Glucose Transfer from Adenosine Diphosphate-Glucose to Starch in Preparations of Waxy Seeds

Abstract. *Preparation of starch granules from the developing seeds of 17 different* waxy *mutants of maize all transfer glucose from adenosine diphosphate-glucose to starch at about one-tenth of the rate of similar preparations from seeds of* non-waxy *maize. The source of most, if not all, of the activity in preparations from* waxy *mutants is a limited number of enzymatically active starch granules from the embryo and maternal tissue of the seed. All starch granules in these tissues appear to be enzymatically active. The endosperm which is the site of most starch synthesis and storage is apparently devoid of transferase activity.*

The *waxy* mutant of maize forms as much starch in the endosperm as does normal or *non-waxy* maize; however, the starch produced in the mutant is entirely amylopectin, while that of the *non-waxy* maize is approximately 75 percent amylopectin and 25 percent amylose. Nelson and Rines (*1*) reported that preparations of starch granules from developing seeds of *waxy* maize lacked the ability to transfer glucose from uridine diphosphophosphate-glucose (UDP-glucose) to starch while preparations from *non-waxy* maize were active. They suggested two different pathways of starch formation with the formation of amylose being by way of the transferase system bound within the starch granule. This is the same enzyme reported by Leloir *et al.* (*2*) in starch granule preparations from beans, potatoes, and sweet corn. Recondo and Leloir (*3*) have shown subsequently that glucose is transferred from adenosine diphosphophosphate-glucose (ADP-glucose) more rapidly than from UDP-glucose and that ADP-glucose is probably the natural substrate for the enzyme system. We have found that preparations of starch granules from *non-waxy* maize transfer glucose from ADP-glucose 2.5 to 3 times as rapidly as from UDP-glucose.

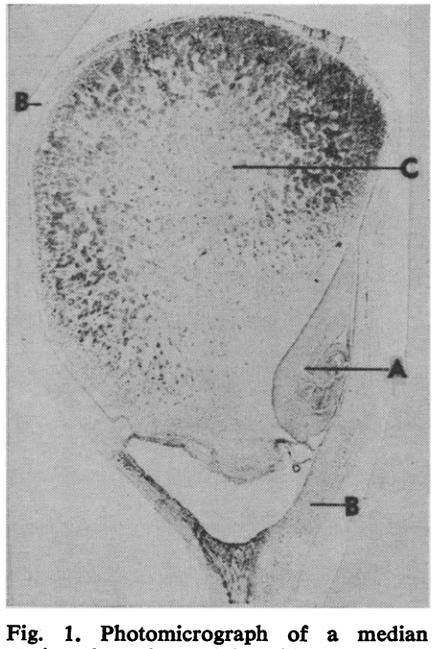

Fig. 1. Photomicrograph of a median section through a 16-day-old *waxy* maize seed stained with a potassium iodide-iodine solution. *A*, Developing embryo; *B*, material tissue; *C*, endosperm.

Frydman (*4*) reported that when ADP-glucose is used as a substrate, preparations of starch granules from seeds of *waxy* maize do show a small amount of activity (about one-fifth of that of a preparation from *non-waxy* maize in the same test). The low activity of *waxy* maize preparations with ADP-glucose as a substrate had been noted in this laboratory also. Preparations of starch granules from 17 different *waxy* mutants that had occurred as separate mutational events all showed a similar amount of transferase activity (about one-tenth of the activity of a *non-waxy* strain used as a standard). This report shows that most, if not all, the enzymatic activity in preparations from *waxy* mutants derives from a limited number of enzymatically active starch granules coming from the sporophytic tissue of the developing seeds. The endosperm which is the important tissue for starch synthesis and storage apparently lacks transferase activity.

About 3 percent of the starch granules in all preparations made from developing *waxy* seeds stain dark blue (indicating amylose) with a potassium iodide-iodine solution. The source of these starch granules is the embryo and maternal tissue of the seeds (Fig. 1). The seed of a corn plant is a mixture of cell types of two different generations. The embryo is sporophytic tissue of the new generation. The pericarp, ovary wall, and integumental remains are wholly derived from the maternal parent and are sporophytic tissue. The morphological nature of the endosperm has been disputed among botanists. In genotype, it is triploid with two genomes from the maternal parent and one from the paternal parent. When median sections are made through *waxy* seeds 16 days after fertilization, and are stained with a potassium iodide-iodine solution, all the starch granules in the embryo and maternal tissue stain the deep blue characteristic of granules containing amylose. All the starch granules in the endosperm, however, stain the brown color typical of granules containing amylopectin, although Lampe (*5*) reported that in 38-day-old *waxy* seeds, there were a small number of blue-staining starch granules in the late-maturing cells at the base of the endosperm.

Weatherwax (*6*) showed the starch granules in embryos of seeds homozygous for the *waxy* mutant to be blue-staining. Only pollen grains (*7*), embryo sac (*8*), and endosperm (*6*) contain starch granules that stain brown (no amylose present).

Since the starch granules of the embryo give a blue color with the iodine stain, it might be expected that these starch granules would have transferase activity. In a 16-day-old seed, the embryo can easily be excised intact, although it is difficult to collect sufficient embryos to process since the embryos are only approximately 1/100th of the seed by wet weight. When starch granules are isolated from the embryos of seeds of *waxy* and *non-waxy* strains, the transferase activity of the preparations from *waxy* strains is somewhat higher than similar preparations from

Table 1. The release of ADP from ADP-glucose in preparations of starch granules. Values for *waxy* and *non-waxy* mutants are expressed as mμmole of ADP per milligram of starch granules. Each value is the average of four determinations of two separate preparations.

Preparation	*Non-waxy*	*Waxy*
Whole seeds	29	3.4
Seeds without embryos	26	2.4
Embryos alone	84	128.0

For assay, the starch-granule preparation, 2.5 mg (except 1 mg of preparation from embryos), was incubated with 25 μl of a glycine-NaOH buffer at *p*H 8.4 containing 0.31 μmole of ADP-glucose, 6.85 μmole of glycine, and 0.17 μmole of ethylenediaminetetraacetic acid. After incubation for 30 minutes at 37°C, 25 μl of a 0.01*M* phosphoenolpyruvate solution and 25 μl of a pyruvate kinase solution containing about 26 enzyme units per milliliter were added. The mixture was incubated for 15 minutes more before being stopped by the addition of 0.15 ml dinitrophenylhydrazine solution (0.1 percent in 2*N* HCl). Thus the total reaction time for the transferase system was 45 minutes. This is a modification of the assay procedure developed by Leloir *et al.* (*2*).

non-waxy seeds (Table 1), and both have considerably higher activity than whole seed preparations from *non-waxy* seeds. Table 1 shows also that although the activity of preparations from *waxy* seeds from which the embryos have been removed is significantly reduced as compared with whole seed preparations, there is still measurable activity. Microscopic examination of such preparations after staining shows that 2.5 percent of the starch granules they contain stain blue, and these are sufficient to account for the transferase activity if it is assumed that the remaining blue-staining starch granules have the same activity as those isolated from the embryos. The maternal tissue surrounding the endosperm cannot be cleanly separated from the endosperm and must be pared away by a series of cuts. In a starch-granule preparation made from blocks of endosperm tissue so obtained from *waxy* seeds, 0.4 percent of the granules stained blue because of remnants of maternal tissue; such preparations also showed much less activity (0.25 mμmole ADP released per milligram of starch).

Table 1 shows a greater absolute loss of activity in enzyme preparations from embryectomized *non-waxy* seeds than from embryectomized *waxy* seeds. The embryos of *waxy* and *non-waxy* seeds constitute the same proportion of the seed by wet weight, and the preparations of *non-waxy* embryos have somewhat lower activity than do the preparations from *waxy* embryos. By using mixtures of starch granules from *non-waxy* embryos and *non-waxy* embryectomized seeds, reconstitution experiments were conducted which failed to show any synergistic effects that could account for the greater activity loss in preparations from *non-waxy* embryectomized seeds. It is possible that during the embryectomy of *non-waxy* seeds, tissue (the endosperm) which contains starch granules with enzymatic activity is exposed, and that there is some loss of activity during collection of sufficient material for processing. Since there are apparently no starch granules with enzymatic activity in the endosperm of *waxy* seeds, the only loss of activity in preparations from embryectomized seeds is that directly attributable to the loss of the embryo.

We have not been able to prepare starch granule samples from the endosperms of *waxy* seeds without some contaminating blue-staining granules from the closely adherent maternal tissue. Thus we cannot demonstrate that starch granules from the endosperm are devoid of transferase activity. But it is demonstrable that whole seed preparations of *waxy* stocks have a low percentage of blue-staining granules that have high transferase activity. Methods of preparation that reduce the number of blue-staining granules (embryectomy, for example) reduce transferase activity almost proportionately. We suggest that all transferase activity noted in whole seed preparations from *waxy* seeds is referable to these enzymatically active starch granules from the embryo and maternal tissue and that the endosperm starch granules do not possess the transferase system. Thus, there must be another system or other systems conditioning the formation of α 1–4 linkages in amylopectin as originally suggested (*1*).

This unique distribution of enzymatically active starch granules in the seeds of *waxy* mutants raises an interesting problem in the regulation of enzyme synthesis. If there is a single structural gene for this enzyme, ADP-glucose-starch glucosyl transferase, then in *non-waxy* plants it functions in both gametophytic and sporophytic tissue but in *waxy* plants, only in the sporophytic tissue. Alternatively, there could be two structural genes for transferase, one of which is active in sporophytic tissue but inactive in gametophytic tissue and the other inactive in sporophytic tissue, but active in gametophytic tissue. Then all the 30 known *waxy* mutants represent mutations in the latter structural gene.

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9. We thank S. N. Postlethwait and Evart Cable for their assistance with the sectioning and photomicrography. Supported by NSF grant GB-1073.

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Photoflashes: A Potential New Tool for Control of Insect Populations

Abstract. *Imported cabbageworms,* Pieris rapae (*L.*), *were reared in cans exposed to light from fluorescent lamps for 10 hours daily. When larvae were exposed to daily electronic photoflashes scheduled 3 to 4 hours after the fluorescent lamp was turned off, pupae failed to diapause. Effective wavelengths are lower than those controlling photoperiodism in plants. The effective energies are about 1 joule per square centimeter.*

Reduction of overwintering insect populations is of practical value in the control of the boll weevil (*1*) and has been a part of good farming practice for years. In the temperate zone many insects survive the rigors of winter in a diapausing state which is controlled by hormones. The possible use of hormones in insecticidal formulations has been considered (*2*). However, the use of photochemical reactions to regulate changes in hormones within the insect has not been considered as a means of control. It would be simpler to reach certain insects with light than with insecticidal formulations, and the

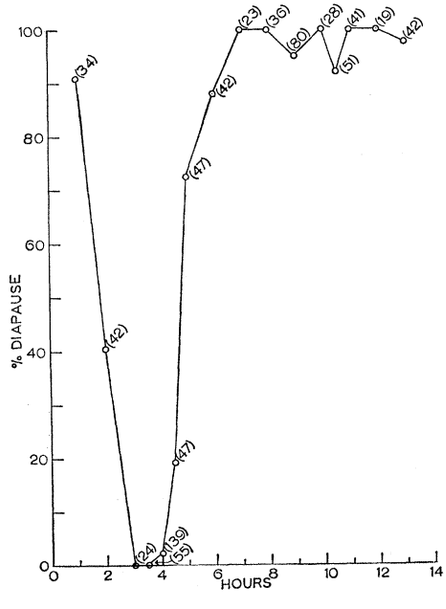

Fig. 1. The incidence of pupae in diapause when photoflashes are applied daily to *Pieris rapae* larvae at varied schedules after 10 hours of light. "Hours" are measured from the end of the 10-hour light period. The parentheses enclose numbers of insects tested at each flash schedule. Larvae were held at 17° to 20°C and fed collard leaves.