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Effects of Cycling Temperatures on Fiber Metabolism in Cultured Cotton Ovules[1]

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ABSTRACT

The effects of temperature on rates of cellulose synthesis, respiration, and long-term glucose uptake were investigated using cultured cotton ovules (*Gossypium hirsutum* L. cv Acala SJ1). Ovules were cultured either at constant 34°C or under cycling temperatures (12 h at 34°C/12 h at 15-40°C). Rates of respiration and cellulose synthesis at various temperatures were determined on day 21 during the stage of secondary wall synthesis by feeding cultured ovules with [^{14}C]glucose. Respiration increased between 18 and approximately 34°C, then remained constant up to 40°C. In contrast, the rate of cellulose synthesis increased above 18°C, reached a plateau between about 28 and 37°C, and then decreased at 40°C. Therefore, the optimum temperature for rapid and metabolically efficient cellulose synthesis in Acala SJ1 is near 28°C. In ovules cycled to 15°C, respiration recovered to the control rate immediately upon rewarming to 34°C, but the rate of cellulose synthesis did not fully recover for several hours. These data indicate that cellulose synthesis and respiration respond differently to cool temperatures. The long-term uptake of glucose, which is the carbon source in the culture medium, increased as the low temperature in the cycle increased between 15 and 28°C. However, glucose uptake did not increase in cultures grown constantly at 34°C compared to those cycled at 34/28°C. These observations are consistent with previous observations on the responses of fiber elongation and weight gain to cycling temperatures in vitro and in the field.

The cotton fiber is a single elongated epidermal cell of ovules of *Gossypium* species. Fiber development is typically divided into two stages, primary wall formation (to accomplish elongation) and secondary wall synthesis (to accomplish fiber thickening) (24). The rate and extent of primary and secondary wall synthesis are of considerable agricultural interest and present intriguing biochemical and developmental problems. Cotton is native to tropical regions and, despite intensive breeding and domestication, is sensitive to cool temperatures. Temperatures less than 16°C essentially halt cotton growth (reviewed in ref. 3), temperatures less than 30°C reduce root and shoot growth in young seedlings (3), temperatures less than 27°C hinder the deposition of cellulose in the secondary wall that is required for fiber maturation (10, 17), and temperatures less than 25°C are low enough to induce resistance to the deleterious effects of subsequent exposure to 5°C in cotton seedlings (20). The research reported here is focused on the period of secondary wall deposition because of the adverse effect of cool night temperatures in northern cotton growing regions on fiber cell wall thickening, which is a major determinant of crop quality and value (10). Although general mechanisms explaining the inhibitory effects of low, nonfreezing temperatures on plant growth have been suggested (14), specific mechanisms by which cellulose synthesis in cotton is decreased are not known. Nor is it known if cellulose synthesis is affected similarly or differently than overall metabolism. One or more factors required for cellulose synthesis could be disrupted by cool temperatures, including (a) production, transport, or uptake of substrate (7, 9); (b) provision of sufficient energy through respiration; (c) function of enzymes due to membrane phase changes (18, 27) or direct kinetic effects (13); and (d) function of the endomembrane system and cytoskeletal elements.

One goal of our research is to understand the physiological basis of cool temperature effects on cotton fiber development. It is possible that identification of relevant mechanisms will allow future genetic engineering of cotton and other agriculturally important plants for improved yield under the diurnal temperature cycles that are frequently encountered in temperate agricultural regions. This mechanistic research has been greatly simplified by use of cultured ovules (2) of *Gossypium hirsutum* L. cv Acala SJ1, which have been shown to be valid models for the field response because secondary wall structure and accumulations of fiber length and weight respond similarly to diurnal temperature fluctuations in vitro and in the field (17). Cultured ovules also offer the advantages of (a) ease of manipulation of temperature conditions in incubators; (b) ability to distinguish temperature effects specific to ovules and fibers from those that depend on "whole plant" physiology; and (c) ability to determine biosynthetic rates by feeding the ovules radiolabeled substrate (6). This article describes the effects of cool temperature cycling on rates of cellulose synthesis, respiration, and long-term glucose uptake in cotton ovule cultures. For comparison, data on the effects of high temperature cycling are also included.

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MATERIALS AND METHODS

Cotton Ovule Culture

Ovules from cotton (*Gossypium hirsutum* L. cv Acala SJ1) ovaries harvested 1 d after flower opening were used for the preparation of floating ovule cultures as previously described (17). To reduce variability between replicate observations, ovules from different ovaries and locules were randomized in culture vessels. The effects of cool temperatures on secondary wall synthesis were determined in cultures grown at 34°C for 18 d and then exposed to cycling temperatures (12 h at 34°C/12 h at 15–40°C). On day 21 (during the exposure to the fourth experimental temperature cycle), ovules were labeled by incubation with [U-^{14}C]glucose as described below. It has been previously established that fibers of cultured ovules grown at constant 34°C are actively engaged in secondary wall deposition between days 12 and 28 (6, 17). The ovules were initially cultured at the control temperature of constant 34°C (1) to allow comparison of fibers close to the same stage of development; cycling from the onset of culture delays development compared to 34°C constant cultures (17). Parallel experiments were performed at the stage of fiber primary wall deposition by culturing ovules for 7 d at constant 34°C, cycling as described above, and labeling on day 10.

Radiolabeling of Ovule Cultures

Ovules were labeled by adding [U-^{14}C]glucose to the culture medium (1.324 μCi/mL; final specific activity 2.45×10^3 Bq/μmol) as described by Montezinos and Delmer (21), except that the final glucose concentration was 10 mM. For labeling, ovules were removed from culture flasks, rinsed free of excess glucose by floating the ovules in Petri dishes containing glucose-free medium, and briefly blotted on paper towels. For dead controls, ovules were heated to 100°C for 10 min, and then carried through all manipulations. Six ovules were transferred to 60 × 15 mm plastic Petri dishes containing 4 mL of labeled medium. The rinse medium and labeled medium were previously equilibrated at the experimental temperature. The Petri dishes were placed in the bottom of 300-mL sealed plastic beakers. Times of labeling sometimes varied for experimental purposes, as indicated in the figure legends, but the validity of a 4-h standard labeling time was established by labeling for 15 min to 6 h at 34, 28, 22, and 15°C.

To test whether cycled ovules resumed control rates of cellulose synthesis and respiration during each period of rewarming, ovules cycled at 15, 22, and 28°C were labeled for 4 h beginning 6 h after rewarming to 34°C. To determine the time courses of recovery of cellulose synthesis and respiration from 15°C, cultures were labeled at 34°C for 45 min during successive hours from 1 to 6 h after return to 34°C from the 15°C part of the temperature cycle. A 34°C constant culture was assayed during the 1st and 6th hours as a control. (A preliminary experiment showed that similar values were obtained for a 34°C constant culture assayed during each successive hour from 1 to 6 h.)

Determination of Cellulose Synthesis

Following labeling, ovules were rinsed in three changes of distilled water then processed differently for various experiments. When ovules and fibers were to be extracted together, which was the typical case, the ovules were frozen in liquid nitrogen, freeze-dried, and weighed. The distribution of radioactivity between ovule tissue and fibers at different temperatures was determined by mechanically separating fibers from ovule tissue before freezing. Differences in labeling between aerial fibers and submerged fibers were determined by plucking small samples from the top and bottom, respectively, of the ovules prior to freezing.

Radiolabeled crystalline cellulose was isolated by extracting the samples in 5 mL of acetic-nitric reagent for 2 h at 100°C (29). Acetic-nitric insoluble material was collected by filtration onto Whatman GF/C glass fiber filters and rinsed five times with distilled water. The filters were added to 2.75 mL of Scintiverse BOA scintillation cocktail (Fisher Scientific) and counted in a Beckman LS 7500 scintillation counter. Cellulose synthesis was expressed as counts per minute incorporated per milligram dry weight of tissue per 4 h (unless a variable time is indicated in the figure legend).

Determination of Respiration

Respiration was determined by trapping $^{14}CO_2$ in 1 N KOH. Two 1.5-mL microcentrifuge tubes, each containing 1 mL of 1 N KOH, were taped at the same height to opposite sides of the sealed beakers. At the end of the labeling period, 250-μL aliquots were removed from each tube and analyzed by scintillation counting as described above. The addition of KOH to the scintillation fluid caused transient chemiluminescence, so samples were not counted until 18 h or more after mixing. The counts per minute from both traps of a single beaker were summed and multiplied by four to reflect the aliquot measured. Respiration was expressed as counts per minute incorporated into CO_2 per milligram dry weight of tissue (as determined above) per 4 h (unless a variable time is indicated in the figure legend).

Measurement of Glucose Uptake

The uptake of glucose by ovule cultures was inferred by monitoring the disappearance of glucose from the culture medium. For these experiments, 25 ovules were cultured in 50 mL of medium (initial glucose concentration of 22 g/L) in a 250-mL flask. To allow comparison of ovules near the same physiological age as in radiolabeling experiments, ovules were incubated for 18 d at constant 34°C before being exposed to cycling temperatures. Since an early period of constant high temperature is not likely to be encountered in the field, ovules were also incubated at cycling temperatures from the beginning of culture to parallel field conditions more closely. Small aliquots (20 μL) of the culture medium were aseptically collected at 3- to 6-d intervals and stored frozen until required for analysis. Glucose concentrations were determined using an enzyme based assay (Trinder assay, Sigma Chemical Co.).

Statistical Treatment

All experiments were repeated at least twice with similar results. As indicated in each table or figure legend, the reported results are of two types: (a) a representative experiment with SDs of 3 to 5 data points indicated; or (b) a compilation of three separate experiments with data points collected from each experiment normalized as percent of control values obtained in the same experiment. For these normalized experiments, percentage groups were compared for similarity or difference by rank order using the Mann-Whitney *U*-test (26). These two alternatives were used because of variation in absolute values of data points between experiments, although the trends observed were the same.

RESULTS

Characterization of Radiolabeling

The distribution of [^{14}C]glucose incorporated into cellulose between ovule and fiber tissue in cultures labeled for 4 h was determined (Table I). The percentages shown in the table are the average for seven replicates at each temperature treatment obtained in three different experiments. After it was determined that most of the label (82–92%) was incorporated into fibers, which were the primary objects of interest, labeled samples were subsequently extracted without separation of the two tissues to minimize labor. The percentages for incorporation into fibers are likely to be conservative due to the difficulty of completely separating fibers from ovules. The average percent incorporation values for 15, 22, and 28°C are in a simple linear relationship with temperature (r^2 = 0.982); the significance if any of this observation remains to be determined.

Distribution of radioactivity between submerged and aerial fibers was determined by separating appropriate subsets before extraction. Submerged fibers incorporated about three times more [^{14}C]glucose into cellulose than aerial fibers (data not shown). Patterns of birefringence of cellulose microfibrils examined by polarization microscopy indicated that fibers of both populations synthesized both primary and secondary walls (data not shown).

Incorporation of [^{14}C]glucose into cellulose became linear within 1 h at 15, 22, 28, and 34°C and remained linear for at least 6 h (data for 15 and 34°C in Fig. 1; data for 22 and 28°C not shown). After 6 h at 34°C constant, less than 15% of the original label had been removed from the medium (data not shown). The release of $^{14}CO_2$ took somewhat longer to reach a steady state (approximately 2 h), and then remained linear for at least 6 h (Fig. 2). The same time depend-

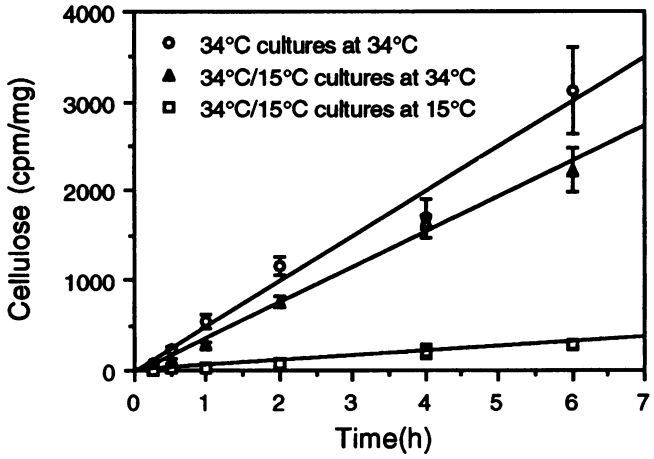

Figure 1. Accumulation at 34 and 15°C of [^{14}C]glucose into crystalline cellulose versus time in 21-d cultured ovules. Ovules were either grown constantly at 34°C and assayed at 34°C (O) or cycled since day 18 at 34/15°C and assayed at 34 (Δ) or 15°C (□). Indicated times of labeling began 4 h after return to 34 or 15°C for the cycled ovules. Each point is the average of three replicate samples from one representative experiment with SDs indicated by the error bars.

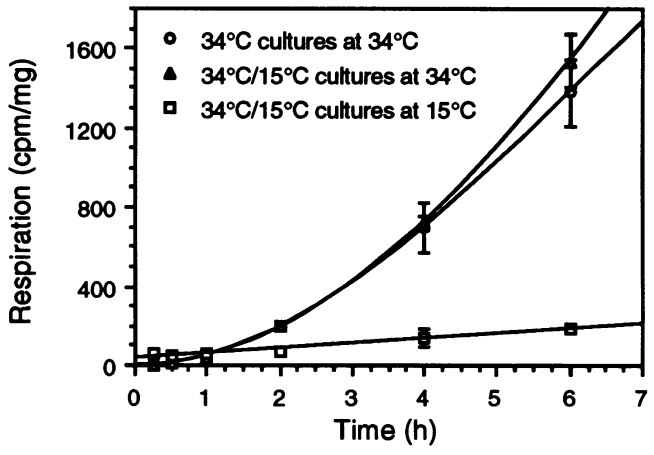

Figure 2. Release at 34 and 15°C of $^{14}CO_2$ versus time in 21-d cultured ovules. Ovules were either grown constantly at 34°C and assayed at 34°C (O) or cycled since day 18 at 34/15°C and assayed at 34 (Δ) or 15°C (□). Indicated times of labeling began 4 h after return to 34 or 15°C for the cycled ovules. Each point is the average of three replicate samples from one representative experiment with SDs indicated by the error bars.

Table I. *Effect of Temperature on the Percent Incorporation of ^{14}C[Glucose] into Cellulose in Ovules and Fibers*

For comparison to the 34°C constant culture, ovules assayed at 28, 22, and 15°C had been cycled for 3 d at those temperatures. Labeling began 4 h after the cultures were exposed to the experimental temperature and continued for 4 h. Raw counts per minute values obtained for separated fibers and ovules were normalized as percent of total incorporation into ovules plus fibers for each temperature treatment. Average percentage values (from seven replicates obtained in three experiments) are shown in the table. The Mann-Whitney *U*-Test was used to compare the percentage values for cycled fibers and ovules to the corresponding values from the 34°C control culture. Average percentage values followed by letters other than a (for ovules) or d (for fibers) represent significantly different groups compared to the 34°C condition for that tissue ($P < 0.05$ for b and e; $P < 0.005$ for c and f).

Assay Temperature	34°C	28°C	22°C	15°C
Percent cpm in ovules	8.1 a	9.4 a	13.7 b	18.3 c
Percent cpm in fibers	91.9 d	90.6 d	86.3 e	81.7 f

ence for attainment of linearity exists in ovules grown constantly at 34°C and those assayed at 34°C after being rewarmed from 15°C for 4 h before labeling commenced (Figs. 1 and 2). A standard labeling time of 4 h was adopted for all experiments in which time was not a variable. Recovery of counts per minute in CO_2 and cellulose for dead controls was negligible in several trials; therefore, uncorrected data are reported.

Effect of Temperature on Cellulose Synthesis and Respiration

The incorporation of ^{14}C[glucose] into acetic-nitric insoluble cellulose after 6 to 8 h of preincubation at the experimental temperature increased between 18 and 28°C, plateaued until about 37°C, and declined at 40°C (Fig. 3). (The variance in preincubation time was due to logistics of carrying out the complex experiment.) The amount of $^{14}CO_2$ released increased between 18 and about 34°C, then plateaued up to at least 40°C (Fig. 4). At least 10 partial replicates of this experiment with different combinations of assay temperatures have confirmed the trends illustrated by this representative experiment.

In parallel experiments performed with 10-d ovules, trends in temperature response of cellulose synthesis and respiration were generally the same as those observed in 21-d cultures. However, more ^{14}C was incorporated into cellulose in 21-d ovules (an 8-fold increase over 10-d ovules) and into CO_2 in 10-d ovules (approximately 2-fold increase over 21-d ovules; data not shown).

Incorporation of [^{14}C]glucose into cellulose and release of $^{14}CO_2$ in cycling cultures (34/28°C, 34/22°C, and 34/15°C) recovered to at least 90% of control cultures (constant 34°C) when returned to 34°C for 6 h (data not shown). The combined results of three separate experiments to determine the time course of recovery from 15°C of cellulose synthesis and respiration are summarized in Table II. Data points obtained for the rewarmed cultures at successive times were normalized as percent of those obtained from a 34°C control culture assayed at the beginning and the end of each experiment. The average percent of control and P values show that for rewarmed ovules, the rate of respiration is the same as controls at all time points ($P > 0.1$), whereas the rate of cellulose synthesis is lower than controls for the first 3 h after rewarming ($P < 0.001$ and $P < 0.05$).

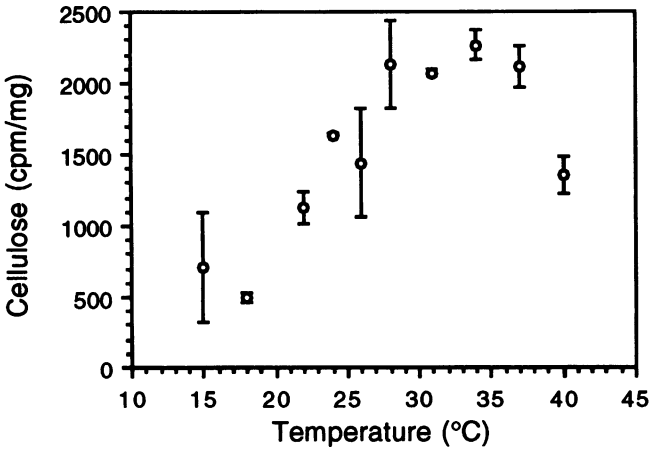

Figure 3. Effect of temperature on incorporation of [^{14}C]glucose into cellulose during 4 h of labeling in 21-d cultured ovules that had been cycled since day 18 at the experimental temperature (12 h at 34°C/12 h at 15–40°C). Labeling on pre-equilibrated medium commenced 6 to 8 h after the beginning of the fourth experimental temperature cycle. Each point is the average of three replicate samples from one representative experiment with SDs indicated by the error bars.

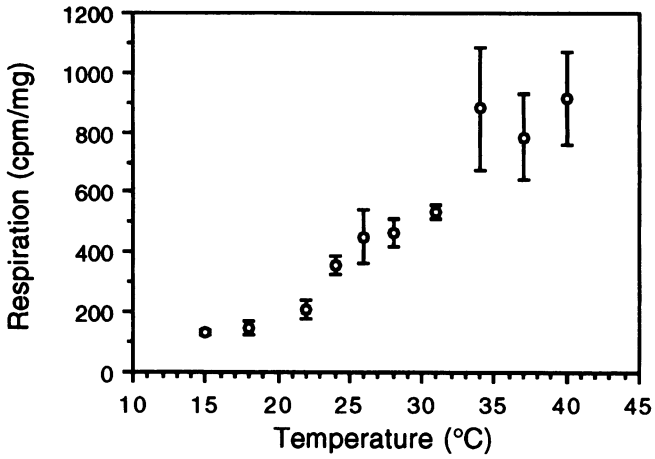

Figure 4. Effect of temperature on release of $^{14}CO_2$ during 4 h of labeling in 21-d cultured ovules that had been cycled since day 18 at the experimental temperature (12 h at 34°C/12 h at 15–40°C). Labeling on pre-equilibrated medium commenced 6 to 8 h after the beginning of the fourth experimental temperature cycle. Each point is the the average of three replicate samples from one representative experiment with SDs indicated by the error bars.

Effect of Temperature on Long-Term Glucose Uptake

The effects of temperature observed in short-term radiolabeling experiments were also evident in long-term (36 d)

Table II. *Percent of Control Cellulose Synthesis and Respiration during Successive Hours after Rewarming from 15 to 34°C*

Experimental data points were normalized as percent of an average control value obtained for a 34°C constant culture assayed at 34°C for 45 min during the 1st and 6th h. Cultures that had been cycled to 15°C were assayed for 45 min at 34°C during successive hours after rewarming to 34°C. The percent of control values for the rewarmed culture at each time were compared for similarity or difference to those from the constant 34°C culture by rank order using the Mann-Whitney *U*-test resulting in the P values indicated. Average percent of control values for nine replicates in three experiments are shown.

	Time after Rewarming				
	1 h	2 h	3 h	4 h	6 h
Cellulose synthesis (%)	54	79	82	88	88
P value	<0.001	<0.05	<0.05	0.1	>0.1
Respiration (%)	114	104	91	94	89
P value	>0.1	>0.1	>0.1	>0.1	>0.1

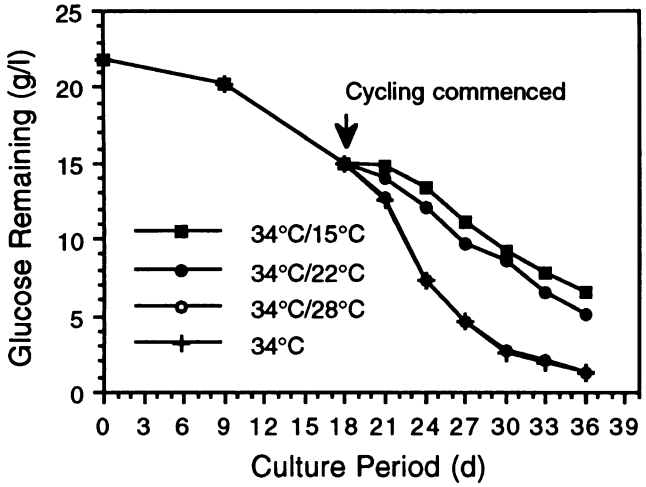

Figure 5. Effect of temperature on glucose uptake in cultures grown at constant 34°C for 18 d and then shifted to cycling temperatures or maintained at constant 34°C as a control. Glucose uptake can be inferred from the decrease in residual glucose in the culture medium as determined by an enzyme-based colorimetric assay. Temperature conditions are indicated in the legend. SDs were less than 6% of the mean of the five sample values averaged for each data point in the graph.

studies on the uptake of unlabeled glucose from the culture medium (Figs. 5 and 6). In cultures grown for 18 d at constant 34°C, shifting to cycling temperatures of 34/15°C or 34/22°C (12 h/12 h) slowed depletion of glucose from the culture medium compared to 34/28°C cycling and 34°C constant growth (Fig. 5). A similar pattern of glucose uptake after day 18 was also observed in cultures that were exposed to cycling temperatures from the day of inoculation (Fig. 6). Even though cycling began on day 0, substantial separation of the glucose uptake curves under different temperature conditions did not occur until after day 15.

DISCUSSION

Despite the well-documented adverse effects of cool temperatures on cotton growth and fiber maturation in the field (10), the causal mechanisms by which fiber development is affected are not well understood. The differential responses of existing cotton cultivars to cool temperatures (10, 12) suggest that further improvement through genetic engineering might be possible if genes related to chilling sensitivity or resistance could be identified. Understanding the mechanism(s) of the responses will aid in reaching that goal, and the research reported here begins to elucidate physiological responses to cool cycling temperatures during fiber development. Previous research in the field showed only that low night temperatures decreased the total accumulation of cellulose over an entire growing season (12). It did not show whether cellulose synthesis was affected similarly or differently than other metabolic processes or whether the rate of cellulose synthesis was depressed at all times or only during the low point of the temperature cycle.

We have been able to address these questions because of the ease with which cultured ovules, which mimic the field response (17), can be experimentally manipulated. The range of assay temperatures (low of 15°C, high of 40°C) was chosen because thermocouple temperature monitoring has shown that cotton fibers inside bolls routinely experience temperatures in this range in major U.S. cotton growing regions (17), and 34°C is the optimal growth temperature of cultured ovules (1). Several background experiments were performed to establish appropriate conditions for radiolabeling of fibers on cultured ovules at different temperatures. Similar to the results of Carpita and Delmer (6) on incorporation of ^{14}C into sugar phosphates and UDP-glucose, incorporation of [^{14}C]-glucose into cellulose and release of $^{14}CO_2$ became linear within 1 and 2 h, respectively (Figs. 1 and 2). Surprisingly, linearity of labeling of CO_2 and cellulose occurred as rapidly at 15°C as at 34°C. Our observation that submerged fibers incorporate more ^{14}C into cellulose than aerial fibers supports the suggestion of Seagull (25) that caution is appropriate in interpretation of experiments in which inhibitors are applied to ovule cultures, since exogenous agents cannot be assumed to reach all portions of the tissue with equal rapidity. However, this differential incorporation does not affect the interpretation of our data in relation to fiber development; both aerial and submerged fibers synthesized secondary walls as judged by patterns of microfibrils observed by polarization microscopy.

Analysis of Temperature-Response Curves for Cellulose Synthesis and Respiration

In 21-d cultures of cv Acala SJ1, cellulose synthesis and respiration responded similarly to temperature changes below 28°C. Both processes occurred at less than 30% of the 28°C rates at 15 and 18°C and increased in a roughly linear manner between 18°C and at least 28°C (Fig. 3 and 4). Above 28°C,

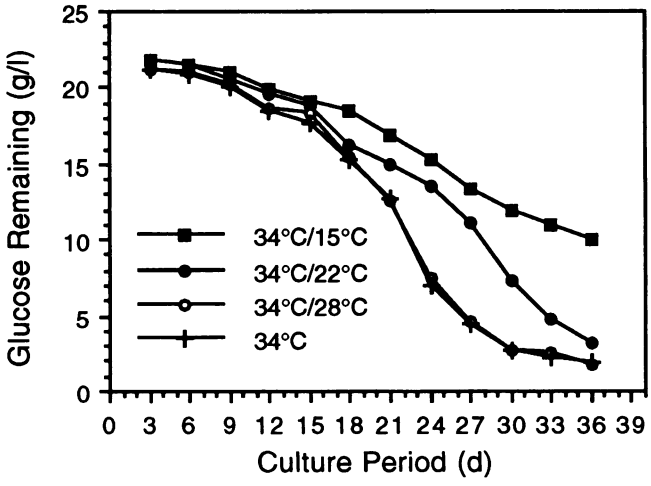

Figure 6. Effect of temperature on glucose uptake in cultures grown under cycling temperatures from the initiation of culture or at constant 34°C as a control. Glucose uptake can be inferred from the decrease in residual glucose in the culture medium as determined by an enzyme-based colorimetric assay. Temperature conditions are indicated in the legend. SDs were less than 6% of the mean of the five sample values averaged for each data point in the graph.

the trends in the measured parameters of the two processes diverged; release of $^{14}CO_2$ continued to increase and remained high at 40°C (Fig. 4), whereas rate of [^{14}C]glucose incorporation into cellulose plateaued up to about 37°C and dropped off at 40°C (Fig. 3). Therefore, if more energy is made available through respiration above 28°C, it is not used for increased cellulose synthesis. Uncoupling of respiration, as has been demonstrated in cotton roots following treatment at 40°C (19), may contribute to this metabolic inefficiency at the higher temperatures.

The temperature-response data indicate that the optimum temperature for rapid, metabolically efficient cellulose synthesis in cotton cv Acala SJ-1 is 28°C. This finding is consistent with field studies that showed that seasonal cellulose yield in many long staple, late maturing cultivars similar to cv Acala SJ-1 is reduced when night temperatures fall below about 27°C (12, 28). The temperature of 28°C falls within the range of 23.5 to 32°C , which has been implicated as optimum for activity of certain enzymes and seasonal biomass accumulation in cotton (4). However, our data show that cellulose synthesis in fibers occurs at its maximum rate up to 37°C. This higher upper limit for optimum activity might be explained by different temperature dependencies of enzymes in cotton bolls compared to other parts of the plant. Given adequate water, cotton maintains leaf temperature between 27 and 32°C by transpirational cooling (5), whereas the temperature inside the developing cotton bolls follows air temperature very closely and often exceeds 32°C (17).

Although the relative rates of cellulose synthesis and respiration are different in 10-d cultures during the period of primary wall synthesis, the temperature responses of the two processes are qualitatively similar to those observed in 21-d cultures (data not shown). Therefore, our data are consistent with past field studies showing that temperatures greater than 15°C are required for elongation to proceed (reviewed in ref. 10) and that night temperatures less than 25°C reduce the rate of fiber elongation (11). They are also consistent with past analysis of accumulation of fiber length over time in fibers developing in vitro under cycling temperature conditions (17).

Respiration and cellulose synthesis also had different recovery patterns upon rewarming (Table II). Respiration consistently recovered to control rates within the 1st h ($P > 0.1$), whereas cellulose synthesis was consistently depressed by an average of 46% in the 1st h ($P < 0.001$). Cellulose synthesis exhibited more variable rates that were 18 and 21% less than the control rate ($P < 0.05$) in the 2nd and 3rd h, respectively. The implication for rate of cellulose synthesis in field plants, which is the primary determinant of fiber maturation, is that the adverse effects of a cool night probably extend into warmer daytime hours. Energy may well be made available through respiration during the day that is not efficiently used to build the fiber wall if the previous night was cool. Therefore, the restriction of available energy due to a cool-temperature reduction in the rate of respiration is not likely to be the only explanation for reduced cellulose synthesis under cool temperatures.

The different temperature dependencies of rates of cellulose synthesis and respiration and the different recovery patterns after rewarming both indicate that cellulose synthesis and respiration respond differently to temperature changes. It is possible that these differences can be explained by particular features of the cellular regulation of cellulose synthesis. The formation of each cellulose microfibril in higher plants is associated with a rosette of six particles (presumably transmembrane proteins) that can be visualized in the plasma membrane after freeze fracture. Numerous glucan chains are polymerized at the site of each rosette, and these crystallize into the composite microfibril. The process of cellulose crystallization can limit the rate of polymerization, presumably by feedback control (reviewed in ref. 15). Therefore, the plateau in the rate of cellulose synthesis from 28 to 37°C, despite increased respiratory activity at the higher temperatures, might be due to reaching the maximum rate of crystallization. Experimental evidence suggests that rosettes must be continually inserted throughout the process of secondary wall deposition, perhaps because each one has a short functional life of about 20 min (16, 23). Rosettes are also very sensitive to cellular perturbation of any kind. For example, great care with preparative techniques must be exercised if rosettes are to be visible after freeze-fracture, an observation that could be related to the difficulty of synthesizing cellulose in vitro from membrane preparations of higher plants (8). Therefore, it is possible that the rosettes become depleted from the plasma membrane during the period of cool temperature and require several hours to be fully replaced by renewed activity of transcription and/or translation and endomembrane processing and transport. In contrast, the enzymes required for respiration might remain in place during the period of cool temperature to be quickly reactivated upon rewarming, a possibility that is consistent with the well-known stability of respiratory enzymes in isolated mitochondria and the centrality of respiration for survival (as opposed to growth) of the plant.

Temperature Effects on Long-term Glucose Uptake

Although intact cotton plants transport carbon as sucrose, all observations reported in this study are for ovules cultured on glucose. Glucose was used instead of sucrose because ovules form large amounts of callus when cultured in sucrose (2; and confirmed by us). Several observations suggest that the choice of carbon source does not affect the labeling results. We have observed "growth rings" induced by cool temperatures in the fiber secondary wall (see ref. 17) regardless of whether glucose or sucrose is used as the carbon source (data not shown). In addition, previous studies using *G. arboreum* have demonstrated similar temperature effects on the incorporation of [^{14}C]sucrose into cellulose. In these studies, the optimum temperature for cellulose production was between 25 to 30°C (22).

The results of long-term glucose uptake experiments (Figs. 5 and 6) correlate with the trends observed in labeling experiments. In cultures grown at 34°C for the first 18 d and then transferred to cycling conditions as in the labeling experiments, subsequent glucose uptake was essentially equal in 34/28°C cycling and 34°C constant cultures, but reduced under 34/22°C and 34/15°C cycling. The general trends in glucose uptake were similar in cultures that were exposed to cycling temperatures from the onset of culture, which would

be more analogous to the field condition. The rapid increase in glucose uptake that occurs after day 15 corresponds to the time of onset of secondary wall deposition (17) when increased amounts of carbon would be required. The equal rates of glucose uptake under 34°C constant and 34/28°C cycling indicate that most of the glucose is removed from the medium to support secondary wall synthesis, because incorporation of [^{14}C]glucose into cellulose, but not release of $^{14}CO_2$, reaches its maximum rate at 28°C.

Possible Mechanisms by which Cool Temperatures Decrease Cellulose Synthesis

The similarity of temperature responses of ovule cultures and field-grown plants indicates that crucial aspects of cool sensitivity are inherent in ovules and/or fibers (17), although augmentation of sensitivity in the field due to temperature effects on whole plant physiology are not excluded by these data. Diurnal differences in availability of a carbon source have been suggested as a causal mechanism for cotton fiber "growth rings" (9; see also ref. 17), but the data reported here were obtained from cultured ovules in which glucose is not limiting during the period of cool temperature. Therefore, even if sufficient carbon source were available to the ovules throughout a cool night, it would not be used efficiently to synthesize the fiber secondary wall. This indicates that further research into causal mechanisms of low temperature inhibition within the ovules and fibers themselves is needed as a prelude to possible future targeted improvement of the temperature response of fiber maturation through genetic engineering.

The effects of cool temperatures on the various steps of cell wall synthesis in cotton are not known. It is not yet clear whether a single rate-limiting enzymic step exists or whether the effect is exerted more generally; e.g. through temperature effects on membrane fluidity. Direct examination of the effects of temperature on the cellulose synthase itself is not yet possible because active preparations of this enzyme have not been successfully isolated from higher plants (8). Although ovule cultures are not dependent on photosynthate transported from leaves, it is possible that cool temperatures reduce the uptake of substrate at the ovule or fiber membrane. This could be the result of feedback inhibition from a later point in the pathway or a direct effect of temperature on the sugar transport mechanism itself. Research is ongoing on the effects of cool temperature on steady-state enzyme kinetics (seeking to determine if a particularly temperature-sensitive enzymic step can be identified), Golgi secretion, protein synthesis, and gene expression. The analysis of changes in gene expression requires not only obtaining experimental data related to the cool temperature response, but also obtaining background data on normal gene expression during the secondary wall phase of fiber development. Complementary studies from other research groups detailing molecular changes during normal secondary wall deposition are just beginning to appear (30).

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