for proline reflects a basic disorder in metabolism which significantly influences the formation of immature collagen in osteogenesis imperfecta cannot be answered from this study. A hydroxylating defect could not be demonstrated by the present techniques. It is noteworthy, however, that proline, one of the principal constituents of the polypeptide chain of collagen and also the precursor of hydroxyproline, should have a low tolerance curve when the amino acid is administered orally (11). GEORGE K. SUMMER

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Longevity of Fusarium oxysporum in Soil Tube Culture

Abstract. In soil tube culture, representatives of three biologic forms of Fusarium oxysporum survived unchanged morphologically for 11 years or more. An isolate of the muskmelon wilt fungus remained viable after 17 years' storage in dry air at a temperature of from 3° to 4°C. The surviving unit was found to be the chlamydospore.

The traditional method of maintaining fungus cultures by making transfers from them at frequent intervals to artificial media is laborious and timeconsuming and is not satisfactory for maintaining organisms unchanged for long periods of time. Other more or less dependable methods have come into use during the last 30 years, but some of them are applicable only to specific organisms.

Maintenance of fungus cultures in tubes of sterilized soil has found acceptance among some plant pathologists. Miller et al. (1) showed that when the muskmelon wilt fungus Fusarium oxysporum f. melonis (L. and C.) Snyder and Hansen and other fusaria are maintained on agar, the "wild type" is rapidly displaced by mutants. He reported success in maintenance of the "wild type" and retention of viability of the melon pathogen after it had been cultured for 15 months in soil tubes. Gordon (2) affirmed the advisability of using soil culture as a means of preserving Fusarium species. Atkinson (3) found that representatives of six of 32 genera of fungi were viable after 5 years in dried soil culture. Members of F. oxysporum were found in the surviving group.

The study reported here was specifically concerned with an investigation of the longevity and the means of survival in soil culture of isolates of F. oxysporum causing wilt of three different hosts.

From 1946 until 1953, stock cultures of Fusarium oxysporum f. melonis were prepared, and from 1948 until 1953, cultures of F. oxysporum, f. lycopersici and f. niveum, the causal agents of wilt of tomato and watermelon, respectively. The following procedure was used. Test tubes of 25-cm³ capacity were twothirds filled with sandy soil containing 10 percent of muck. The mixture was saturated with water, and the tubes were plugged and autoclaved. The fungi were single-spored, after 3 days' growth, from platings of infected plant tissue on 2-percent potato dextrose agar. When monosporous cultures were 3 or 4 days old, a small weft of mycelium from the periphery of the colony was transferred to the soil tubes. The resultant cultures were allowed to incubate in the laboratory at room temperature in a diffuse diurnal light for from 2 to 4 weeks before they were transferred to a refrigerator for storage at from 3° to 4°C.

Recently, the viability of many of the stock cultures was determined by plating soil suspensions (0.01 to 0.04 g) on modified Martin's peptone agar а medium, as described by Snyder et al. (4). The results are given in Table 1.

Table 1 shows that the three biologic forms of the soil-borne, wilt-producing fungi showed varying capacity for survival. One isolate from muskmelon showed exceedingly high survival after 13 years, and another, appreciable survival after 17 years. A few cultures contained no viable units. Some cultures

were found to be contaminated with bacteria. The presence of the latter, however, did not appear to be the reason for nonsurvival of the fungus, because several bacterial-contaminated cultures showed high viability of the pathogen after 13 years.

Microscopic examination of culture plates inoculated with soil suspensions revealed that the fungus was surviving as chlamydospores. These findings are in agreement with those of Warcup (5)and Nash et al. (6), who isolated other species of Fusarium directly from the soil and found the surviving unit to be a chlamydospore.

general, chlamydospores In are spherical, varying in diameter from 9 to 14 μ (average, 12.6 μ). The wall of the chlamydospore varies from smooth to slightly warty. Chlamydospores are found to contain from one to three oil globules. The chlamydospores occur singly and are usually firmly embedded in particles of organic matter, and often it is necessary to crush the particles before plating to determine the characteristics of the chlamydospore. On germination, chlamydospores have been observed to produce a single germ tube.

Growth of chlamydospores on various culture media showed that isolates of the three biologic forms of Fusarium oxysporum survived the long storage periods morphologically unchanged. Furthermore, comparative tests with fresh isolates showed that stored cultures of these three fungi survived without loss of pathogenicity.

Table 1. Survival of *Fusarium oxysporum* after storage in soil tubes at 3° to 4° C.

Isolate	Number of years in storage	Number of surviving units per gram of soil		
F.	oxysporum	f. melonis		
Miller*	17	200		
McKeen	15	1,200		
Waters	13	3,300		
Waters	13	134,000		
Robinson	121/2	7,400		
Quick	121/2	4,400		
Wigle	121/2	200		
Setterington	121/2	74,000		
Murray	8	32,000		
Larabee	8	300		
F_{i}	. oxysporum f.	lycopersici		
Miller*	17	0		
McKeen	12	95,600		
Tecumseh	10	9,000		
F	. oxysporum	f. niveum		
Waters	11	33,000		
Tingen	11	22,000		
*T1		A L X X MORE		

*These cultures were prepared by J. J. Miller at the Harrow Laboratory in 1944.

The results of this study (7) not only demonstrate the usefulness of a long-term preservative method of maintaining biologic forms and races of F. oxysporum but indicate that maintenance of the fungi as the original "wild type" is due to survival as a dormant propagule.

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- Photoperiodic Response of an **Albino Mutant of Einkorn Wheat** in Aseptic Culture

Abstract. An albino mutant of einkorn wheat which lacks plastid pigments was cultured in a test tube on nutrient agar medium containing 8 percent sucrose, under long and short photoperiods. The plants showed typical photoperiodic responses to long and short days, suggesting the presence of a pigment system, other than plastid pigments, which is sensitive to dim radiation.

In studies of photomorphogenesis, it would be desirable to separate the individual pigments or pigment systems from the whole-light-absorbing pigments in the tissues or organs concerned, since each pigment is expected to have a different function in the process of photomorphogenesis. Thus, albino or abnormally colored plants which lack some of the pigments as a result of spontaneous or artificial mutations may serve as useful experimental materials. Under field conditions, however, it is difficult to sustain the growth of albino plants until flower initials are formed. I succeeded in growing spring wheat to flower initiation in total darkness on nutrient agar media in test tubes (see 1).

The experiment reported here was conducted to test the response of the albino plants of einkorn wheat to long and short photoperiods by means of aseptic culture in test tubes.

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The material used was an x-rayinduced mutant strain of einkorn wheat (2). In germination, the strain segregates normal green and albino seedlings in the ratio of 3 to 1. The plastid pigments of seedling leaves were analyzed according to the method of Koski and Smith (3). Neither chlorophylls nor carotenoids were found under the experimental conditions described elsewhere (4) (Fig. 1).

The culture medium, which contained modified White's minerals (1), 8 percent sucrose, 1 percent dried brewer's yeast in suspension, and 0.8 percent agar, was poured into test tubes (180 by 18 mm) in the amount of about 10 ml per tube. All the test tubes were then autoclaved at 1.2 kg above atmospheric pressure for 15 minutes.

The photoperiodic treatments were given by automatic artificial illumination from 20-watt fluorescent daylight tubes and a 40-watt incandescent lamp. The luminosity at plant level was about 1200 lux in 8 hours for a short day. For a long day, the illumination was supplemented to the extent of about 100 lux by using the incandescent lamp only for the remaining 16 hours.

The plants were grown at a temperature of $25 \pm 2^{\circ}C$ for 8 hours at the higher intensity and at a temperature of $20 \pm 2^{\circ}C$ for the 16 hours of lowerintensity illumination.

The seeds heterozygous for albino were sterilized with 10-percent chlorinated lime for 30 minutes and washed with sterilized distilled water. Then they were placed on the nutrient agar medium in the test tubes. About 10 days after sowing, the germinated albino and green segregants, which were kept at $25 \pm 2^{\circ}$ C in light, were steeped in the agar medium in order to have a better chance of absorbing the nutrients through the leaf surface.



Fig. 1. Absorption spectra of ether extracts from leaves of normal green (solid circles) and albino (open circles) seedlings grown at 26°C.

All the plants were then subjected to the photoperiodic and temperature conditions mentioned above. Observation was made on days 67 and 107 after germination.

Table 1 shows that the albino plants, which are free of the plastid pigments, respond to the photoperiods just as the normal green plants do. Albino plants subjected to the long-day treatment produced flower primordia, and flower initiation was inhibited by the short day.

The sensitivity to light of the albino plant is, however, somewhat less than that of the normal green plant. The number of leaves formed on the main axis prior to flower initiation, which is generally considered a criterion of flowering response, is significantly greater in the albino plants than in green plants under the inductive long photoperiod.

The net accumulation of dry matter in the shoot is greater under the long photoperiod than under the short day

Table 1. Cultures of normal green and of albino wheat plants in test tubes under long- and short-day photoperiods.

Туре	Plants observed (No.)	Plants with flower initials (%)	Leaves formed on main axis (No.)	Length of stems (mm)	Flowering stages*	Dry wt of shoots (mg)
			Long days (N, 67)		
Green	16	100	7.94 ± 0.17	128.6 ± 6.0	3.1 ± 0.1	43.1 ± 3.1
Albino	16	100	8.63 ± 0.12	45.1 ± 8.0	3.0 ± 0.2	41.3 ± 2.2
			Short days (N, 67)		
Green	12	0	$> 8.33 \pm 0.19$	†	0	31.9 ± 1.7
Albino	12	0	$> 8.80 \pm 0.29$	†	0	31.6 ± 1.9
			Short days (N, 107)		
Green	7	0	$>11.00 \pm 0.57$	*	0	73.3 ± 10.1
Ibino	11	0	$>12.09 \pm 0.30$	Ť	0	54.8 ± 5.9

* The stage was arbitrarily assigned, from 0 to 5, to correspond to the completely vegetative state and heading, respectively (6). † All plants were rosetted, and stem elongation was not