Competition Between Ergots of *Claviceps purpurea* and Rye Seed for Photosynthates

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We are indebted to B. M. Cunfer, Georgia Experiment Station, University of Georgia, for supplying the culture of *Claviceps purpurea* and to Rita M. Bennett for technical assistance.

Accepted for publication 17 March 1982.

ABSTRACT

Bacon, C.W., and Luttrell, E.S. 1981. Competition between ergots of *Claviceps purpurea* and rye seed for photosynthates. Phytopathology 72:1332-1336.

Competition between developing ergots (sclerotia) of *Claviceps purpurea* and developing seed for ^{14}C-labeled photosynthates translocated from leaves of intact infected rye plants was compared with translocation of ^{14}C-labeled photosynthates into seeds of healthy plants. The study included the 6-wk period between anthesis and maturation of the rye seed on plants grown under field conditions. The assimilation of the ^{14}C-labeled photosynthates by seeds and ergots during this period was determined by measuring total sugars, sugar alcohols, amino acids, organic acids, lipids, proteins, RNA, and DNA. Competition for photosynthates followed the early establishment of the stromatic foot, the absorbing organ of the parasite, which remained active during the entire 6 wk even though the sclerotial tissue located above had matured in 3–4 wk and no longer incorporated photosynthates. The flow of nutrients into the sclerotium decreased as it developed acropetally. Seeds and sclerotia of diseased rye plants incorporated more $^{14}CO_2$ than did seed of healthy plants, which reflected a larger and more efficient energy sink. Seeds from healthy plants completed development at the end of the 6-wk period as indicated by dry weights and decline in the uptake of photosynthates. However, development of competing seeds on infected plants was retarded until the fourth week when high levels of translocation and increases in dry weight resumed after a concurrent decline in photosynthate translocated to competing ergots.

Additional key words: Secale cereale, infection, parasitic development, host-parasite relationships, $^{14}CO_2$ assimilation.

The sclerotia (ergots) of *Claviceps purpurea* (Fr.) Tul. produced in individually infected florets of rye (*Secale cereale* L.) and other grasses are complex fungal structures that replace the host ovary and proceed through an ordered sequence of development (13). Initially the fungal replacement tissue consists of a stromatic foot seated in the base of the floret and an apical stroma involuted into labyrinthine chambers lined with conidiophores that produce conidia suspended in honeydew. The sclerotium proper then develops from a palisade of meristematic hyphae across the top of the foot and pushes the conidial stroma (*Sphacelia*) upward on its tip. The cylindrical sclerotium differentiates into a dark outer rind and a medulla composed of a compact pseudoparenchyma of thick-walled storage cells traversed by plectenchymatous strands of conducting tissue. The sclerotium, therefore, represents a large, complexly structured body of fungus tissues sharply delimited from uninvaded host tissue by a narrow interface in which hyphae surround living host cells and establish a stable connection with the vascular system of the plant. This host-parasite system has obvious advantages for the study of translocation from host to parasite. It was used by Mower and Hancock (16) to study translocation of ^{14}C-sucrose from cut stems of ryegrass into the fungal stroma during the period of honeydew formation. They demonstrated the operation of a sucrose sink in the fungal tissues resulting from conversion of sucrose into carbohydrates characteristic of the fungus. By supplying excised heads of rye with $^{14}CO_2$ Dickerson et al (6) found that sclerotia of *C. purpurea* are more efficient than developing seeds in competing for photosynthates.

The present study compares translocation of photosynthates from leaves of intact rye plants grown under field conditions into the seeds of healthy plants and into ergots and competitor seeds in heads of infected plants. The comparison extends throughout the normal 6-wk period from anthesis to maturation of the grain. A preliminary report has been published (2).

MATERIALS AND METHODS

Growth of plants. Experiments were conducted in 1976 and 1979 with the rye cultivar Athens abruzzi. Seeds were planted in 15-cm-diameter pots in mid-November of the previous year and were grown outdoors over the winter. Inflorescences were inoculated at anthesis in April. The foliage was pulsed with $^{14}CO_2$ 1, 2, 3, 4, and 5 wk after inoculation. Ovaries and ergots were excised for analysis 24 hr after pulsing. In 1976 plants in each pot were divided into two groups. The inflorescences in one group were covered with a plastic bag, whereas the inflorescences in the second group were sprayed with inoculum and then covered with a plastic bag. Inoculations were made on 1, 19, and 21 April. In 1979, inoculated and control plants were in separate, paired pots, and all inoculations were made

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on 30 April.

Inoculations. *Claviceps purpurea* (ATCC 34656) was grown on slants of the sporulation medium of Kybal et al (9) in 250-ml medicine bottles for 2 wk. Inoculum was prepared by adding water to the culture bottle, shaking the bottle, and filtering the spore suspension through cheesecloth. Plants in the process of blooming were brought into the laboratory the night before and placed in an east window to receive the morning sun. Florets were marked as they opened during the morning by blackening the awns with India ink. Inoculum was misted onto the inflorescences at noon, and the inflorescences were covered with plastic bags with the inner surface misted with water. The bags were removed the next morning, and the plants were returned to their original outdoor location.

Generation of $^{14}CO_2$. Translocation of ^{14}C-labeled photosynthates into tissue was studied by supplying $^{14}CO_2$ to infected and noninfected plants. Plants were placed in a Pyrex glass chromatography chamber (30 × 30 × 60 cm), and the height of the plant was adjusted such that the flag leaf of each inflorescence was contained within the chamber while the inflorescences projected out of the chamber. The chamber lid was sealed with silicone grease, and the holes through which the inflorescences projected were sealed with modeling clay. At time zero, $^{14}CO_2$ was generated by injecting 0.5 ml of 90% (w/v) lactic acid into a $NaH^{14}CO_3$ solution with a syringe inserted through a port capped with a rubber stopper. The lactic acid was delivered from the syringe through a teflon tube to a 5-ml beaker containing 0.5 ml of $NaH^{14}CO_3$. The $NaH^{14}CO_3$ and lactic acid solution were mixed with a magnetic stirrer during the 2-hr exposure period, and the plants were illuminated laterally from an incandescent light and laboratory overhead fluorescent lights for a total of 5,381 lux at 29 C, internal chamber temperature. After the 2 hr of exposure, an ethanolamine-ethylene glycol monomethyl ether solution (1:2, v/v) was introduced into the chamber to absorb excess $^{14}CO_2$, and the plant was removed from the chamber within 15 min. The plants were allowed to assimilate the labeled photosynthate for 24 hr, after which the seed and fungus were dissected as described below. In all experiments, 300 μCi of $^{14}CO_2$ was released into the chamber and the total specific activities of the $NaH^{14}CO_3$ were 55.5 mCi/mmole for the first year, and 47.5 mCi/mmole for the second year. No residual radioactivity was found in the acidified $NaH^{14}CO_3$ solutions, and analysis of the ethanolamine solution indicated that 96–98% of the $^{14}CO_2$ was incorporated during each exposure period.

Tissue sampling. Developing ovaries and sclerotia were excised from the marked florets under a dissecting microscope. In 1976, at weeks 1–4, the sclerotia were cut off at the top of the foot to ensure that no host tissue would be included in the sample of fungus tissue. At week 5, the sclerotium was divided transversely into four segments, the foot, basal, middle, and apical regions; each segment was analyzed separately. In 1979, the sclerotium was divided into four segments at weeks 3–5. Each tissue sample was placed in a tared vial, dried at 100 C for 24 hr, weighed (dry weight), then freeze-dried and kept at −20 C until analyzed. Growth of fungus and seed was expressed as average dry weight per sclerotium or seed.

Sample extraction and analysis. Dissected freeze-dried tissue (15–20 mg) was pooled from each inflorescence during a sampling period. Usually enough tissue was available for at least three replications for each sampling period of each year. Replicates did not vary more than 7%, and results are presented as averages. The incorporation of ^{14}C activity into sugars, amino acids, organic acids, lipids, proteins, RNA, and DNA was determined on fractions prepared from tissue according to the procedure described previously (3). Total sugars in the sugar fraction were determined by the anthrone method (12). Total lipids were determined by the chromic acid procedure using steric acid as a standard (17). Glucose and total sugar alcohols were specifically determined by the *o*-toluidine procedure (8) and by the sodium metaperiodate procedure (11), respectively. Total amino acids were determined by the ninhydrin method (15), and organic acids by titration.

A 0.5-ml portion of each fraction (representing 1.5–2.0 mg of the initial tissue) was tested for radioactivity. Water-miscible fractions were counted in the modified scintillation fluid of Bray (3), and water-immiscible fractions were counted in a toluene-based scintillation fluid. Radioactivity of the sample was measured in a Packard model 3255 Tri-Carb liquid scintillation spectrometer. All counts were quench-corrected by external standards ratio.

RESULTS

It took 6 wk for healthy rye plants to develop seeds that no longer incorporated $^{14}CO_2$ assimilates (Fig. 1A). Seeds on 5- and 6-wk-old plants were visibly dry and hard; therefore, the inability to incorporate labeled assimilates reflected the final stages in the maturation of the seeds. Ergot sclerotial development was essentially completed within 4 wk, as indicated by dry weight and decline in total incorporation (Fig. 1B and Table 1). Incorporation of assimilates into seeds of parasitized rye plants continued throughout the 6-wk period, with the higher percentage of incorporation occurring during the fifth and sixth weeks (Fig. 1C). Of the total amount of labeled $^{14}CO_2$ administered to plants during a pulsing period, 2- and 3-wk-old sclerotia incorporated more than 60% and competing seeds incorporated less than 20% (Table 1). It

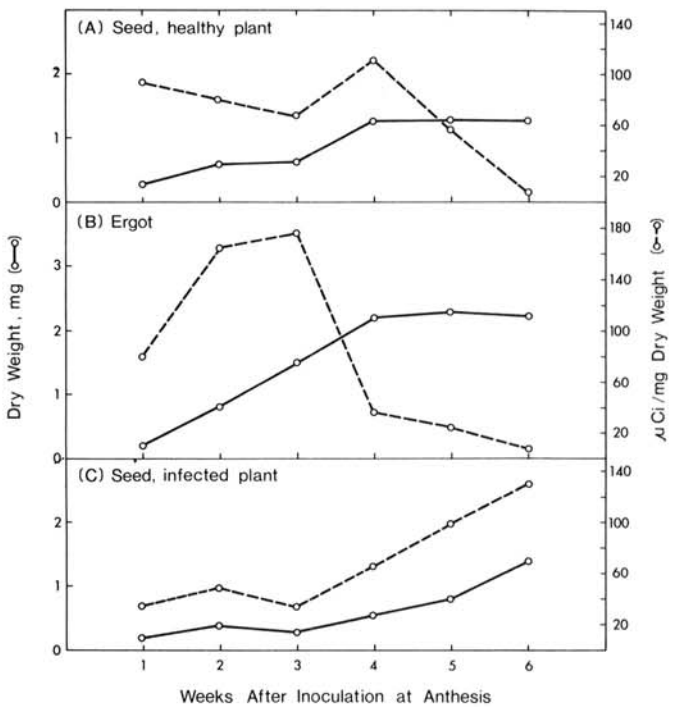

Fig. 1. Development of rye seed and sclerotia of *Claviceps purpurea* (milligrams dry weight per seed or ergot) and total incorporation of ^{14}C-photoassimilates into fungus tissue, and into rye seeds from parasitized and nonparasitized plants grown in 1976.

TABLE 1. Percentage total ^{14}C incorporated into seeds collected in 1976 from infected and healthy plants and into ergot sclerotia at weekly intervals following anthesis

Week	Percent of total (300 μCi) ^{14}C incorporated[a]		
	Seed, infected plant	Seed, healthy plant	Ergot sclerotia
1	11	31	26
2	16	30	68
3	10	24	83
4	21	36	13
5	32	21	9
6	44	0	2

[a]The plants were exposed to ^{14}C for 2 hr, which was followed by an assimilation period of 24 hr.

was only after the fourth week, when the percentage incorporation into the sclerotia dropped, that incorporation was directed into competing seeds. Seeds of uninfected plants incorporated a maximum of 36% of the $^{14}CO_2$ administered during the 6-wk testing period.

The distribution of ^{14}C label incorporated into the various classes of compounds in 3-wk-old seeds was less for seeds on infected plants for each class of compounds except lipids (Table 2). A 68% decrease in incorporation of ^{14}C-assimilates in seeds from parasitized plants was found when competing seeds were compared with seeds from nonparasitized plants. The decrease in level of total sugar due to infection was 74%, with similar decreases occurring in protein and RNA fractions, while decreases in free amino acids and organic acids were slightly higher. Similar decreases in these classes of compounds and increases in total lipids were obtained for seeds of parasitized plants from weeks 1 to 4 following infection by *C. purpurea*. The incorporation of assimilates into the various fractions of seeds from parasitized plants was reduced until the sclerotia matured between weeks 3 and 4 (Table 3 and Fig. 1C). Seeds from 6-wk-old nonparasitized plants were not actively taking up ^{14}C-assimilates, whereas seeds on 6-wk-old parasitized plants incorporated ^{14}C at levels comparable to those of 2-wk-old seeds of nonparasitized plants (Table 3). Throughout the development of the ergot disease, competing seeds incorporated a much higher level of label into the lipid fraction than did seeds of nonparasitized plants at a comparable age. Competing seeds were further characterized by higher levels of ^{14}C-photosynthates incorporated into the free amino acid pool, organic acids, and protein fractions during the first week of parasitism.

Sclerotia utilized a higher percentage of $^{14}CO_2$-assimilates during development than did competing seeds. Three-week-old sclerotia incorporated ^{14}C into sugars and lipids throughout the four regions of the sclerotia, but 6-wk-old sclerotia incorporated ^{14}C less actively in all except the foot region (Tables 4 and 5). There was no apparent difference between the translocation of labeled material in the foot region of 3- and 6-wk-old sclerotia. However, there were differences in the accumulation of the total amount of these compounds in the foot region; 3-wk-old foot tissue had a higher level of lipids and sugars than did 6-wk-old foot tissue. The base and middle segments of 6-wk-old sclerotia showed quantitative differences in total synthesis of sugars and lipids than did the foot. For example, these two regions accounted for 94% of the total sugars and 98% of the lipids in 6-wk-old sclerotia. While the tissues of the base, middle, and apex were no longer active in incorporating photosynthates into the various fractions, these pools were still present in the foot and were quantitatively higher than the foot segment of 3-wk-old sclerotia of *C. purpurea*.

TABLE 2. Distribution of ^{14}C isolated from 3-wk-old seeds collected in 1976 from diseased and healthy plants and the decrease in incorporation by seed from infected plants

Fraction	μCi/mg dry wt		Decrease due to infection (%)
	Infected plant	Healthy plant	
Total ^{14}C-label	32.9	102.1	68[a]
Free amino acids	2.3	15.4	85
Total sugars	12.8	50.1	74
Lipids	7.9	2.0	...[b]
Organic acids	2.8	14.0	80
Protein	4.5	18.4	73
RNA	0.5	1.1	73
DNA	0.0	0.4	...[b]

[a] Percentage of total radioactivity incorporated into seeds from infected plants to that incorporated by seeds of healthy plants minus 100%.
[b] The amount of incorporation into the lipid fraction was an increase over seeds from healthy plants and incorporation into DNA was not detected in seeds from infected plants.

DISCUSSION

Parasitism by *C. purpurea* on rye produces an effective diversion of the flow of nutrients from the competing seeds. The flow of nutrients is initiated within 1 wk after entry of the fungus into the rye floret, and continues to the final stage of sclerotium development. Cells of the foot at the interface with the host cells of

TABLE 3. Distribution of ^{14}C isolated from rye seeds collected in 1976 from infected and healthy plants and from sclerotia of *Claviceps purpurea*

Fraction	Week, μCi/mg dry weight of:								
	Seed from								
	Infected plant			Healthy plant			Sclerotia		
	1	2	6	1	2	6	1	2	6
Total ^{14}C-label	43.6	48.6	131.2	90.7	109.8	0	82.6	163.9	8.7
Total sugars	15.9	13.2	78.1	66.0	40.2	0	31.5	39.8	2.0
Total sugar alcohols	0	0	0	0	0	0	28.0	46.2	1.2
Glucose	4.6	6.1	15.6	18.2	26.3	0	12.3	33.3	0.2
Free amino acids	8.8	10.0	12.7	2.2	18.3	0	1.0	13.1	1.2
Organic acids	5.9	12.4	14.3	1.8	19.3	0	1.1	10.2	0.7
Lipids	4.9	3.2	4.6	0.9	1.9	0	3.4	14.1	2.1
Protein	1.8	1.7	4.8	0.8	2.2	0	1.2	1.2	0.3
RNA	0.5	0.9	0.8	0.5	1.4	0	1.9	1.4	0.1
DNA	0.0	0.2	0.0	0.3	0	0	1.1	1.8	0

TABLE 4. Distribution of ^{14}C incorporated into soluble carbohydrates and lipids among the four regions of ergot sclerotia following the supply of $^{14}CO_2$ to plants 3 wk after anthesis during the 1979 growing season

Fraction	Foot		Base		Middle		Apex	
	μg/mg dry wt	μCi/mg dry wt	μg/mg dry wt	μCi/mg dry wt	μg/mg dry wt	μCi/mg dry wt	μg/mg dry wt	μCi/mg dry wt
Total sugar alcohols	37.0	5.2	70.0	54.6	57.0	28.2	15.0	6.2
Total sugars	80.0	1.0	150.0	16.7	109.0	4.2	40.0	4.1
Glucose	3.2	1.3	11.2	9.0	0.6	2.2	2.1	3.1
Free amino acids	4.0	0.7	7.6	12.8	10.1	4.1	3.2	1.2
Organic acids	19.3	0.3	29.0	10.7	30.2	3.1	17.1	3.1
Lipids	3.1	0.2	18.2	10.1	26.8	2.1	2.0	1.1

TABLE 5. Distribution of ^{14}C incorporated into soluble carbohydrates and lipids among the four regions of ergot sclerotia following the exposure of rye plants to $^{14}CO_2$ 6 wk after anthesis during the 1979 growing season

Fraction	Foot		Base		Middle		Apex	
	μg/mg dry wt	μCi/mg dry wt	μg/mg dry wt	μCi/mg dry wt	μg/mg dry wt	μCi/mg dry wt	μg/mg dry wt	μCi/mg dry wt
Total sugar alcohols	10.0	5.6	233.0	0.4	341.0	0	26.0	0
Total sugars	20.0	0.9	571.0	0.2	670.0	0	60.0	0
Glucose	1.4	1.1	44.2	0.1	55.6	0	3.0	0
Free amino acids	1.1	0.9	19.3	0.1	29.6	0	0.1	0
Organic acids	6.2	0.2	124.6	0.2	144.0	0	19.2	0
Lipids	0.9	0.1	30.8	0.1	28.8	0	5.1	0

the rachilla are active in the uptake of $^{14}CO_2$ assimilates and remain active for 6 wk. Of the total amount of labeled sugars incorporated into the 6-wk-old parasite, 88% was in the foot tissue; only 4.6% was in this area at 3 wk. The occurrence of more total sugars and lipids in 3-wk-old than in 6-wk-old foot tissue reflects the larger pool sizes required for maintaining and supplying the rapidly growing and differentiating tissues located above. The fact that amounts of labeled compounds incorporated in 3- and 6-wk-old foot tissues during a pulsing period were essentially the same indicates that this tissue remains physiologically active in the uptake of food and that the contact with the plant is still functional. The lower specific activities of the pools in 6-wk-old tissues of the base, middle, and apex compared to the total levels of compounds accumulated indicates that while the pools still exist, the translocation of photosynthates to these regions is reduced as they mature. The apex is the oldest and most mature region of the sclerotium. The sclerotium elongates by addition of tissues at the base, the region most active in the uptake of photosynthates. This pattern of nutrient flow is supported by morphological studies showing that sclerotia develop acropetally (4,6,13).

Translocation is considered to be a source and sink mechanism (16,19). There may be control mechanisms that serve to maintain translocation so that developing sclerotia are not at a disadvantage as a result of callose formation (7). The source and sink mechanism would decrease during the final stages of ergot development. The early establishment of the ergot foot suggests its importance in the translocation process, and since the morphology of the foot cells does not change during development (13), it may be argued that this tissue remains a metabolic sink as characterized by its uptake of photosynthates and the biosynthesis of compounds. Sclerotia at 4–6 wk are uniformly dark purple to black, and during this latter stage of development they abscise easily across the top of the foot, leaving the foot embedded in the floret. Sclerotia at 6 wk of age contain lipids, sugar alcohols, glucose, and other carbohydrates as major constituents. Similar compounds were reported in dormant sclerotia where carbohydrates serve as an energy source during dormancy, whereas lipids are utilized during germination and development of clavae from the sclerotium (5,10). Translocation into seeds of parasitized plants increases following the differentiation and maturation of the sclerotium, and a reduction in the accumulation of storage compounds, which reduces the upward movement of photosynthates. Consequently, the flow of translocates is restricted to the foot tissue. Thus, while supplies of photosynthates may be adequate, innate factors limit translocation by controlling development of the sclerotium, resulting in a decrease in the capacity of the ergot tissue to utilize photosynthates. Rye seeds depend on the flag leaf, the two leaves immediately below the flag leaf, and the glumes to supply carbohydrates for development (14). Possibly these locations are more efficient sources from which photosynthates move more rapidly. Nevertheless, there is evidence that in cereals an increase in the size of a sink (ie, number of seeds) increases production of carbohydrates by leaves and subsequent translocation to seeds (20). Therefore, the increased $^{14}CO_2$-assimilation observed in parasitized plants over healthy plants may reflect the greater size and efficiency of the sink.

Declines in the accumulation of compounds during a 5- to 6-wk maturation period of rye seed have been reported on field-grown plants (1,14). This substantiates the maturation period observed in this study. Seeds on diseased plants continue development beyond this period. Seeds on diseased plants contain high levels of lipids throughout development; this suggests that lipids are preferentially synthesized under stress conditions such as the carbohydrate restriction induced by the fungus. As a consequence of continued development, competing seeds might proceed to maturity. However, the final yield from diseased plants might reflect the extent to which competing seeds complete development. Furthermore, our observations during the first year, and those reported earlier by Seymour and McFarland (18) was that the yield of normal seeds from diseased plants also depends on the number of blasted florets induced by the fungus, probably during the early stages of fungus penetration.

The fact that parasitized inflorescences incorporated more $^{14}CO_2$ than those from nonparasitized plants suggests that the fungus increases the assimilation of CO_2 by the plant and interferes with early translocation of photoassimilates to developing competing seeds. Thus, the ergot disease of rye increases the capacity of the plant to produce carbohydrates, and competition by the fungus for photoassimilates occurs primarily during the first 3–4 wk which allows for the subsequent flow of nutrients to competing seed after the sclerotium has matured.

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