Polarizing Microscopic Study of the Origin and Growth of Extinction Bands in Native Cotton Fibres

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Native cotton fibres in never-dried and dehydrated states show under crossed polarized light characteristic extinction bands along their lengths. The cause of the origin and growth of these bands is still unresolved. This paper reviews critically the published literature in the light of the present author's experimental observations on the origin and growth of extinction bands. Some plausible mechanisms for the origin of these bands from our observations with a polarizing microscope on developing cotton fibres of known ages are discussed in detail. The classical hypothesis of reversing spirals of cellulose fibrils in the secondary layers is refuted to explain the origin of the extinction bands.

Keywords: Cellulose fibrils, Cotton fibre, Extinction bands

1 Introduction

Cotton fibre has a fibrillar structure and the bulk of its cellulose is laid in the secondary diurnal growth layers\(^1\)\(^-\)\(^9\). These growth layers have been shown to arise as a result of diurnal temperature cycles during the growth period rather than due to changes in illumination cycles as shown earlier\(^9\)\(^-\)\(^20\). Even cotton fibres grown under constant illumination and temperature, though without discernible growth layers, show extinction bands and their cellulose also has been shown to be fibrillar\(^21\).

Cellulose fibrils in the individual secondary layers form a spiral about the fibre axis\(^1\),\(^2\),\(^4\),\(^22\)\(^-\)\(^26\). Some workers\(^27\)\(^-\)\(^34\) believe that the orientation of these fibrils in the successive secondary diurnal growth layers to be similar, whereas others believe that the individual secondary growth layers can both be left-handed and right-handed and also could differ in spiral structure, cellulose chain length and crystallite sizes\(^1\),\(^4\),\(^20\),\(^27\)\(^-\)\(^33\),\(^35\)\(^-\)\(^39\). Some workers\(^1\),\(^2\),\(^9\),\(^25\),\(^26\),\(^35\),\(^40\)\(^-\)\(^44\) have argued that in each layer the sense of the helix reverses several times along the length of the fibre. These so called reversal points are identified as extinction bands in a polarizing microscope under crossed field. Several electron micrographs of fragments of secondary layers of cotton fibres by negative staining, shadow casting techniques and by surface replicas have however not shown any clear evidence of the existence of an abrupt change in orientation in secondary layers\(^13\),\(^35\),\(^45\)\(^-\)\(^52\). Parallelly-laid cellulose fibrils only are however seen. Some evidence of cross-orientations in successive secondary layers deposited in definite wave patterns in developing cotton fibres have been recently shown by freeze-fracture replica technique in a transmission electron microscope\(^45\).

Extinction bands have been mapped by several investigators\(^1\),\(^16\),\(^28\),\(^29\),\(^33\),\(^38\),\(^39\),\(^49\),\(^53\)\(^-\)\(^55\), and their frequency is reported to be influenced by genetic\(^32\),\(^53\),\(^56\)\(^-\)\(^58\) and environmental\(^11\)\(^-\)\(^13\),\(^30\),\(^33\),\(^38\),\(^59\) factors and by irrigation\(^53\). The number of extinction bands has been shown to be more in \textit{Gossypium barbadense} varieties than in the \textit{Gossypium hirsutum}, \textit{Gossypium arboreum}, and \textit{Gossypium herbaceum} species of cotton fibres\(^22\),\(^28\),\(^35\),\(^55\)\(^-\)\(^62\). Positions of extinction bands are again shown to be as weak points by some workers\(^53\)\(^-\)\(^68\), but as strong points by some others\(^65\),\(^69\)\(^-\)\(^72\). If the reversal points are weak points, their increased number in \textit{Gossypium barbadense}\(^32\),\(^60\) varieties cannot be easily reconciled with higher fibre strengths of the fibres of this species\(^35\),\(^61\),\(^70\). The negative correlation between the number of extinction bands and the strength of cotton fibres observed by some workers\(^63\) may be misleading because the strength data on these fibre varieties were taken by them from a general booklet\(^63\) of fibre characteristics, rather than from the actual measurements on the same fibres used for counting extinction bands.
Reversals of cellulose fibrils in individual secondary growth layers are believed by some to coincide exactly or nearly so. If however true, the intensity of blackening of extinction bands should progressively increase with the deposition of each secondary diurnal growth layer. No such report exists, nor have our own observations reported shown any such effect. Superposition of reversals in individual secondary growth layers therefore appears to be only speculative.

In a system like the cotton fibre, where internal dimensions are changing with each deposition of diurnal-secondary growth layer, such a possibility seems doubtful. Reversals in secondary growth layers are again different from the occasional reversals in external convolution twists on cotton fibres, and yet coincidence in tensile fractures with reversals in morphological studies with a scanning electron microscope and a polarized light microscope have been shown. However it has been clearly demonstrated that such a coincidence is limited to only the plane of observation and should not be mistaken as a morphological or structural coincidence. The evidence in respect of the hypothesis of reversibility of cellulose fibrils in secondary growth layers, therefore, continues to be derived from the polarizing microscope although, not without doubts.

X-ray diffraction patterns taken by Wakeham et al. of single fibres at the reversal position have shown a high degree of orientation and crystallinity. A comprehensive review of structural reversals and their relation with mechanical properties has been published.

Several attempts have been made over the years to explain the origin and growth of extinction bands, and yet the exact cause and manner are not definitively known. In the earlier publications, extinctions have been attributed to the presence of pits in the cell-wall. Kuhn, as quoted by Balls, ascribed them to the mystic gyration of the protoplasm in the drying cell, and Balls explained it as due to the movement of the growth centre within the fibre, during secondary cellulose deposition. Iyengar, observing moist fibres from ripe cotton bolls, found the reversals to appear midway between the adjacent bends of the fibre. Each flattened portion develops into a structural reversal; more often than not the bend is also a reversal. However, none of these explanations is convincing in the light of experimental observations.

Whatever may be the real cause of the origin of the extinction bands in cotton fibre, it is clear that these are formed during the secondary thickening period. It is also now definite that the cellulose fibrils and their orientation in individual secondary growth layers or in association with several underlying layers have a bearing on the origin of extinction bands. How and under what conditions the extinction bands should appear, need explanation.

In this study, we have made attempts to explain the origin of the extinction bands in the light of some of our observations on developing cotton fibres of known ages from the dates of flowering, with the help of a polarizing microscope.

2 Experimental Procedure

A large number of flowers in a Gossypium hirsutum variety 'Bikaneri Narma' were tagged for a week. Green cotton bolls of known ages were collected in double-distilled water in a stoppered bottle. A few drops of chloroform were added to prevent bacterial growth. Fibres were carefully unlocked under water by cutting open the green bolls, and were examined in wet condition on a Carl-Zeiss polarizing microscope under crossed polarizer and analyzer condition.

3 Results and Discussion

Fibres from 6,7-day-old bolls showed complete extinction from the tip to the base under all orientations in the crossed field, which is a characteristic of an isotropic fibre. Photographing these fibres was rather difficult for lack of sufficient transmission of light through fibres. The first result presented in Fig. 1 is a photograph of fibres collected from 14-day-old boll after flowering, as seen under a crossed-polarized light. The body of these fibres is dark and remains so even if the specimen stage is rotated, indicating that the fibres are essentially isotropic. Only the wall edges are seen illuminated and this is apparently an edge effect.

The brightness in the body of the fibres generally increases with age and the clear distinction between the edges is lost after about the 20th day from flowering as seen from Figs 2 and 3. The first dark extinction band was seen in a fibre from a 21-day-old boll. Fibres

![Fig. 1-14-day-old fibres of Bikaneri Narma cotton variety under crossed-polarized field](image-url)
collected from bolls on later days showed the appearance of more bands and the interband spacings became proportionately smaller for fibres from bolls collected still later. The appearance of new bands between the existing ones would be expected to result in this decrease of band spacing. Similar observations were made by Betrabet et al.30,54,58,60. The spacings were generally not periodic, although in some fibres statistically regular spacings were observed. Randomness in extinction band spacings has also been shown by several workers1,9,33,54,79 and also a fall in the number of extinction bands per millimetre in the second picking of fibres, which Iyengar78 attributes to the rise in the atmospheric temperature with the advance of the season. This decrease in the number of extinction bands in fibres of second picking may be due to the shorter boll maturation period (secondary thickening period) offered by the place of growth to the bolls from late flowers, owing to the advancing season. Raes et al.66, however, observed a statistically significant periodicity in extinction band distribution and they presumed this to be inherent in the generation of extinction bands. Figs 4 and 5 show some illustrations of the extinction band spacings. It is therefore clear from the above observations that the formation of the extinction bands takes place during the secondary wall thickening period. A definite correspondence between the number of days of secondary thickening period and the number of growth layers has been seen5,20,53. It is also indicated that the period of secondary thickening continues for well over 30 days depending upon the environment. A prolonged range of secondary thickening periods (boll maturation periods) for Gossypium barbadense cotton from 51 to 74 days has been observed for locations in Egypt and Sudan, and this has been ascribed to the differences in air temperatures compared to the other economic zones81,82. Schubert et al.83 and Itoh84, however, indicate an overlapping of secondary thickening period to some extent over the elongation phase, from their studies with an electron microscope on developing cotton fibres. Since in general the length of the fibres is determined before the secondary thickening begins, an interesting close correspondence between the mean fibre lengths per unit temperature cycles for the secondary thickening period and the inter-reversal distance for the fibre of Gossypium hirsutum species reported in the published litera-
change in the direction generally accepted and seen, the evidence for abrupt fibrils in the primary walls of cotton fibre has been formtion of extinction ands. Others consider that the fibrils are deposited in S and Z spirals in successive secondary layers. Yet others believe that the spirals are deposited as the S spiral only. The spiral angle in all the successive secondary growth layers, irrespective of the species of cotton, is believed by some to be constant at 22°. Others believe that the spiral angle progressively decreases towards the core of the fibre. Increase in refractive index for $n_l$ with respect to $n_s$ in a fibre refractometer has been attributed to decreasing spiral angle and to variations in spiral arrangement among different fibres or segments of the same fibre. Duckett and Ramey, however, believe that there is a rapid decrease in spiral angle in the outer diurnal layers, after which a constancy in spiral angle is approached.

In the context of the discussion above, the most important question that needs to be answered is: in the absence of abrupt spiral reversals in direction, how can the extinction bands appear in a crossed polarised light? To answer this question, the following mechanisms are considered and discussed.

### 3.1 Mechanism of Formation of Extinction Bands

The polarizing microscopic studies have shown that the transverse extinction bands statistically increase in number with each diurnal layer deposited during the thickening of the cell wall, and that the conditions for origin of extinction bands are actually formed during this period of a developing cotton fibre. Although many workers believed, there are differences in the cross-sectional dimensions of cellulose microfibrils from primary and secondary walls of cotton, Willison and Brown found no differences in the dimensions of fibrils from the freeze-fractured replicas of these walls in developing cotton fibres using a transmission electron microscope. They however found the individual microfibrils and bundles of microfibrils in secondary layers deposited in definite wave patterns with definite amplitude, wavelength and relative phase differences.

With this knowledge, there are several possibilities in which the mechanism of the origin of extinction bands may be worked out. These are discussed below.

#### 3.1.1 The spirally deposited cellulose fibrils in each individual secondary layer may be oriented parallel to the fibre axis at extinction points.

In considering this possibility, we are required to revert to the classical hypothesis of the cellulose fibrils abruptly changing their sense of orientation from left to right and while doing so, they align themselves in an almost parallel position to the fibre axis and produce an extinction band. It is however very difficult to imagine a whole cylindrical diurnal layer changing abruptly in orientation during its deposition. Also, it is difficult to conceive of such patterns in cellulose deposition being copied in toto in the same location in successive secondary layers deposited, particularly in a dynamic system such as cotton whose internal dimensions are changing with each secondary layer deposited. Here therefore two conditions arise. If the reversals in successive secondary layers deposited were to coincide with the reversals of the first ever secondary layer, then the number of the extinction bands seen along the entire length of the fibre must be determined in full number in the very first secondary layer deposited, and the successive layers merely copy them by exact superposition. Experimental evidence and our own observations reported in the early part of this paper however have shown that the number of extinction bands increases after the 21st day of flowering. Secondly, if we assume that the reversals in every individual secondary layer deposited do not coincide with each other but are established separately, or marginally ahead of the ones in previous layers, the whole fibre should appear dark under crossed polarized light or at least the extinction bands should diffuse into very wide zones. None of these two possibilities has been experimentally observed and therefore this postulate in explaining the origin of the extinction bands also seems implausible.

#### 3.1.2 Formation of isotropic zones in the secondary layers along the length of the fibre.

Assuming that the spiralling cellulose fibrils in increasing orientation from the outermost to the innermost secondary layers create totally isotropic conditions at periodic intervals along the fibre through small cross-sectional matrix of the fibre, one can see extinction bands at such isotropic locations under crossed polarized light. Such bands arising out of the isotropic disposition of cellulose fibrils between superposing secondary layers must necessarily stay in their positions without change in intensity under all orientations of fibre in crossed polarized field. This is however not experimentally true since the extinction bands alternate from dark to bright on rotating the stage of the microscope. The conditions of isotropy
between layers does not therefore seem to be the cause for the origin of the extinction bands in cotton fibres.

3.1.3 Formation of conditions for complete extinction may be a localized phenomenon between two successive or intermediate secondary layers in the upper half or the lower half of the cylindrical fibres, when observed vertically in a two-dimensional plane in a crossed polarized field. If this be true, and in view of the fact that the secondary layers are not uniform in thickness along the cross-section of the fibres\(^5,6,48,87\), it implies that cotton fibres at extinction points in one plane of observation should produce no extinction when observed orthogonally. Our observations of extinction bands in several cotton fibres in such mutually orthogonal views, on a specially designed axially rotating goniometer, show that the extinction bands stay in their positions along the entire cross-section of the fibre on rotation about the fibre axis. The formation of the extinction bands is therefore not a localised phenomenon between a few intermediate superposing secondary layers but is disposed through the entire circular cross-section of the fibres.

The above three arguments leave only one possibility on the origin of the extinction bands as discussed below.

3.1.4 Freeze-fractured replica of secondary layers of developing cotton fibres\(^45\) have shown that the individual cellulose microfibrils and bundles of microfibrils are deposited in definite wave patterns with a definite amplitude, wavelength, and phase differences between them. These wave patterns made by the bundles of microfibrils contribute to the variable orientation of the anisotropy revealed by polarized light studies. These bundles of wavy fibrils within an individual secondary layer or in co-operation with a number of underlying or overlaid layers form a narrow region of crystallized matrix in which the orienting bundles of cellulose fibrils are held parallel or nearly parallel to the fibre axis, thereby producing extinction bands. If however these bundles had been laid exactly parallel, the extinction bands would have been expected to change from dark to bright at each 90° rotation in a crossed polarized field. Close observation of extinction bands shows that they alternate from dark to bright at each 20-27° rotation of the stage of the microscope. This means that there is a cross-oriented crystallized matrix of cellulose fibrils at the extinction bands. Such orientations may therefore be possible between the successive secondary layers.

The little periodicity observed in the distribution of extinction bands must therefore be associated with the periodic wave patterns in which the cellulose fibrils are deposited within the secondary layers and their periodic in-phase superposition. Assuming that the wave patterns of the deposited cellulose fibrils in every secondary layer remains the same, the wave patterns in the freshly laid layers would be laid slightly ahead of the waves in the preceding layers owing to the reduced diameter of the fibre. As a consequence, the number of extinction bands would be expected to be more in the upper half of the developing cotton fibre along its length than in the lower half. Experimental observations of increased frequency of extinction bands in the upper halves of cotton fibres\(^5\) support our hypothesis on the origin of extinction bands in native cotton fibres.

4 Conclusion

We refute the classical hypothesis that the reversing cellulose spirals in secondary layers of cotton are the cause of the origin of extinction bands. The origin of these bands is therefore a phenomenon between secondary layers deposited in a developing cotton fibre. Since the duration of the deposition of the secondary layers depends greatly on several environmental and growth conditions, besides the genetic factors, varietal characterization of cotton fibres based on the frequency of extinction bands is almost impossible. Prolonged secondary deposition periods for the varieties of the long-staple \textit{Gossypium barbadense} species\(^82,83\) also explain why the fibres of this species have an increased number of extinction bands seen on them. Also, since the extinction bands arise as a result of the ordered regions in the matrix of the cotton fibres, their increased number should correspond to higher strengths of the fibres as has been observed by some workers\(^4,22,26,55,60\).

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