Lipid Changes During Pre-Germination and Germination of *Striga asiatica* Seeds

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Witchweed (*Striga asiatica* L. Kuntze) seeds were incubated at 28 C in a moist environment for a 14-day period, after which seeds germinated only when exposed to specific natural or synthetic germination stimulants. Changes in lipid composition were determined during germination of witchweed seeds and during early seedling growth. Witchweed seeds contained 37.5% (w/w) oil. Increased levels of monogalactosyl-diacylglycerol and phosphatidylglycerol suggested the enlargement or multiplication of plastids after witchweed seeds had germinated. In contrast to the usual course of events in seeds with high oil reserves, witchweed seeds did not hydrolyze triacylglycerol rapidly during or after germination. These findings indicated that triacylglycerol in germinating witchweed seeds was conserved for subsequent use during haustorial formation and host invasion.

Witchweed, *Striga asiatica* L. Kuntze, is a chlorophyll-containing root parasite from the Scrophulariaceae family (1). Four species of *Striga, S. asiatica, S. gesnerioides, S. euphrasioides* and *S. hermonthica* are common in tropical and temperate areas of the world, and are the most widespread of all parasitic seed plants. Crops such as sorghum, corn, sugarcane and rice are particularly susceptible to witchweed (1). Corn yields may be reduced by as much as 90% in infested fields (2). Such infestation of graminaceous crops is particularly serious in Africa and India where food shortage problems already exist. The control of witchweed is made difficult by the extremely small seed size (0.2 to 0.4 mm in length), the prodigious number of seeds produced (3) and by seed viabilities of up to 20 years (4). In the U.S., control of witchweed is governed by strict federal and state quarantines (5). These efforts have limited the spread of the parasite to the Carolinas and Florida.

There is a lack of information concerning the physiological changes that occur after maturation and during germination of witchweed seeds. It is known that the seeds are dormant when dispersed and that a period of after-ripening followed by a pre-germination period of up to 14 days under water-saturated and moderate temperature conditions is required to break dormancy (6). After these requirements are fulfilled, a specific germination stimulant elicits rapid radicle protrusion usually within 18 to 24 hr. Root exudates from host as well as certain non-host plants contain the germination stimulant (7). Strigol, a natural germination stimulant, has been isolated from cotton root exudates (8). In addition, a series of synthetic germination stimulants characterized by one or more lactone rings, such as GR 7 and GR 24, have been found to induce germination of witchweed seeds (9). When germinated, a further chemical signal from a suitable host plant is required to initiate haustorial formation and active invasion of the host (10).

Germination and initial seedling growth are dependent on metabolism of carbon reserves. In many plant species, triacylglycerol (TG) serves as the major carbon reserve for seed germination (11). In species such as witchweed and castor bean, TG is sequestered in membrane-bound oil bodies or oleosomes that accumulate in the endosperm surrounding the embryo. During germination TG is hydrolyzed to free fatty acids and glycerol by lipase activity at the surface of the oleosomes. Through beta oxidation and gluconeogenesis, the reactive products are eventually converted to sucrose, which is translocated to the embryonic axis and metabolized in support of seedling growth and development (12). Most oilseeds metabolize most TG within seven to eight days after germination is initiated (11).

TG metabolism in witchweed may be a key factor in seedling establishment and/or invasion of host plants. This study provides the first documentation of changes in lipid composition in germinating *Striga asiatica* seeds. Changes in polar membrane lipid indicate rapid formation or increase of plastids after witchweed seeds germinate. However, *S. asiatica* seeds utilize TG slowly and to a lesser extent during germination than other oilseeds.

MATERIALS AND METHODS

Seed pre-germination/germination. To prevent release of witchweed into the environment, this study was conducted under isolated conditions at the North Carolina State University Phytotron in Raleigh, North Carolina. Seeds (obtained from USDA, APHIS, Whiteville, NC) were surface-sterilized with a 2% v/v sodium hypochlorite solution for 15 seconds and then rinsed three times with sterile deionized water. Seed lots (200 mg each) were placed in 100 × 15 mm petri dishes and submerged in 20 ml sterile deionized water before incubation at 28 C and 100% relative humidity. After a two-day pre-germination period, the water in each petri dish was decanted and replaced with sterile deionized water. This procedure removed seed germination inhibitors that may have leached from the seeds (13). Petri dishes were returned to the incubators until day 14 of pre-germination, at which time the water was again decanted and replaced with a 10^{-12} M solution of GR 24 (from USDA, Southern Regional Research Center, New Orleans, Louisiana). Germination occurred within 18 to 24 hr. The 22-day seedling samples remained in the incubator for seven days after germination.

Lipid analysis. Witchweed seeds (1 g dry weight) were harvested at 0, 14, 15 or 22 days after initial imbibition in water and were oven-dried at 60 C for four days. Total oil content was measured by wide-line NMR with a Newport Mark III analyzer (Newport of

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North America, Inc., Villanova, Pennsylvania) equipped with a type 10 magnet and 2.5 ml probe. Oil measurement was based on comparison of NMR response of the weighed sample with the response of known weights of pure liquid vegetable oil.

Total polar lipids (TPL) and triacylglycerol (TG) were extracted and separated according to Carver et al. (14) with the following modifications: 1 g (dry weight) of seeds was homogenized in 10 ml chloroform-methanol (2:1, v/v) using a Virtis homogenizer fitted with a Micro Ultra Shear blade (Virtis Company, Inc., Gardner, New York). The homogenate was filtered with an additional 10 ml volume of methanol. Deionized water (10 ml) was added to the filtrate, which was then held at 4 C for 10 min to allow phase separation. The chloroform phase was collected and dried under nitrogen at 50 C.

TPL and TG were separated from the lipid extracts on thin layer plates of Silica Gel GHR. The developing solvents were petroleum-diethyl ether-acetic acid (80:20:1, v/v/v). Chloroform-methanol-ammonium hydroxide (70:20:1.5, v/v/v) was used to separate individual components of the TPL fraction. All glycerolipids were identified by co-chromatography with standards and visualized under ultraviolet light after spraying plates with 0.2% w/v 2,7,dichlorofluorescein in 95% ethanol. Bands corresponding to the respective polar and neutral lipids were scraped from the TLC plates and extracted by sequential elution with 2.5 ml each of chloroform-methanol (2:1, v/v), chloroform-methanol (1:2, v/v) and methanol. The lipid extracts were dried under nitrogen at 50 C. Fatty acid methyl esters were prepared by methanolysis with Meth-prep II (Applied Science Labs, Deerfield, Illinois). Amounts of each glycerolipid were determined by quantitative GLC analysis of fatty acid methyl ester with a Hewlett Packard 5880A gas chromatograph equipped with a flame ionization detector. Methyl myristate (Supelco, Inc., Bellefonte, Pennsylvania) was included in each sample as an internal standard. The glass column was packed with 10% Silar-10 C on Gas-chrom Q, 100/120. Operating conditions were: 195 C (oven), 250 C injection port and detector, and a nitrogen carrier gas flow of 35 ml/min. All data were reported as the mean of two replications.

RESULTS AND DISCUSSION

Seed germination usually is completed upon radicle emergence (11). However, the germination process in witchweed seed encompassed three distinct periods. The first period, pre-germination, was defined by a 14-day imbibition/incubation interval during which time radicle protrusion was not achieved. The second period, germination, was defined by a one-day interval where radicle protrusion occurred after the stimulant GR 24 was administered. The third period, post-germination, was defined by a seven-day interval from days 15 through 22. The post-germination period normally would end with initiation of haustorial formation upon receipt of a second stimulus from the host plant. However, due to quarantine restrictions this experiment had to be terminated during post-germination.

Glycerolipid content of *Striga asiatica* seeds as determined by NMR, accounted for 37.5% (w/w) of the seed dry weight. In comparison with some agronomic oilseed species, the oil concentration of witchweed seed was quite high, comparable to rape and sunflowerseeds, which contain 40% oil (w/w) (15).

TABLE 1

Changes in Total Polar Lipids (TPL) and Triacylglycerol (TG) Concentration of Ungerminated and Germinated *Striga asiatica* Seeds

Periods	Treatment interval (days)	Glycerolipid	
		TG	TPL
		(% Total fatty acid)	
Initial	0	93.6	6.4
Pregermination	0–14	94.3	5.7
Germination	14–15	94.8	5.2
Post-germination	15–22	82.1	17.9
$LSD_{0.05}$		10.1	10.0

TG content in *S. asiatica* seeds decreased significantly, but only during the post-germination period (Table 1), whereas in many other species a rapid decrease in TG concentration occurs during germination (11). In flax, watermelon and West African oil palm seed, nearly half of the TG could be hydrolyzed in 90 hr (16–18). TG declined 55% within eight days in germinating soybean cotyledons (19); in tobacco seedlings, lipid reserves were consumed totally by the ninth day (20). Over the 22-day study period, no significant change was observed in the double-bond index for TG (mean = 152.6 ± 0.3). The fatty acid composition of TG averaged 11.4 ± 0.3% (16:0); 0.7 ± 0.3% (16:1); 4.4 ± 0.1% (18:0); 16.5 ± 0.1% (18:1); 65.8 ± 0.5% (18:2), and 1.2 ± 0.3% (18:3).

TPL concentration increased significantly in *S. asiatica* seedlings only during the post-germination period (Table 1). Total fatty acid content of the TPL fraction increased 3.2-fold between 15 and 22 days (Table 2). Increased TPL content has been observed in other germinating seeds (21) and was associated with membrane synthesis in the developing seedling. In the present study, amounts of individual fatty acids in the TPL fraction did not change significantly from initial water imbibition through 15 days. However, during the post-germination period there was a 2.8-fold increase in total saturated

TABLE 2

Fatty Acid Content of the Total Polar Lipids (TPL) From *Striga* Seeds and Seedlings

Days incubated	Fatty Acid						
	16:0	16:1	18:0	18:1	18:2	18:3	Total
	μmol fatty acid/g dry weight						
0	29.4	4.2	7.1	14.3	25.0	4.7	84.7
14	22.2	3.2	4.9	12.0	23.5	4.2	70.0
15	17.2	2.8	4.5	10.9	26.6	3.6	65.5
22	47.3	10.2	13.8	37.1	89.7	14.0	212.0
$LSD_{0.05}$	20.9	6.4	6.9	21.3	53.7	8.3	116.1

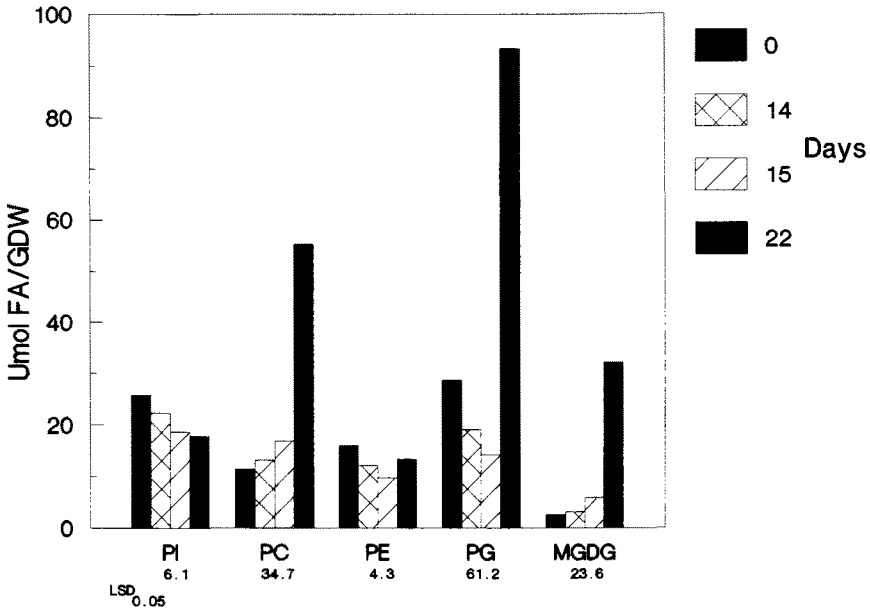

FIG. 1. Classes of polar lipids isolated from *Striga asiatica* seeds (0 and 14 days) and seedlings (15 and 22 days).

fatty acid (16:0 plus 18:0), a 3.4-fold increase in monoene fatty acids (16:1 plus 18:1) and diene fatty acid (18:2), and a 3.9-fold increase in triene fatty acid (18:3). Phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and monogalactosyl diacylglyceride (MGDS) were the major constituents of TPL in seeds and seedlings of *S. asiatica* (Fig. 1). The change of TPL content during the post-germination period was attributed to increase levels of PC (five-fold), PG (three-fold) and MGDG (twelve-fold).

Plastid production has been observed in castor bean endosperm during germination (22). One of the major roles played by plastids during seed germination is the synthesis of fatty acids and glycerolipids for new membranes (23). MGDG and PG represented major lipid constituents in chloroplasts (24) and plastids (25). The major fatty acid constituents of MGDG and PG in witchweed seeds and seedlings (Table 3) were similar to those reported for developing leaf proplastids (25). Hence, the increase in MGDG and PG contents in witchweed seeds during germination, in combination with a general increase in unsaturation of fatty acids, suggested that plastid formation and/or enlargement was initiated after *Striga asiatica* seed germination.

In conclusion, TG represented a major reserve metabolite in witchweed seeds. While there was apparent hydrolysis of TG in witchweed, TG concentration remained constant until the post-germination period. Even then, the relatively slow rate of TG hydrolysis indicated that the energy and carbon requirement for radicle emergence and seedling growth in *Striga* was low. The next major developmental phase/stage for witchweed following germination is the formation of haustoria. The energy and carbon requirement for differentiation of the radicle tips into haustoria, which invade the host, should be greater. Thus, the residual TG in *S. asiatica* after germination and initial seedling growth appears to be conserved until the seedling receives a host signal for haustorial formation and the subsequent invasion of the host. The sequence of events in witchweed seed germination documented in this report differed

TABLE 3

Fatty Acid Composition of Monogalactosyl Diacylglyceride (MGDG) and Phosphatidylglycerol (PG) Isolated From Dry Seed and 7-Day-Old *Striga asiatica* Seedlings

Days incubated		Fatty Acid					
		16:0	16:1	18:0	18:1	18:2	18:3
		Mole %					
0	MGDG	3.6	0.4	1.4	10.9	54.5	29.2
	PG	31.4	9.1	9.1	14.6	31.7	4.1
22	MGDG	3.1	0.2	0.5	10.5	62.0	23.7
	PG	27.7	10.6	7.9	21.1	30.5	2.2

from that of seed without pre-germination or host invasion requirements. Further study of the metabolism of lipids during germination and haustorial formation, in particular, of *Striga asiatica* seedlings, is required in order to better assess the contributions of lipids to these developmental events.

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