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Phytochrome Destruction

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ABSTRACT

Phytochrome destruction begins immediately following actinic irradiation of 4-day-old, dark-grown oat (*Avena sativa* L., cv. Garry) shoots grown in open containers. When grown in closed containers, otherwise identical oat shoots exhibit a delay of about 40 minutes between irradiation and the onset of destruction. This delay can be attributed to accumulation of ethylene by several criteria, including elimination of the delay by mercuric perchlorate. These data provide an explanation for otherwise contradictory observations concerning the presence of a delay prior to the onset of destruction.

Phytochrome destruction is the loss of both spectrophotometrically and antigenically detectable phytochrome in etiolated plants as a consequence of the photoconversion *in situ* of Pr to Pfr (10). The onset of phytochrome destruction following a brief actinic irradiation has been reported to occur both with (*e.g.* 5, 6, 11) and without (*e.g.* 4–6, 8) an intervening time delay. In at least two instances, this delay was shown to occur only in very young tissue that had not yet developed the capacity to destroy phytochrome (5, 6). In other instances, an apparent discrepancy exists, with no explanation for why some investigators observed a delay while others did not.

We present here evidence that at least some of these contradictory observations are a function of whether plants are grown in closed or open containers and that the effect of the closed containers is to permit ethylene accumulation.

MATERIALS AND METHODS

Oats (*Avena sativa* L., cv. Garry) were germinated and grown for 4 days in darkness at 25 ± 1 C on moist cellulose packing material (Kimpak 6234, Kimberly Clark) in open cafeteria trays (35 × 45 cm) (100 g oats), plastic buckets (27 × 30 × 12 cm) (75 g oats), or enameled metal pans (20 × 31 × 19 cm) (40 g oats). The buckets or pans were occasionally covered by aluminum foil or Celgard 2400, a polypropylene film 25 μm thick with 38% porosity and pores typically 20 nm in diameter (Celanese Plastics Co.). In some experiments, two plastic trays (31 × 7 × 1 cm), each containing a strip of Kimpak saturated with 100 ml of 5% mercuric perchlorate, were suspended in a plastic bucket. Humidity in the growth room was kept near saturation. Except for actinic irradiations, tissue was handled under green safelights (9).

Actinic red light was obtained from six unfiltered 40-w Gro-lux fluorescent lamps (Sylvania) spaced at 10-cm intervals and placed 20 cm above the tissue to be irradiated. The photostationary equilibrium, which was established in about 5 sec, was found to be the same as for 660 nm light obtained with a Balzer B-40 interference filter.

Phytochrome was assayed spectrophotometrically utilizing 0.60-g shoot samples and a custom built, dual wavelength spectrophotometer (666 *versus* 727 nm) as described by Kidd and Pratt (5). The spectrophotometer, with a 9658R photomultiplier (EMI Gencom Inc.), had been modified for automatic operation. Within an experiment, the same number of shoots were used to prepare each sample to minimize biological variability.

Regression lines, correlation coefficients, standard errors, and tests of the null hypothesis that two regression coefficients are estimates of a common slope were computed as described by Steel and Torrie (12).

RESULTS

Phytochrome destruction began immediately after a 4-min red irradiation of etiolated plants grown on open trays while an appreciable lag was observed using plants that were grown in plastic buckets covered with aluminum foil (Fig. 1). In both cases, destruction continued until about 25% of the initial phytochrome level remained, exhibiting pseudo-first order kinetics. A regression line for data (to 160 min) obtained with plants grown in open buckets extrapolates to the 100% photoreversibility value at 4 min, with a $t_{1/2}$ of 90 min. A regression line for points between 50 and 160 min, using plants from closed containers, extrapolates to 100% photoreversibility at 40 min, with a $t_{1/2}$ of 70 min. Comparison of the slopes for regression lines of points before ($t_{1/2}$ = 466 min) and after ($t_{1/2}$ = 70 min) 50 min, using plants from closed containers, indicates that the differences between them are highly significant (t_{13} = 8.10; $P < 0.0001$). Plants grown in aluminum foil-covered, enameled metal pans gave the same results as those grown in covered plastic buckets (data not shown).

Plants grown in plastic buckets covered with Celgard exhibited no delay before the onset of destruction (Fig. 2). A regression line of these data points extrapolates to 100% photoreversibility at −5 min, with a $t_{1/2}$ of 110 min, while points beyond 40 min for the time course determined with plants grown in buckets covered with aluminum foil give a regression line that extrapolates to 100% photoreversibility at 39 min. With trays containing mercuric perchlorate suspended in the closed buckets, no lag prior to destruction was observed (Fig. 2). A regression line through these points extrapolates to 100% photoreversibility at 1 min, with a $t_{1/2}$ of 130 min. Slopes obtained by regression analyses of points during the first 45 min and through the second 45-min interval are not different (t_{10} = 0.141; $P < 0.5$). Mercuric perchlorate had no apparent effect on germination, growth rate, or morphology.

When peas (*Pisum sativum* L., cv. Alaska) were grown in aluminum foil-covered plastic buckets in a manner identical to the oats, they exhibited morphology indicative of ethylene accu-

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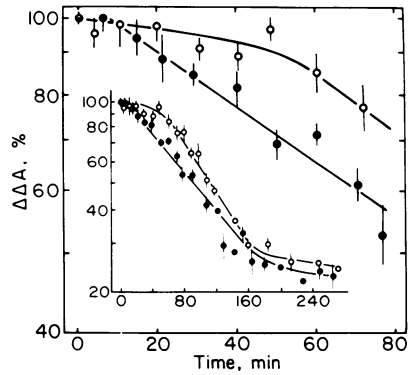

FIG. 1. Phytochrome destruction in etiolated oat shoots grown in aluminum foil-covered plastic buckets (○) or on cafeteria trays (●). Photoreversibility (100% = 0.049–0.078 *A*) was measured as a function of time at 25 C after a 4-min red actinic irradiation. Inset shows destruction time courses over a longer time period. Time courses represent average ± SE of 5 (●) or 7 (○) independent experiments.

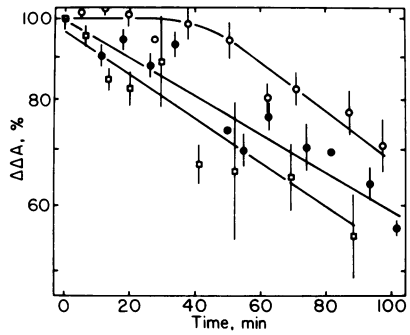

FIG. 2. Phytochrome destruction in etiolated oat shoots grown in plastic buckets covered with aluminum foil (○), covered with Celgard (□), or covered with aluminum foil but with trays containing mercuric perchlorate enclosed (●). Photoreversibility (100% = 0.040–0.043 *A*) was measured as a function of time at 25 C after 1-min actinic red irradiation. Time courses represent the average ± SE of 3 (□, ●) or 7 (○) independent experiments.

mulation: very short shoots (1 *versus* 8 cm for controls grown in open buckets); swollen bases; and loss of positive geotropism, with roots growing upward above the cellulose pad (3).

DISCUSSION

The apparent inconsistencies concerning whether or not a delay follows photoconversion of Pr to Pfr and precedes the onset of phytochrome destruction may be ascribed, in at least some instances, to growth of plants under conditions that restrict gas exchange (Fig. 1). Some investigators who have reported a delay (*e.g.* 2, 11) grow plants in closed containers while those who have observed no delay (*e.g.* 4, 5) typically use open containers. It may be concluded that the effects of growing plants in closed containers is probably a consequence of the accumulation of ethylene because: (*a*) oats are known to emanate ethylene (7); (*b*) peas, grown under conditions that lead to a delay prior to the onset of destruction in oat shoots, exhibit ethylene-induced morphogenic responses, indicating that ethylene accumulates in the buckets covered with aluminum foil; (*c*) the use of a covering material (Celgard) that permits gas exchange eliminates the delay (Fig. 2); and (*d*) mercuric perchlorate, at a concentration that quantitatively adsorbs ethylene from the atmosphere (1), eliminates the delay in oats grown in closed containers (Fig. 2). That the plants, not the buckets, are responsible for inducing the delay in destruction is demonstrated by the occurrence of an identical delay using plants grown in enameled metal pans. It is unlikely that a gas other than ethylene, *e.g.* CO_2 or another gaseous hydrocarbon, is responsible for the effect described here because of the specificity of mercuric perchlorate (13).

Although ethylene is apparently responsible for some reported delays prior to the onset of destruction (11), it is not involved in all reports of a delay. For example, demonstrations that very young plants do not possess the capacity to destroy phytochrome (5, 6) utilize tissue grown in open containers and therefore should not reflect the problem pointed out by these experiments. Use of open containers may still pose a problem if other sources of ethylene contamination are present, in which case flowing gas systems with ethylene scrubbers may be necessary.

Since closed containers should be expected to accumulate ethylene during plant growth, and since ethylene has a significant effect on the ability of plants to destroy phytochrome, destruction experiments utilizing plants grown in closed containers require reevaluation. Since the rate of loss of ethylene during experimental manipulation of plants grown in closed containers probably varies between experimental and control plants and from one experiment to the next, the data presented here reinforce the argument that growth of plants in closed containers should be avoided.

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