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Studies on Sequential Parasitism by *Orobanche* and *Cuscuta* on *Petunia hybrida*

CHOLINE KINASE AND PHOSPHOLIPID[1]

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ABSTRACT

Parasitism by *Cuscuta* and *Orobanche* on *Petunia hybrida* resulted in decreased choline kinase activity and phospholipids in the host shoots. The *Cuscuta*-infected host roots suffered a decline in phospholipid concentration with no appreciable change in enzyme activity, whereas the roots of the *Orobanche*-infected plants exhibited a substantial increase in phospholipid concentration despite a marked lowering in enzymic activity. Superimposition of infection by *Cuscuta* on *Orobanche*-infected plants resulted in an increase in both enzyme activity and phospholipid in host shoots; the host roots recorded a decline in phospholipid, although enzyme activity was increased. As compared to the filaments infecting singly, *Cuscuta*, in sequential infection, registered an increase in phospholipid concomitant with a fall in enzyme activity, whereas the root parasite revealed a lowered enzyme activity and a slight decrease in phospholipid. It is hypothesized that a physiological response to infection by root parasite was an accumulation of phospholipids at the region under infection, and to that by shoot parasite was an uptake of phospholipids by the parasite from the host; this was effected not by *de novo* synthesis but rather by mobilization from distal regions.

This laboratory has been engaged now for over a decade and a half in studies on the biochemical effects on plants infected by some angiosperm parasites; such studies have generally been made under conditions of infestation by single parasites (8, 15). In the present investigation, studies were undertaken on the biochemical alterations consequent on infection by *Cuscuta* (the shoot parasite) of *Petunia hybrida* which was already under the active influence of infection by *Orobanche* (the root parasite). This experiment permitted a comparison of infection of the same host separately, and in combination, with the shoot and root parasite. Some of the studies made with these plants have already appeared; for example, the decreased dry solids, protein, and phosphorus contents in *Orobanche*-infected plants was counteracted by secondary infection by *Cuscuta* (11). Singh (13, 14), studying starch and nucleic acid metabolism, observed that the influence of the two parasites was not confined to the region immediately infected but was transmitted over the entire host plant, sometimes being more prominent in the distal region. The two parasites, when infecting together, in addition to modifying the response by the host in a single infection, appeared to exert an effect on each other.

While fractionating phosphate compounds in the tissues of angiosperm parasites, we observed that *Cuscuta reflexa* had an unusually high phospholipid content. It was of interest to search for a possible interrelationship in the phospholipid contents between the host and the parasite tissues. Choline kinase, which is a key enzyme in the *de novo* biosynthesis of choline phospholipids, was also assayed in the parasite and host tissues in order to elucidate a physiological/functional relationship between the two.

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MATERIALS AND METHODS

Petunia hybrida × Hort. ex Vilm. served as the host for *Orobanche cernua loefl.*, the root parasite, and for *Cuscuta campestris* Yunck., *Cuscuta indecora* Choisy, and *C. reflexa* Roxb., the shoot parasites.

Seedlings of *P. hybrida*, raised in nursery beds, were transplanted singly into a number of pots filled with garden soil, half of the pots having been inoculated with the seeds of *O. cernua*. Ten days following the emergence of scapes of *O. cernua* from soil surface, the infected pots were separated into four groups, one to serve for single parasitism by *Orobanche*, while the other three were infected separately with *C. campestris, C. indecora,* and *C. reflexa* by the technique already described (15). Simultaneously, the pots which were not inoculated with *Orobanche* were divided into four batches, one to serve as control, without any infection, and the other three infected singly with the three species of *Cuscuta*. After a lapse of another 3 weeks when dodder growth was well established, harvesting was commenced batchwise and completed within a period of 12 days. The parasite tissue was manually freed. For every analysis, the tissue material from six pots was pooled and thoroughly randomized.

Thirty, 10, and 5% (w/v) homogenates, respectively, of freshly harvested (7) *Cuscuta, Orobanche,* and *P. hybrida* were prepared in a Waring Blendor in 10 mM tris-HCl buffer (pH 9) containing 15 mM $MgCl_2$ and varying concentration of freshly neutralized cysteine-HCl (50 mM for parasite and 10 mM for host tissue).

Choline kinase in the tissue homogenates was assayed as reported earlier (12). One unit of enzyme activity was the disappearance of 1 μmol of choline in 60 min at 30 C under conditions of assay.

The phospholipid fraction, isolated according to the phosphate fractionation technique of Schmidt and Thannhauser (10), as modified by Volkin and Cohn (16), was estimated as phosphorus colorimetrically (1).

Protein was determined in trichloroacetic acid precipitates of the homogenates according to Lowry *et al.* (6), with modifications as suggested by Khanna *et al.* (4).

RESULTS

Calculations in terms of unit dry solids enabled better appreciation of the changes in enzymic activity in relation to the amount of phospholipid in the tissues (Table I). The data for dry solids have already appeared (11).

ENZYME ACTIVITY AND PHOSPHOLIPID IN PARASITE AND HOST TISSUES

Parasite. The root parasite invariably possessed far higher choline kinase activity but much lower phospholipid concentration than the shoot parasite. Of the three species of shoot parasite, *C. reflexa*, when infecting singly, exhibited maximal enzymic activity and phospholipid concentration. The enzyme activity in *Cuscuta* species was comparatively of a much lower order than that in the host tissues, control or infected.

Host. The enzyme activity in the shoots of the control, the root-infected and the doubly infected plants generally exceeded that in the roots; with the exception of the plants infected by the root parasite, the phospholipid concentration followed a similar pattern. The *Orobanche*-infected plants stood out in that the roots accumulated phospholipids. On the contrary, in the case of plants infected singly by the shoot parasite, while enzyme activity of the roots surpassed that in the shoots, the phospholipid concentration was higher in the shoots.

INFLUENCE OF SINGLE PARASITISM: RESPONSE BY HOST DUE TO INFECTION BY

Shoot Parasite. Infection was generally attended by a consistent fall in the enzymic activity of the shoots. The roots tended to resist shoot parasitism by registering only marginal changes in their levels of enzymic activity. The phospholipid concentration in the shoots and roots also registered a decrease except in the case of *C. reflexa*-infected plants which exhibited a complete retention of phospholipid in the shoots and a preferential retention in the roots.

Root Parasite. There was a considerable lowering of the enzymic activity in both the shoots and roots, being particularly acute in the case of the roots. Whereas the phospholipid concentration in the shoots registered a 22% decrease, that in the roots was significantly (43%) increased, even exceeding the level in the shoots (calculations in terms of fresh weight of tissue showed a significant increase of 47%).

It appeared that generally similar alterations in enzymic activity and phospholipid concentration were demonstrable only in the infected shoots, irrespective of whether infection was by shoot or root parasite.

INFLUENCE OF DOUBLE PARASITISM

Response by Parasite. With a single exception, the choline kinase activity in the shoot and root parasites infecting together was at a reduced level than when they parasitized the host separately; such a response was more pronounced in the case of the shoot parasites. The phospholipid, on the other hand, registered an increase in the shoot parasites and, with an exception, a decrease in the root parasite.

Response by Host. The shoots as well as the roots of the *Orobanche*-infected plants revealed a remarkable recovery of the enzyme activity as a result of superimposition of shoot parasitism, the extent of increase being higher in the case of the roots; however, the phospholipid concentration of the shoots was generally increased, but that of the roots was significantly and consistently decreased. In other words, the increase in the enzyme activity of the roots was concomitant with a depletion of phospholipids.

DISCUSSION

It was apparent from the foregoing data that the phospholipid concentration in the host plant was higher near the site of infection in parasitism by *Orobanche* and in that by *C. reflexa*. Our results have an analogy with the observations made by Kupervich and Ustinova (5) who reported that infection stimulated the flow of P and C compounds to the infected area. *Cuscuta* species appeared to wield a greater influence than *Orobanche* on the phospholipid concentration of the host when infecting together because in combined infection, *Cuscuta* did not permit the accumulation of phospholipids in the roots which would have otherwise occurred during infection singly by *Orobanche*.

The nominal variations in the enzyme activity of the roots of the *Cuscuta*-infected plants were accompanied by significant decreases in the phospholipid concentration, pointing to the possibility that this lowering was not due to retarded phospholipid biosynthesis in the roots, but more probably was the result of translocation of phospholipid from the root to the parasite via the shoot. This is even more so since the shoot parasites possessed nearly 2- to 6-fold higher phospholipid concentration, despite a very low order of enzyme activity, than the host shoots. Similarly, lowered choline kinase activity in the roots of the *Orobanche*-infected plants, associated with an increased phospholipid concentration, points to the likelihood of the occurrence of translocation of phospholipid.

This postulated translocation of phospholipid may involve either the intact molecule or a moiety of phospholipid (probably the phosphorylated form of choline, and, to a certain extent, of ethanolamine), most likely via the xylem vessels. The transloca-

Table 1. *Influence of Parasitism, Singly and in Sequential Combination, by the Root and Shoot Parasite on Choline Kinase and Phospholipid of P. hybrida*

For growth characteristics, fresh weight, dry solids, protein, and total phosphorus, see reference 11.

	Choline kinase activity, units per g dry solids				Phospholipid-phosphorus, ug per g dry solids			
	Host		Parasite		Host		Parasite	
	shoot	root	Cuscuta	Orobanche	shoot	root	Cuscuta	Orobanche
Control Host:	311	229			92.8	51.8		
Host Infected Singly by:								
C. campestris	185	235	59.7		29.2	27.5	166	
C. indecora	146	191	56.0		43.9	17.1	160	
C. reflexa	130	252	111		91.3	44.3	197	
O. cernua	134	42.5		238	72.1	74.3		83.3
Host Infected by O. Cernua And:								
C. campestris	212	198	29.2	130	92.8	51.7	214	71.4
C. indecora	156	89.6	16.5	241	57.4	32.2	177	52.2
C. reflexa	245	89.1	25.1	106	78.1	68.5	284	100

tion appeared to occur toward the site of infection singly by the shoot or root parasite and toward the shoot parasite after superimposition of shoot parasitism on *Orobanche*-infected plants; the *Cuscuta* species, in order to recoup their phospholipids, probably drew not only upon the host shoots, but also upon the roots (where *Orobanche* had induced a high order of phospholipid accumulation) and also, to an extent, on root parasite, thus accounting for the increase in phospholipid concentration after superimposition had occurred. The host, thus, could have served as a bridge for translocation of phospholipids from *Orobanche* to *Cuscuta*. Such a translocation would have an analogy with the translocation of sucrose (3), hormones (2), and viruses (9) from some parasites to some hosts.

Orobanche, infecting by itself, has proved to be a more drastic parasite than *Cuscuta* (8). Our results indicate that superimposition of infection by *Cuscuta* could induce near normal lipid metabolism in the *Orobanche*-infected host plants. Also, bearing in mind that *Cuscuta* counteracted the effects of primary parasitism by *Orobanche* in the case of the gross tissue constituents as well (11), superimposition of the shoot parasite on the *Orobanche*-infected plants may prove a beneficial technique in combating the detrimental effects of root parasitism.

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