, with standard deviation. The significance of the observations was evaluated by Student's *t*-test.

There was a loss in dry weight (statistically significant) per plant, especially marked in the root system, as a result of infection. Although only a few parasites were visible above ground at the time of harvest, a large number of submerged growths were present. The dry weight of total parasite growths per host was nearly the same as that of infected host (shoot + root). There occurred significant loss of protein from the shoot system and more so from the root system of the infected plants. The total protein in the parasite growth was nearly the same as that of host (shoot + root), but there was marked fluctuation among the

[1] M. SHAW, *Ann. Rev. Phytopathol.* **1**, 259 (1963).
[2] C. E. YARWOOD, *Ann. Rev. Plant Physiol.* **18**, 419 (1967).
[3] S. G. JENSEN, *Phytopathology* **58**, 204 (1968).
[4] A. LIVNE, *Plant Physiol.* **39**, 614 (1964).
[5] D. SMITH, L. MUSCANTINE and D. LEWIS, *Biol. Rev.* **44**, 17 (1969).

TABLE 1. EFFECT OF INFECTION OF *Petunia* BY *Orobanche*

	Host shoot		Host root		Parasite
	Control	Infected	Control	Infected	
Dry wt, g/host or parasite growth/host	53·4 ± 9·4	30·5 ± 18·8	5·73 ± 1·52	1·72 ± 0·62	30·1 ± 18·5
Protein, g/host or parasite growth/host	2·24 ± 0·87	0·87 ± 0·40	0·11 ± 0·05	0·026 ± 0·01	0·82 ± 0·85
Respiration, μl/100 mg/hr					
Fresh tissue	1·59 ± 0·18	1·61 ± 0·30	0·847 ± 0·085	1·342 ± 0·197	1·29 ± 0·30
Dry tissue	15·1 ± 2·0	17·44 ± 4·57	7·29 ± 1·46	10·08 ± 1·44	10·17 ± 3·45
Chlorophyll in leaf, μg/100 mg					
Fresh tissue	58·81 ± 8·09	48·09 ± 4·46			
Dry tissue	637·9 ± 91·2	484·4 ± 118·7			
Protein	62·43 ± 6·98	63·06 ± 8·82			

The details of sampling and the determination of respiratory rate and chlorophyll were as reported in text. For dry weight determinations on whole plants, 10 plants each of control and infected were harvested; for protein determination nine each of control and infected hosts and the parasite growth from eight hosts were employed. For respiration measurement and chlorophyll determination six samples each were used. Dry weight was expressed for host shoot and root in terms of weight in g/single plant. For the parasite it was expressed as the weight of parasite growth/single host plant. Chlorophyll in leaf, in μg, was expressed in terms of 100 mg fr. wt. or dry wt. of leaf or 100 mg total protein in leaf.

samples. These results suggested that the hosts were in an advanced stage of infection at the time of harvest.

The infected roots respired at a higher rate than the control roots. The observed difference was significant at 0·1 per cent level on basis of fresh weight and at 2 per cent level on the basis of dry weight. The rate of respiration of parasite tissue was not significantly different from that of infected rootlet whether on fresh weight or dry weight basis. The tissues were not examined microscopically; however, in view of the fact that 1-cm length of root was rejected on either side of the swelling at the junction of parasite attachment to host, the root tissue used in the respiration studies may be considered uncontaminated with cells of parasite tissue. The advantage in having parasite-free tissue cannot be over-emphasized, considering the fact that studies on infection by the cellular microbes have been complicated by the presence of parasite tissue in the test samples of host. No statistically significant change occurred in the rate of respiration of leaves of infected plants, calculated in terms of fresh weight and dry weight. It may be pointed out that the tissue chosen (leaf) was located at a considerable distance from the site of infection (root) and the physiological effect of parasitism may have been diluted in this region.

There was a decrease in content of chlorophyll, calculated on the basis of fresh weight and dry weight. The decrease was significant at 5 per cent level of significance. In terms of tissue protein, there was no change in the chlorophyll content. Assuming that the chlorophyll content could be taken as a measure of the photosynthetic efficiency of the plants, the results did not support a concept that increased respiration was accompanied by increased photosynthetic activity. Deficiency of minerals is known to decrease the content of chlorophyll.[6] In view of the massive growth of the obligatory parasite, it is likely that conditions of mineral deficiency were induced in host. Since chlorophyll expressed in terms of protein content of leaves is the same in the control and the infected plants, the more likely explanation is that the effect of the parasite has been to decrease the amount of available nitrogen, which in turn has affected both protein and chlorophyll synthesis.

EXPERIMENTAL

Growing of Plants

Seedlings of *Petunia hybrida* X Hort, ex Vilm. were transplanted singly in pots during the latter part of the month of October 1969. Half the pots contained soil which had been heavily inoculated with seeds of *Orobanche cernua* Loefl. The aerial growth of the parasite commenced towards the last week of January and continued into the months of February and March.

Sampling

The infected and control hosts were harvested in pairs on consecutive days. The host shoot system and the root system, severed at the level of the soil, and the parasite growth were carefully and completely washed free of dirt and soil and surface dried with cloth and weighed. Special care was taken to collect the parasite growths which had got detached during uprooting of host.

Respiration

(*a*) *Leaves*. Leaves occupying the same position in the various branches were harvested. The leaves were neither too old nor too young and were located in the middle region of the branch; 2–3 leaves were collected from every branch. Discs were punched with a cork borer and 200-mg samples were transferred to Warburg flasks.

(*b*) *Roots*. Rootlets were chosen with attached *Orobanche* and cut at a distance of about 1 cm on either side of the swollen region where the parasite made contact with host. The severed rootlets at either side of the infection zone were washed thoroughly to remove any sand particles and surface dried between filter paper folds. These were cut transversely into about 1-mm lengths for a distance of about 5 mm from the original cut. 200 mg amounts were transferred to Warburg flasks.

(*c*) *Parasite*. Parasite tissue at a distance of 2–3 mm from the point of attachment to rootlet was sliced thin (about 0·2 mm) with a razor blade and 200 mg amounts were removed to Warburg flasks.

Measurement of Respiration

The oxygen uptake was measured in conventional Warburg manometers, using 3 flasks for each set of measurements. Each flask contained 3·0 ml. 50 mM phosphate buffer, pH 4·6. The centre well contained 0·2 ml 20% KOH and filter paper. After 15 min equilibration period at 37°, oxygen uptake was measured at 10 min interval for a total of 1 hr.

At the end of the experiment, the tissue from each flask was taken out and washed thoroughly with distilled water and the dry weight determined.

Respiration was expressed in μl. O_2 uptake/100 mg fr. and dry wt./hr.

Chlorophyll Estimation

10 g each of randomized leaves was used for the determination of chlorophyll by the method of Arnon.[7] Chlorophyll contents were expressed in μg/100 mg fr. wt. and dry wt. and /mg protein.

[6] D. E. Bottrill and J. V. Possingham, *Biochim. Biophys. Acta* **189,** 80 (1969).
[7] D. I. Arnon, *Plant Physiol.* **24,** 1 (1949).

Protein Estimation

Protein content was determined by the method of Lowry *et al.*,[8] with the modifications suggested by Khanna *et al.*[9]

Dry Weight

Weighed samples of randomized root, shoot, leaves and parasite were dried to constant weight at 70°.

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[8] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193,** 265 (1951).
[9] S. K. KHANNA, R. L. MATTOO, P. N. VISWANATHAN, C. P. TEWARI and G. G. SANWAL, *Indian J. Biochem.* **6,** 21 (1969).