ENDOTHIA PIGMENTS. I[1]

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The genus Endothia is characterized by a yellow or orange stroma and all known species produce a yellow or buff color in the mycelium and upper layers of the substratum when grown on starchy culture media. In connection with cultural studies of this genus Shear and Stevens[2] first called attention to the fact that certain species when grown on cornmeal or other starchy media produced a bright color, "perilla purple," while the others produce no such color.

Continued study enabled them to divide the genus on the basis of this color production. This division does not, however, coincide with the classification based on morphology. On the basis of spore form the genus is arranged as follows:[3]

Section 1.—Ascospores short—cylindrical to allantoid, continuous or pseudo-septate.

E. gyrosa (Schw.) Fr.

E. singularis (Syd. & Syd.) S. & S.

Section 2.—Ascospores oblong—fusiform to oblong-ellipsoid, uniseptate when mature.

E. fluens (Sow.) S. & S.

E. fluens mississippiensis S. & S.

E. longirostris Earle.

E. tropicalis S. & S.

E. parasitica (Murr.) And. & And.

Of these species the first three uniformly produce perilla purple on such media as cornmeal, oatmeal or rice flasks while the others have consistently failed to produce this color. It is noteworthy that *E. fluens* is included in the group which produces the purple color while *E. parasitica* is not. These two species are so similar morphologically that at one time leading mycologists considered them iden-

[1] Published by permission of the Secretary of Agriculture.

[2] Shear, C. L., and Stevens, Neil E. Cultural characters of the chestnut-blight fungus and its near relatives. U. S. Dept. Agr. Bur. Pl. Ind. Circ. 131: 3–18, 1913.

[3] Shear, C. L., Stevens, Neil E., and Tiller, Ruby J. *Endothia parasitica* and related species. U. S. Dept Agr. Bull. 380. 1917.

tical. They are found within the same areas, the United States and Japan, and on the same hosts, Castanea sp. Yet *E. fluens* is a saprophyte while *E. parasitica* is one of the most uniformly destructive fungous parasites known. This is perhaps the only case yet recorded of two closely related fungi, growing on the same host, one of which is a virulent parasite and the other a saprophyte. The two species grow readily and can be easily distinguished on artificial culture media. It is obvious that cultural or physiological differences between *E. fluens* and *E. parasitica* are of great interest.

It was to study the production of the various colors in species of the genus that the work described in the present paper was taken up. Some attention has been paid to the coloring matter produced by *E. parasitica*. Pantanelli[4] considers the pigment to be a lipochrome but records no experimental work in proof of this statement. Anderson[5] disagrees with Pantanelli on this point. He considers the pigment to be an aurine and quotes unpublished work by Mr. C. T. Thomas to substantiate his view. It was hoped in the present investigation to obtain more evidence on this disputed point.

In taking up the study of the pigments various solvents were tried to see which was most favorable for the extraction of the pigment from the mycelium and the mass of rice upon which the fungi were grown. It was found that the coloring matter of all the species was soluble in ethyl alcohol, and a considerable portion of it readily soluble in ether. Accordingly extracts were made of the culture media and mycelium, with alcohol, at room temperature. The alcohol was evaporated and the residue extracted with ether. The ether extract was then filtered, the ether distilled and the pigments taken up in alcohol again. All tests were made in alcoholic solution unless otherwise noted. The coloring matter was found to be yellow when acidified with either hydrochloric, sulphuric, nitric, phosphoric, or acetic acids. When the acid solution was treated with dilute alkali, sodium, potassium or ammonium hydroxides, or sodium or potassium carbonates, it became a deep red. Apparently all the fungi elaborated pigments which were bright yellow when acidified and red when made alkaline. While the alcoholic extracts from all the fungi

[4] Pantanelli, E. Sul parassitismo di Diaporthe parasitica Murr. per il Castagno. Rendiconti della R. Accademia dei Lincei, Classe di Scienze, Fisiche, Matematiche e Naturali. V. 20: 366–372, 1911.

[5] Anderson, P. J. The morphology and life history of the chestnut-blight fungus. Bull. Penn. Chestnut Tree Blight Comm. 7: 1–43, 1913.

reacted in the same general way, there were various nuances of red in the extracts from the different fungi.

A study of the alcoholic extracts from pure cultures on rice[6] of *E. parasitica*, *E. fluens*, *E. fluens mississippiensis*, *E. tropicalis*, *E. gyrosa*, *E. singularis*, and *E. longirostris* was made with a spectro-

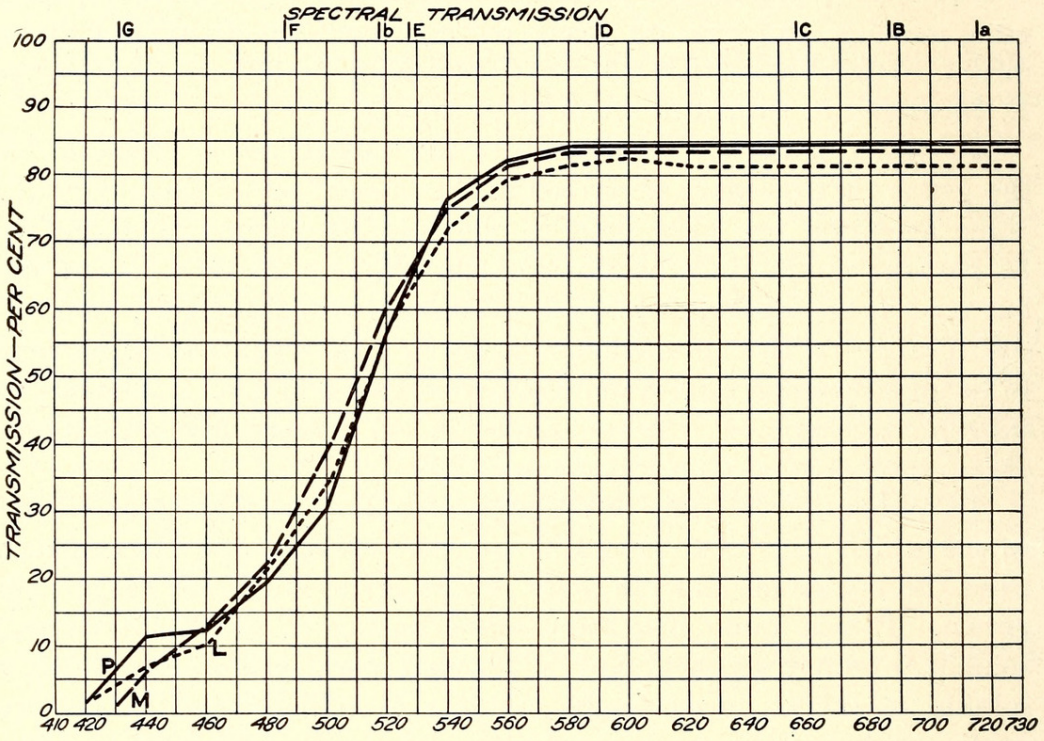

FIG. 1. Curves of percentage of spectral transmission of acidified alcoholic extracts from pure cultures on rice of *E. parasitica* (P), *E. longirostris* (L), and *E. fluens mississippiensis* (M). The curves were plotted with the percentage of light transmitted as ordinates and wave-lengths of light in $\mu\mu$ as abscissae.

photometer. With this apparatus measurements were made from which the percentage of light of various wave-lengths transmitted by the solution was calculated. These data were used in plotting the curves of spectral transmission.[7] The alcoholic extracts of stromata of *E. singularis* from chaparral oak, *E. gyrosa*, from beech, and *E. parasitica* from chestnut were prepared by separating the stromata

[6] Throughout this study the fungi were grown on rice flasks prepared according to the method published by Shear and Stevens. Loc. cit., p. 13.

[7] This part of the work was made possible through the kindness of Mr. C. G. Peters, of the Bureau of Standards, who made the measurements and calculations.

from the bark and extracting the mass with alcohol. The curves of spectral transmission of these extracts are included in figures 3 and 5.

From the curves shown in figures 1 to 3 it is noticeable that the transmission spectra of the acidified alcoholic extracts of pure cultures of the species of Endothia studied group themselves into three classes.

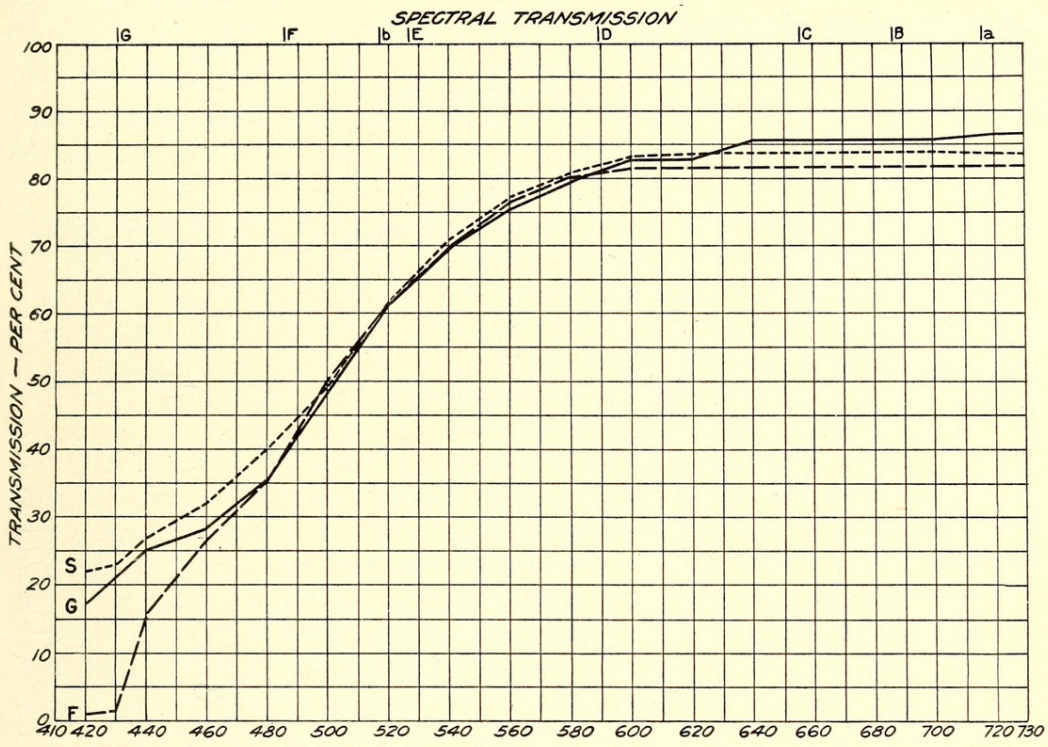

FIG. 2. Curves of percentage of spectral transmission of acidified alcoholic extracts from pure cultures on rice of *E. fluens* (F), *E. gyrosa* (G), and *E. singularis* (S). The curves were plotted with the percentage of light transmitted as ordinates and the wave-lengths of light in $\mu\mu$ as abscissae.

The first of these includes *E. parasitica*, *E. longirostris*, and *E. fluens mississippiensis*. The curves for these three fungi agree rather closely in most cases, the region of greatest variation being in the shorter wave lengths transmitted. The distinctive feature of these curves is that they indicate that practically all the violet rays are absorbed. Only a small portion of the blue is transmitted while most of the yellow, orange and red rays pass through.

The curves of spectral-transmission for *E. fluens*, *E. singularis*, and *E. gyrosa* make up the second group and are shown in figure 2. An inspection shows that the greatest variation in these curves is again in

the shorter wave-lengths. There is some transmission of the violet rays, more of the blue, and a gradual increase in the percentage transmitted through the green and yellow to the orange. From this region through the red the percentage of transmission is practically the same for all wave lengths. The curves of this type are different from those of group I in that more of the violet and blue are transmitted and somewhat less of the yellow.

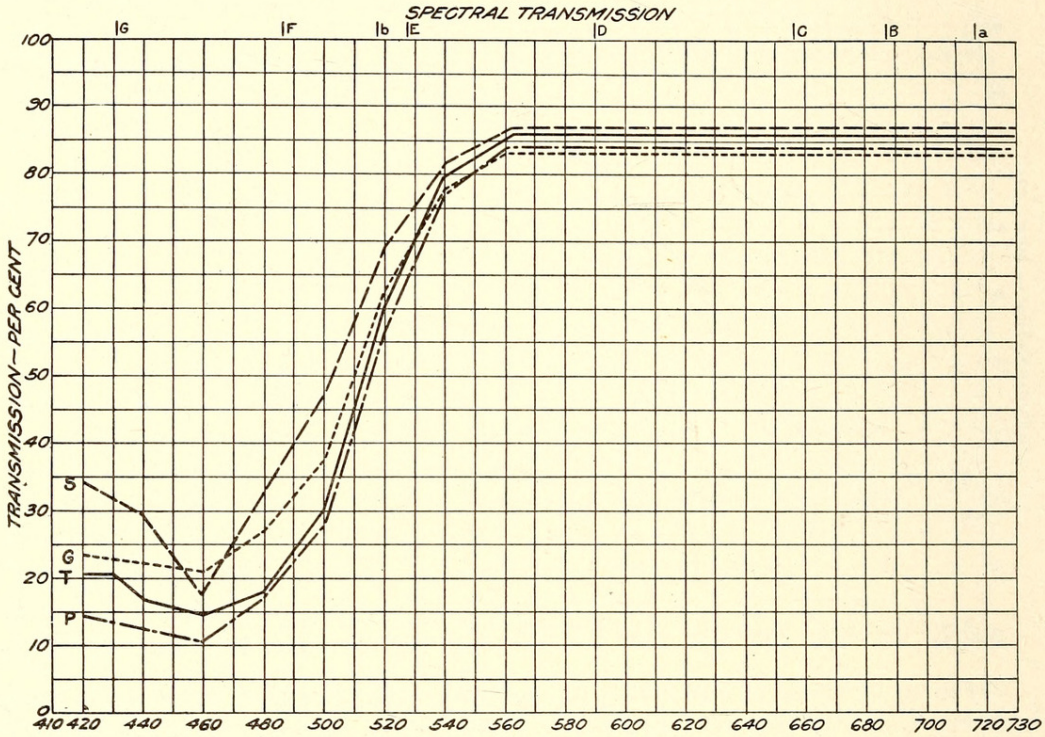

FIG. 3. Curves of percentage of spectral transmission of acidified alcoholic extracts of *E. tropicalis* (T) from pure culture on rice, *E. parasitica* (P) from stromata from chestnut, *E. gyrosa* (G), from stromata from beech, and *E. singularis* (S) from stromata from chaparral oak. The curves were plotted with the percentage of transmission of light as ordinates and the wave-lengths of light in $\mu\mu$ as abscissae.

Figure 3 shows the curves derived from the percentage of light of the different wave-lengths transmitted by the alcholic extract of *E. tropicalis* in pure culture and also the curves for the alcoholic extracts of the stromata of *E. singularis* grown on oak, the stromata of *E. gyrosa* grown on beech and the stromata of *E. parasitica* grown on chestnut. The curve obtained for the extract from *E. tropicalis* is typical of the group. This fungus was grown on artificial culture

media and thus is the only one in this group directly comparable with those of the other two groups.

The curves of this group are different from those of type I in that more of the violet and blue are transmitted. They are separated from those of group II by the fact that their minimum percentage of transmission is in the blue. A higher percentage of wave-lengths shorter than 460 $\mu\mu$ and a slightly higher percentage of the yellow is transmitted than in any of the others. The chief difference in these three groups is in the degree of absorption of the shorter wave-lengths of light by the solutions.

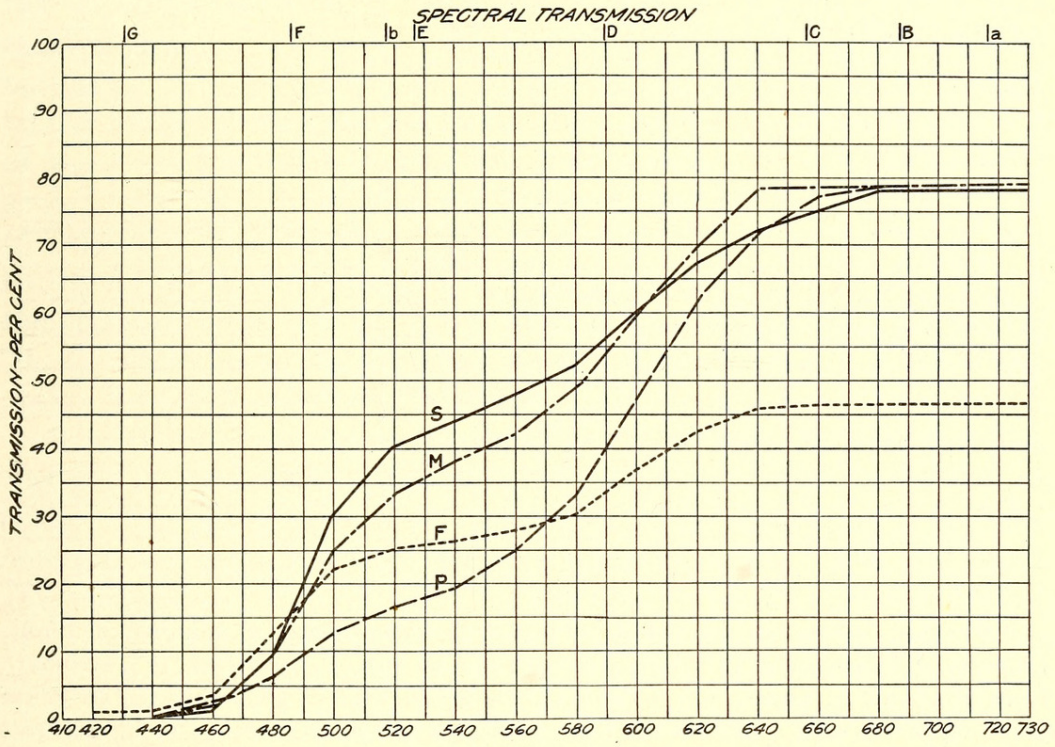

FIG. 4. Curves of percentage of spectral transmission of the alkaline alcoholic extracts of pure cultures on rice of *E. parasitica* (P), *E. fluens*, (F), *E. fluens mississippisensis* (M) and *E. singularis* (S). The curves were plotted with the percentage of light transmitted as ordinates and wave-lengths in $\mu\mu$ as abscissae.

The curves derived from the percentages of spectral transmission of the alcoholic extracts were considerably different when these solutions were made alkaline. They group themselves into two classes and the curves within each class are more widely divergent than in the acidified solutions. As in the acidified extracts one striking dif-

ference between these two groups is in the transmission of the shorter wave-lengths. In figure 4, which shows the curves of spectral transmission of *E. parasitica*, *E. fluens*, *E. fluens mississippiensis*, and *E. singularis* grown in pure culture, it is apparent that very little of the light of a wave-length below 480 $\mu\mu$ is transmitted. From this point the percentages of transmission increase with increase in wave-length.

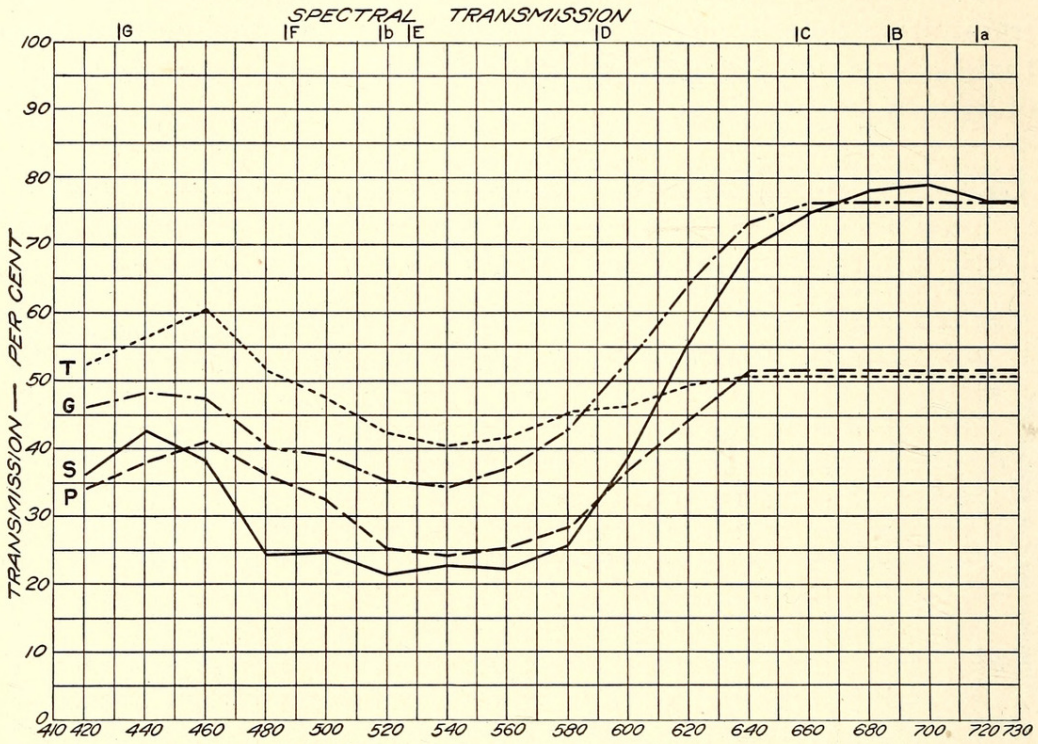

FIG. 5. Curves of percentage of spectral transmission of alkaline alcoholic extracts of *E. tropicalis* (T) from pure cultures on rice, *E. parasitica* (P), stromata from chestnut, *E. gyrosa* (G), stromata from beech, and *E. singularis* (S) stromata from chaparral oak. The curves were plotted with the percentage of light transmitted as ordinates and wave-lengths of light in $\mu\mu$ as abscissae.

The curve of the percentages of transmission of the alkaline extract from the mycelium of *E. tropicalis* in pure culture shown in figure 5 is an example of the other type of curve. With this solution about 52 percent of the light of a wave-length of 420 $\mu\mu$ passed through and 60 percent of the wave length of 460 $\mu\mu$. This is the maximum transmission of this solution.

The other three curves shown in figure 5 were derived from the percentages of light transmitted by the alkaline alcoholic extracts from

E. gyrosa, *E. singularis*, and *E. parasitica*, stromata on beech, chaparral oak, and chestnut respectively. These curves are the same type as that obtained for *E. tropicalis*, but in the case of the extracts from *E. gyrosa* and *E. singularis* considerably more light was transmitted in the longer wave-lengths.

The chief difference between these two groups, as shown in figures 4 and 5 is in the percentage of violet and blue light transmitted by the extracts.

In the foregoing pages it has been shown that there were three types of curves of the percentage of spectral transmission in the acidified alcoholic extracts of these seven species of fungi. This fact seemed to indicate the presence of several different pigments in the cultures. The differences in spectral transmission might of course be due either to the presence of different pigments, or to the presence of the same pigments in different proportions. Investigations were accordingly carried out to see if there was any common pigment and to determine if possible the presence of other pigments. As it was hardly possible to investigate carefully the pigments formed by all the species, forms typical of the three groups were chosen for study. Those selected were *E. parasitica* as a type of group I, *E. fluens* from group II and *E. tropicalis* from group III.

The fungi were grown on rice in Erlenmeyer flasks until the medium was well covered with mycelium and showed a considerable amount of color. This usually required from three to six weeks. The culture medium and mycelium were then removed from the flasks and dried at a temperature of about 60° C. The dried mass was ground, placed in a percolating funnel and extracted with cold neutral alcohol. The extract thus obtained was concentrated under reduced pressure until nearly all the alcohol was driven off. The residue was washed into a precipitation jar with water and from 10 to 15 volumes of water added. A reddish yellow precipitate resulted. This precipitate was collected on a filter and extracted with ether until the solvent came through nearly colorless. It was noticeable that a residue always remained on the filter and was especially large in the preparation from *E. fluens*. The ether extract was concentrated and four volumes of petroleum ether added to it. A yellow amorphous precipitate of pigment settled out. This pigment was separated by filtration, dried, redissolved in ether and precipitated out with petroleum ether. Pigments were obtained from all three fungi which, judged by their

appearance, their solubility in ether and alcohol and the fact that they were practically insoluble in water, were very similar. No crystalline compound was obtained by this method.

An acetyl derivative was prepared from this yellow pigment by dissolving a quantity in acetic anhydride to which some anhydrous sodium acetate had been added and boiling under a reflux condenser for two and one half hours. It was allowed to cool and was poured into a beaker of cold water and allowed to stand with frequent stirrings for ten or twelve hours. The precipitate which had formed was separated from the solution, dissolved in absolute alcohol and cleared with animal charcoal. It crystallized from the alcoholic solution in yellow needles. After recrystallizing three times the melting point was determined and found to be between 240° and 243° C. uncorrected. The acetyl derivatives were prepared from the yellow pigment from all three fungi and were apparently identical. They had the same appearance, solubility, and melting point. There was, however, a considerable difference in the yield of acetyl derivative from the different pigments. The largest yield in proportion to the quantity of pigment used was obtained from the pigment from *E. tropicalis;* the smallest was from the yellow precipitate from *E. parasitica.*

The acetyl derivative was broken down and the original pigment recovered by dissolving the crystals in concentrated acetic acid, with heat, and then adding a drop or so of concentrated sulphuric acid and warming again. Several volumes of water were then added and the pigment was precipitated out. The mixture was filtered and then washed with water and dissolved in alcohol. The acetyl derivatives of this yellow pigment from all three species of Endothia were broken down in the same way. The three alcoholic solutions were treated with a number of common reagents and reacted in exactly the same way in all cases. It is evident that the three species of Endothia produce the same pigment when grown on rice flasks and, as these three species are typical of the three groups mentioned earlier in this paper, it is highly probable that all the species of Endothia studied produce this pigment. This pigment will be designated pigment *A* throughout the rest of this paper. It is soluble in acetic acid, ether, carbon-tetrachloride, and a number of other organic solvents. It is slightly soluble in petroleum ether. It dissolves with a green color in sulphuric acid and in concentrated nitric acid. When acidified it is yellow and is insoluble in water. When made alkaline it has a color

approaching crimson magenta. The dry pigment is soluble in dilute aqueous solutions of sodium or potassium or ammonium hydroxides, or sodium or potassium carbonates.

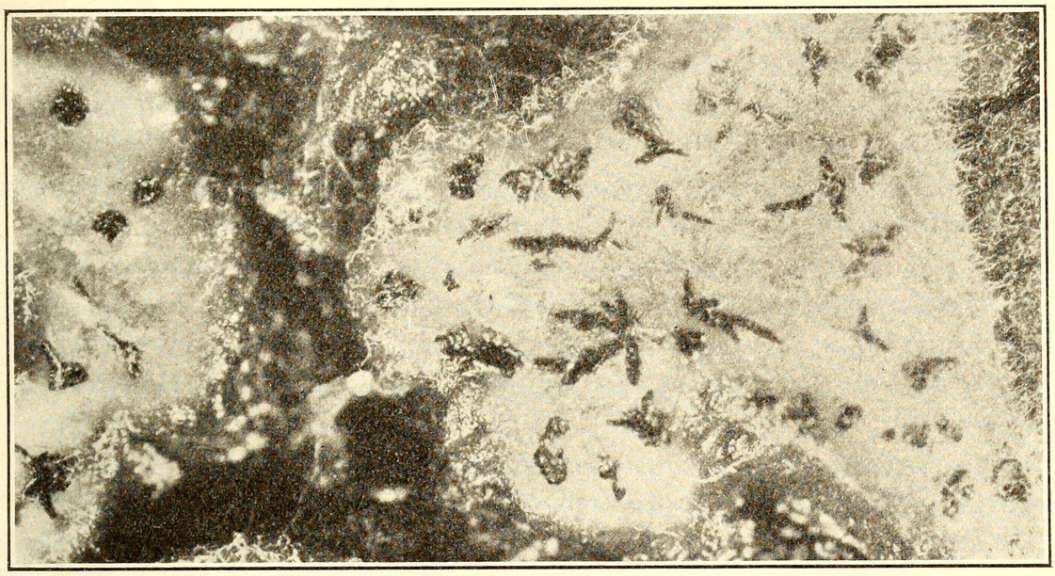

FIG. 6. Photomicrograph of crystals of pigment *B* on the mycelium of *E. fluens* grown on rice. (The writers are indebted to Dr. Erwin F. Smith for this photograph.)

As was mentioned earlier, not all pigments extracted from the ground-up rice and mycelium with alcohol were readily soluble in ether. The residue remaining after the ether extraction in the case of the pigment mixture from *E. fluens* was considerable. The cultures of this fungus on rice, as has been pointed out, show a brilliant purple color in the medium while the mycelium on top is yellow. In the present study a red pigment frequently crystallized out on the mycelium of *E. fluens*, fig. 6. These crystals were not observed in cultures of *E. parasitica* or *E. tropicalis*. It was noticed also that when the concentrated alcoholic extract was treated with water the water was a brilliant red.

These facts seemed to indicate that part of the pigment *A* was alkaline and soluble in water and slightly soluble in ether, or that some other pigment was formed by this species of Endothia along with pigment *A*. The red watery solution obtained from the first precipitation of the yellow pigment was evaporated to dryness and taken up in hot dilute alcohol. The dark red solution thus obtained was con-

centrated in a desiccator and a red crystalline precipitate was formed. The residue from the first precipitation of the pigment of *E. fluens* after the extraction with ether was treated with alcohol. The greater part of this residue dissolved. When this solution cooled a red crystalline precipitate was formed similar to that obtained from the watery extract mentioned above. The crystals were red glistening plates and were optically active. After two recrystallizations an acetyl derivative was prepared according to the method already described. The compound crystallized out of absolute alcohol in colorless needles. It was recrystallized twice and dried. It melted at 196° to 197° C. uncorrected. A portion of the acetyl derivative was broken down with sulphuric acid after the method followed with the acetyl derivative of pigment *A*, and a pigment was obtained which had much the same appearance as the original red pigment, which will be designated as pigment *B* in this paper.

The properties separating pigment *B* from pigment *A* are: It forms a different acetyl derivative, is only slightly soluble in ether, insoluble in toluene, carbon-tetrachloride, petroleum ether, and concentrated nitric acid. It is soluble in water and may be crystallized from a water or dilute alcohol solution. When a dilute solution is treated with a drop of ferric chloride it becomes darker, assuming a greenish raw-sienna color. The alcoholic solution when made slightly alkaline closely approaches orange vermillion in color. Crystals of the red pigment found on the mycelium of *E. fluens* were removed and tested with various reagents. The reactions were apparently the same as those just described.

Pigment *B* has not been discovered in cultures of either *E. parasitica* or *E. tropicalis* grown on starchy culture media. It may be elaborated by these two fungi, but if so it occurs in such small amounts as to render detection exceedingly difficult.

It was mentioned in the description of the work with pigment *A* that the yield of acetyl derivative obtained from the yellow precipitate from the extract from *E. parasitica* was very small as compared to the yield from a similar amount of the yellow pigment from *E. tropicalis* or *E. fluens*. It was also shown that the alcoholic solution of the pigment from *E. parasitica* grown on rice has a considerably different spectral transmission than that from *E. tropicalis*. These considerations made it seem quite possible that another pigment might be present in the solution in addition to pigment *A*.

The alcoholic solution remaining after the acetyl derivative of the pigment from *E. parasitica* had crystallized out and had been separated was treated with about four volumes of water. A flocculent yellow precipitate was formed. This was filtered off and washed with water. The dry pigment was amorphous and of a bright yellow color. No acetyl derivative was formed even on long boiling with acetic anhydride and sodium acetate. When the pigment was dissolved in alcohol and treated with dilute alkali, the color closely approached rose doré. It is evidently another pigment and the properties which separate it from pigment *A* are as follows: It does not form an acetyl derivative; it has an entirely different color when treated with alkali; it is insoluble in cold petroleum ether and dissolves in cold concentrated nitric acid with an orange red color and is red when dissolved in cold sulphuric acid. It is readily distinguished from pigment *B* by its solubility in ether and nitric acid and insolubility in water and very dilute alcohol, also by its appearance when dry and when in acid or alkaline solution. This pigment, which will be referred to in this paper as pigment *C* is also found in extracts from *E. fluens*. Its presence has not yet been demonstrated in the extract from *E. tropicalis*.

From the experimental work just described it is evident that there are at least three different pigments formed by species of this genus, pigment *A*, apparently common to all species, pigment *B* found in *E. fluens* and probably also in the other species having a similar spectral transmission of the acid alcoholic solutions and pigment *C* which is present in the two groups typified by *E. fluens* and *E. parasitica*.

It is of course quite possible that these three pigments are closely related chemically and may be derivatives of the same substance. They are similar in many particulars. All three are composed of carbon, hydrogen and oxygen. That is, on incineration they leave no ash and tests for nitrogen,[8] phosphorus, sulphur and the halogens showed that none of these elements were present.

The comparative solubilities of these three pigments are shown in Table I. These tests were all at room temperature and indicate whether or not the pigment is appreciably soluble in the reagent used.

From the table it is evident that these pigments, especially pigments *A* and *C*, are readily soluble in a considerable number of organic

[8] Fresenius, C. R. Quantitative Chemical Analysis. Cohn's translation of the sixth German edition, 2: 4–7. 1911.

solvents. They are not in all cases soluble in the same reagent, however, and these points of difference are of use in distinguishing and separating the pigments.

TABLE I

Solubility of Pigments A, B and C, in Various Reagents at Room Temperature

Reagent	Pigment		
	A	B	C
Acetic acid	Soluble	Soluble	Soluble
Acetic ether	"	"	"
Acetone	"	"	"
Ethyl alcohol	"	"	"
Methyl alcohol	"	"	"
Amyl-alcohol (normal)	"	"	"
Ether	"	Slightly soluble	"
Toluene	"	Insoluble	"
Benzol	"	Soluble	"
Carbon-tetrachloride	"	Insoluble	"
Chloroform	"	"	"
Carbon-bisulphid	"	"	"
Petroleum ether	Slightly soluble	"	Insoluble
Sulphuric acid	Soluble	Soluble	Soluble
Nitric acid (conc.)	"	Insoluble	"
Water	Insoluble	Soluble	Insoluble

The statement by Pantanelli that the coloring matter of *E. parasitica* is a lipochrome seems hardly to be corroborated by the evidence brought out in these experiments. Lipochromes according to Zopf[9] and Samuely[10] break down readily when exposed to light and air. They are soluble with a green color in concentrated sulphuric and nitric acids. When saponified by boiling with dilute sodium hydroxide they are insoluble in alcohol. According to Zopf they are soluble in petroleum ether and insoluble in water.

The pigments obtained in this study did not break down when exposed in solution to light and air. Solutions were kept in the laboratory for more than a year without apparent deterioration. Pigment *A* was the only pigment which gave a green color when dissolved in concentrated nitric or sulphuric acids. Pigment *B* was insoluble in this last mentioned reagent. Boiling in a dilute solution of sodium hydroxide did not, apparently, affect the solubility of the pigments in alcohol. Pigment *A* was the only pigment soluble in cold

[9] Zopf, W. Die Pilze. Page 144. Breslau, 1890.

[10] Samuely, F. Abderhalden's Handbuch der Biochem. Arbeitsmethoden II: 758, 1910.

petroleum ether. Pigment B was soluble in water. It is obvious then that these pigments are lacking in many of the properties of lipochrome and there is little reason at present for assuming that they belong in this rather indefinite group.

There is some evidence to support the conclusion of Anderson that the pigment in *E. parasitica* is aurine. According to Rota's[11] system for the classification of coloring matters these two pigments might be classed as aurin.

Aurin is the trade name applied to a red pigment obtained by heating phenol and oxalic acid with sulphuric acid. According to Dale and Schorlemmer,[12] it was applied to this preparation as prepared by Kolbe and Schmitt.[13] Dale and Schorlemmer found this preparation to be a mixture of compounds and succeeded in separating out what they considered pure aurin. The name aurin is retained by Schultz and Julius[14] and by Allen[15] for para-rosolic acid which according to Allen has a formula $C_{19}H_{14}O_3$. This dye, however, is insoluble in benzol and carbon-bisulphide and by boiling with sodium hydroxide and zinc dust it is decorolorized. This is not true of the pigment from *E. parasitica*. Moreover, a comparison of pigments *A* and *C* in both alkaline and acid solutions with solutions of commercial aurin shows that they are not the same color.

Other points of difference might be mentioned. It is, however, apparent that while aurin and the pigments from *E. parasitica* have some properties in common the conclusion that they are the same is unwarranted. Whether any of the three pigments considered in this paper are similar in structure to aurin is a problem which needs further investigation. It lies within the scope of this paper to take up the chemical and physical properties of these pigments only in so far as is necessary for separating and distinguishing them from each other. Their chemistry is under investigation and will be considered in a later paper.

[11] Wiley, H. W., et al. Official Methods of Analysis of the Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bull. 107. 1910.

[12] Dale, R. S., und Schorlemmer, C. Ueber das Aurin. Ber. Deutsch. Chem. Ges. 4: 574–576. 1871.

[13] Kolbe, H., u. Schmitt, C. Rother Farbstoff aus dem Kreosot. Annalen der Chemie und Pharmacie. **119**: 169–172. 1861.

[14] Schultz, G., u. Julius, P. Tabellarische Übersicht der Künstlichen Organischen Farbstoffe. Dritte Auflage. 124, 1897.

[15] Allen, Alfred, H. Commerical Organic Analyses. ed. 3. 3: 310–311. 1902.

Early in this work it became apparent that all the members of the genus studied elaborated pigments which were bright yellow when acidified and red when alkaline. This suggested the possibility that if *E. parasitica* could be grown on a sufficiently alkaline medium it might produce the purple color considered characteristic of *E. fluens*. The writers were able to suppress the production of the purple color in cultures of *E. fluens* by the addition of 10 cc. n/10 sulphuric acid to each 100 cc. culture flask. Cultures of *E. parasitica* were made to produce a wine color in the culture media by the addition of 2 grams of calcium carbonate to each 100 cc. flask before sterilizing.

While not particularly significant, these tests furnish another example of the necessity of carefully standardizing culture media used in critical comparative study of fungi. This is especially true since no character is more commonly used to distinguish species of fungi in pure culture than the production or nonproduction of color changes in the mycelium or culture media. Under carefully controlled cultural conditions the ability to produce color on certain media may be a distinguishing character of great value, as in the work of Appel and Wollenweber[16], Grossenbacher and Duggar,[17] Thom,[18] and others.

In studying the growth of species of Endothia on liquid media it was noticed that *E. parasitica* produced a red coloration in old cultures when the medium contained peptone. This was found to be due to the ammonia liberated by the growth of the fungus acting on the yellow pigment. Anderson (loc. cit., p. 14) mentions the fact that old cultures of *E. parasitica* often become purple or wine colored and attributes this change to the fact that the fungus in its growth on the agar gradually causes it to become alkaline, thus changing the pigment from yellow to purple. The writer's investigations indicate that these conclusions were probably correct and that the change to an alkaline reaction may have been due to the formation of ammonia in the cultures.

In the experimental work described it has been shown that three

[16] Appel, O., and Wollenweber, H. W. Grundlagen einer Monographie der Gattung Fusarium (Links). Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft. 8: 1–207. 1910.

[17] Grossenbacher, J. G., and Duggar, B. M., A contribution to the Life-history, Parasitism, and Biology of *Botryosphaeria ribis*. N. Y. Geneva Agr. Exp. Sta. Tech. Bull. 18. 1911.

[18] Thom, Charles. The *Pencillium luteum-purpurogenum* group. Mycologia 7: 134–142. 1915.

different pigments are elaborated by the fungi in this genus. It has been shown further that the curves of the percentage of spectral transmission of the acidified alcoholic extracts of the pigments from the seven different fungi group themselves into three distinct classes. An investigation of the pigments produced by typical fungi from each of these three classes, *i. e.*, *E. parasitica*, *E. fluens*, and *E. tropicalis*, show that there is one pigment common to all three groups but that each of the three fungi is characterized by some one of the three pigments.

E. tropicalis apparently elaborates only pigment *A* in quantity, although a very little of pigment *C* may be present. *E. parasitica*, the type of the group which contains also *E. longirostris* and *E. fluens mississippiensis*, secretes pigment *A* in small amounts, but pigment *B* was not found at all. Pigment *C* is characteristic of this group. The group containing *E. fluens*, *E. gyrosa*, and *E. singularis*, of which *E. fluens* is considered typical, is apparently the only one of the three which secretes all the pigments. Pigment *B* is found only in this group, and is thus characteristic of the group. This pigment is soluble in water and is the cause of the "perilla purple" color in cultures of this fungus. It frequently forms crystals on the mycelium (fig. 6).

It is evident from this work that the curves of percentage of spectral transmission shown in figures 1 to 5 are in most cases curves of mixtures of these pigments, and that the difference in the curves of spectral transmission for the three groups is due to the fact that different pigments predominate in the alcoholic extracts from the fungi of these three groups. It is probable that further investigation with quantitative methods would show that the variation in the curves for different members of the same group was due to the presence in varying proportions of the pigments characteristic of that group.

It is of interest to note that the grouping of the species based on the spectral transmission of the acidified alcoholic extracts of the mycelium shows no apparent agreement with the division based on morphology, host, or geographical distribution. *E. tropicalis*, which apparently produces only pigment *A*, differs to be sure from all the other fungi examined in host and geographical distribution, being known only from Ceylon on Elaeocarpus. It is, however, rather closely related morphologically to *E. parasitica* and other members of this group.

The group characterized by a curve of spectral transmission in-

dicating the presence of pigments *A* and *C* contains only forms having oblong fusiform to oblong ellipsoid ascospores, and with somewhat similar stromatic characters. The members differ widely, however, in host and geographic relations, *E. longirostris* being a tropical form known at present only from Porto Rico and French Guiana. *E. parasitica* and *E. fluens misissippiensis* occur on the same hosts, Castanea sp. and Quercus sp., but *E. parasitica* is the destructive chestnut blight organism known already from China, Japan, and the United States, while *E. fluens mississippiensis* is a weak saprophyte which has been found in only four localities in the United States.

The group characterized by a spectral transmission curve indicating the presence of all three pigments contains species widely different in morphology and distribution. *E. gyrosa* and *E. singularis* have cylindrical ascospores and are found only in the United States, *E. singularis* only on the chaparral forming species of Quercus in Colorado and New Mexico. *E. gyrosa* is found on species of Quercus, Fagus, Castanea, and Liquidambar, and is widely distributed in this country, though abundant only in the southeastern portion. *E. fluens*, on the other hand, is a cosmopolitan species found in the United States on Castanea and on Quercus in Europe and Asia on a variety of hosts. In their stromatic characters also these species are widely different. The stromata of *E. singularis* are large and irregular, being 3–5 mm. wide by 2–4 mm. high, and disintegrate into a powdery mass when the wall is ruptured. The stromata of *E. fluens* are much smaller, being only .75–3 mm. in diameter by .5 to 2.5 mm. high and very compact.

On the other hand, the two most closely related fungi of the genus, *E. fluens* and *E. fluens mississippiensis*, fall in different color groups. In fact, it may well be that the production of pigment *B* by *E. fluens* is the chief character which distinguishes it from its variety. The varietal name was proposed by Shear and Stevens to designate a form which they were unable to separate from *E. fluens* on morphological grounds, but which showed constant differences on culture media.

The fact that the red pigment in *E. fluens* is not found in *E. parasitica* grown on the same media and under the same conditions is of especial interest since the two species are so much alike morphologically and grow on the same host, yet differ so widely in their relation to their hosts, *E. parasitica* being, as has already been pointed out, the uniformly destructive chestnut blight parasite, while *E. fluens* is a harmless saprophyte.

The fact deserves more than passing notice also that what is apparently the same pigment is produced by all the known members of a genus, which, although small, includes species from four continents, from both temperate and tropical climates and occurring on rather unrelated hosts.

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