Review

Comprehensive Description of *Fusarium graminearum* Pigments and Related Compounds

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Abstract: Several studies explore in depth the biochemistry and genetics of the pigments present in *Fusarium graminearum* but there is a need to discuss about their relationship with the mold’s observable surface color pattern variation throughout its lifecycle. Furthermore, they require basic cataloguing and description of their major features known so far. Colors are a viable alternative to size measurement in growth studies. When grown on yeast extract agar (YEA) at 25 °C, *F. graminearum* initially exhibits a whitish mycelium, developing into a yellow-orange mold by the sixth day and then turning into wine-red. The colors are likely due to accumulation of the golden yellow polyketide aurofusarin and the red rubrofusarin, but the carotenoid neurosporaxanthin possibly play also a major role in the yellow or orange coloration. Torulene might contribute for red tones but it perhaps ends up being converted into neurosporaxanthin. Culmorin is also present but it does not contribute for the color, though it was initially isolated in pigment studies, and there is the 5-deoxybostrycoidin-based melanin, but it occurs mostly in the teleomorph’s perithecium. There is still a need to chemically quantify the pigments throughout the lifecycle, analyze their relationships and how much each impacts *F. graminearum* surface color.

Keywords: *Fusarium graminearum*; color; pigments; polyketides; carotenoids

1. Introduction

*Fusarium graminearum* (teleomorph: *Gibberella zeae*) is a pathogen of maize, wheat, rice and barley responsible for the disease known as *Fusarium* head blight (FHB) and mycotoxin contamination [1,2]. FHB destroys the grain starch and protein and was responsible for losses over $2.7 billion in United States between 1998 and 2000 [2]. The mold’s most common mycotoxins are nivalenol (NIV) and deoxynivalenol (DON) [3], usually occurring together and frequently associated with gastrointestinal disorders among other health impairments [4]. Yet, there are other toxins as relevant, such as zearalenone (ZEA) [5], an estrogenic compound capable of causing abortion and other reproductive complications [6,7].

There are very few studies comprehensively describing and relating *F. graminearum* surface colors and its pigments, their properties and biosynthetic or genetic origin, though some were isolated during 1930-60 [8-12]. There was also considerable chemical analysis on *Fusarium* pigmentation in late 1970s and early 1980s but never trying to relate the compounds with the mold’s observable biological phenomena [13]. Recent sequencing of *F. graminearum* genome and development gene replacement tools allowed major progress in genetic and biochemical studies [13]. Now, it is known that red pigmentation of *F. graminearum* is due to deposition of aurofusarin in the walls [2,14], but it is likely to be rather the combination of several pigments [15,16].

Pigmentation is part of the mold growth process and it can be used as a tool for growth studies as an alternative to the expansion in size [17]. This approach can help overcoming spatial constraints in fungal studies or applications such as the limited size or particular shape of a Petri dish or bioreactor, or even predict toxin production solely by analyzing the mold surface color. For instance,
mutants with absence of the pigment aurofusarin seem to produce an increased amount of ZEA [2], and histone H3 lysine 4 methylation (H3K4me) is important for the transcription of genes for biosynthesis of both DON and aurofusarin [18]. Thus, there is some connection between the production of major Fusarium mycotoxins and pigments.

This review aims to identify the major F. graminearum pigments described in the literature and summarize what is known so far about them so that future researchers will be able to more comprehensively relate their dynamics and the mold’s color change.

2. F. graminearum colors throughout its lifecycle

It is first important to know that there is no single set of colors to describe F. graminearum throughout its lifecycle. The surface colors change depends on several variables such as strain, maturity, nutrients, temperature, pH, water activity, light exposure and aeration [8,10,16,17]. Ashley, et al. [8] mentioned early studies identifying pH as the main determinant of Fusarium colors, “so that one and the same culture may be orange or yellow colored at an acid reaction, the color changing to red or blue when the medium becomes alkaline”. Medentsev, et al. [19] said that the biosynthesis of naphtoquinones (major secondary metabolites, including pigments) is the mold’s main response to stress. F. graminearum has different types of pigments, all with distinct properties [8,15,20-22], from which we have to expect numerous combinations and the resulting chromatic attributes. For instance, the teleomorph was found to have violet pigmentation in their perithecia [23]. Thus, it is impractical to summarize all possibilities. For this reason, this description will simply focus on the mold grown on yeast extract agar (YEA) at 25 °C as an example (Figure 1). The isolate was obtained from the Catalogue of the Japan Collection of Microorganisms (JCM), where it is registered as the teleomorph Giberella zeae (Schwabe) Petch, and it was isolated by Sugiura [24] from rice stubble in Hirosaki, Aomori Prefecture, Japan.

![Figure 1. F. graminearum surface colors on its (a) 3rd day, (b) 6th day and (c) 16th day.](image_url)

YEA is a highly nutritive medium containing agar as solidifier, peptic digest of animal tissue and yeast extract, thus rich in nitrogenous compounds, vitamin B and other nutrients [25]. Furthermore, yeast extracts do not seem to affect the quality or level of aurofusarin, a major pigment, by any Fusarium species [26]. This is important because it is desirable to use the mold’s original coloration in the studies without changing much because of the nutrients available.

F. graminearum colors change in a very consistent and predictable pattern [17]. At a glance, the mold germinates as a pale mycelium and starts to acquire a yellowish coloration between its 3rd and 4th day. It attains its full orange tone on the 6th day and then shifts to dark wine red by the 16th day. The color distribution is heterogeneous: it forms a radial gradient with the center more intensely colored and increasingly pale surroundings. Kim, et al. [1] described the F. graminearum as a “yellow to tan mycelia with the white to carmine red margins”, certainly depending on the condition it grows. A dominant red color tends to become more evenly distributed as the fungus ages, and alternated concentric layers of white and red ring close to the center become increasingly more evident. The
white rings are hairy and seem to be formed of colorless hyphae. The medium’s color change (Figure 1c) from pale to yellow is due to accumulation of aurofusarin [14].

Recent analysis based on red, green and blue (RGB) channels taken for *F. graminearum* photographs shows that the three color components are positively correlated and all exhibit a 3rd degree polynomial trend when measured throughout the first 20 days of its lifecycle, and it makes the colors a potential tool to replace size-based measurements for growth studies and to predict toxin production [17].

3. Major *F. graminearum* pigments

Most of what is known about pigmentation of *F. graminearum* comes from studies on *F. culmorum*, *F. aquae ductuum*, *F. fujikuroi* and *F. oxysporum* and eventual confirmation that the pigments occur across species [15]. Such studies aimed primarily to enhance pigment production for the dye industry as an alternative to synthetic counterparts [27]. A pioneer study by Ashley, *et al.* [8] identified aurofusarin, rubrofusarin, culmorin and their derivatives among the pigments. Since then, other have been mentioned, including perithecial melanin [28] and carotenoids [15]. The most relevant carotenoids from *F. graminearum* are perhaps neurosporaxanthin and torulene the most relevant [14,15,21].

There are two more aspects to consider before listing *F. graminearum* pigments or related compounds:

- Culmorin is colorless but it is included in this review because it was isolated together for the first time during studies of *Fusarium* pigmentation;
- Bikaverin and fusorubin are *Fusarium* pigments [19,29,30] but they are not included in the following list because there is very little evidence about their occurrence and impact on the coloration of *F. graminearum*.

3.1. Aurofusarin

Aurofusarin had been obtained chemically and isolated from *F. culmorum* before Baker, *et al.* [10] extracted and purified from a strain of *F. graminearum* Schwabe. It is a dimeric metabolite belonging to the naphthoquinone group of polypetides [13,31], described as a golden yellow-orange or red micro-crystalline pigment in form of prism [8,13,20,32], with C30H18O12 as its molecular formula (Figure 2), 570.5 g/mol as molecular weight and the melting point over 360 °C [8,33]. It is now assigned as 13191-64-5 under the Chemical Abstracts Service (CAS) [33]. Aurofusarin is the only *F. graminearum* pigment produced under deficiency of nitrogen, phosphorus, oxidative stress, and inhibition of respiration [19].

![Figure 2. Structure of aurofusarin. Source: Glentham Life Sciences [33].](image-url)
activity (a) have also impact on aurofusarin biosynthesis, as they were found to be directly proportional [3].

The species *F. acuminatum*, *F. avenaceum*, *F. crookwellens*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. pseudograminearum*, *F. sambucinum*, *F. sporotrichioides* and *F. tricinctum* all produce aurofusarin [13]. Different strains of *F. graminearum* produce different quantities under similar conditions [34], besides *Fusarium* species, it can also be isolated from *Hypomyces rosellus* and *Dactylium dendroides* [35]. It is negatively correlated with vegetative growth [1], thus the pigment is expected to be more abundant in differentiated structures. Aurofusarin certainly increases the organism’s competitive saprophytic ability due to its antibiotic properties, but it does not help the fungus colonizing the host crops and do not protect the fungus against radiation [2]. The pigment can be extracted using benzene-acetone (4:1) and purified by chromatography on silica gel impregnating with oxalic acid [12,20].

Aurofusarin biosynthetic pathway involves several genes in clusters and at least five enzymatic steps with rubrofusarin as intermediate [36]. For instance, its synthesis requires the intervention of polyketide synthase genes [37] in a 30 kb cluster (Figure 3) including PKS12, AurR1, *aurJ*, *aurF*, *gip1*, and *gip2* [36,38] within at least 11 reading frames (FG02301.1–FG02330.1) plus a facilitator transporter gene (FG02331.1) [39]. Among the genes, AurR1 and *gip2* are believed to be the cluster’s transcription factors [1,13], the putative laccase *gip1* has been described as the potential responsible for the dimerization of rubrofusarin into aurofusarin [36,40]. More recently, two more genes (*gip3* and *gip8*) were also shown to be important for the dimerization [38]. Frandsen, *et al.* [36] mentioned two more orphan genes (*aurZ* and *aurS*), and *AurT*, an aurofusarin pump, responsible of transporting aurofusarin and rubrofusarin across the plasma membrane.

![Figure 3. Biosynthetic gene cluster for aurofusarin in *F. graminearum*. Based on Hoffmeister and Keller [39].](https://example.com/figure3.png)

Though aurofusarin was described before 1937 [8], it is only regarded as food and feed contaminant since the new millennium started [41]. There is considerable literature about the issue [31,41]. It is frequently found in several commodities throughout virtually all climatic regions in the world, some times at concentrations as high as 2,046 µg/kg to 10,200 µg/kg [31]. Beccari, *et al.* [34] detected 10,400–140,000 µg/kg in Italian samples of durum wheat, Ezekiel, *et al.* [42] mentioned concentrations above 800 µg/kg in chicken feed, and Nichea, *et al.* [43] detected median concentrations of 71.4 µg/kg (2011) and 80.7 µg/kg (2014) in native grass of Argentina, intended for grazing cattle. The latter was found co-occurring with zearalenone.

Aurofusarin has antibiotic properties against some fungi [32] and it is a mycotoxin “on its own right” [31]. It was found to be cytotoxic to mammalian cells [31,44], impair growth of red tilapia, inhibit considerably the function of antioxidants and vitamins A, E, lutein, zeaxanthin and fatty acids even at concentrations below 30 µg/kg [42] during the early stages of avian lifecycle, reduce of meat and egg quality by affecting aminoacids and fats [41], exhibit genotoxic effects and cause oxidative stress, and it is known to increase the toxicity of other *Fusarium* mycotoxins such as trichothecenes [45]. It was found to be the only *F. graminearum* toxin significantly causing egg deterioration [32,41].

### 3.2. Rubrofusarin

Rubrofusarin (CAS: 3567-00-8) is a crystalline polyketide red-orange pigment [8,20] usually found in the form of needles [10,46]. Demicheli, *et al.* [47] described it as a powder. Rubrofusarin belongs to the class of naphthopyrones and resembles the aurofusarin monomer [13], consisting of a mono methyl ether [8] (Figure 4). Indeed, the biosynthesis of both pigments seems connected because different levels of aeration produce distinct proportions of rubrofusarin and aurofusarin [22].
Stout, et al. [9] and Tanaka et al. [48] were the first investigators presenting its structure in 1962. According to them, rubrofusarin’s molecular formula is C_{15}H_{12}O_{5} and the melting point is 210–211°C. The molecular weight is 272.3 g/mol [46]. Rubrofusarin is insoluble in water but it is soluble in ethanol and dimethyl sulfoxide (DMSO) [47]. Its color does not respond to pH, unlike aurofusarin [8]. Furthermore, it has chelating properties, forming complexes with Mg^{2+}, Al^{3+}, Fe^{3+}, Ni^{2+} and Cu^{2+} in solid state and aqueous medium [49-51].

Several studies mention rubrofusarin or derivatives isolated from different sources, usually fungi [52] or plant roots or seed [51], for pharmacological purposes. Ashley, et al [8] published a pioneer report on isolation of rubrofusarin from F. culmorum and F. graminearum. From the fungus Guanomyces polytrix, Mata, et al. [53] isolated rubrofusarin B, a variation of the compound in which methyl ether replaces the hydroxy group at position 6 [54]. Another rubrofusarin producing mold is Aspegilus niger, also known for producing ochratoxins and consequently causing the Balkan nephropathy [55]. Regarding plants, Rangaswami [56] isolated the compound from Senna tora in India, and Oliveira et al. [57] obtained the rubrofusarin glycoside from the softwood of S. macranthera in Brazil. Senna comprises a diverse genus of native leguminous throughout the tropics [47]. Berchemia polyphylia var. leioclada, a woody deciduous plant abundant in China, produces at least three rubrofusarin glycosides [58]. Other rubrofusarin producing species are Paepalanthus bromelioides [59] and Flavoparmelia euplecta [60]. Moreover, it is reasonable to think that virtually all aurofusarin producing organisms also have the potential to produce rubrofusarin because the latter pigment is an intermediate of aurofusarin biosynthetic pathway [36].

F. graminearum synthesizes rubrofusarin through a polyketide chain intermediate by condensing seven acetate units [61]. Deletion mutants of AurR1 and PKS12 cannot synthesize rubrofusarin, exactly as it happens to aurofusarin [13]. Furthermore, FG12040 protein is also responsible for rubrofusarin synthesis, and the process is inhibited in mutants without the gene cch1, known as calcium ion channel encoder [62]. Rugbjerg, et al. [63] confirmed the intervention of the genes mentioned and the metabolic pathway by reconstructing it using Saccharomyces cerevisiae. They “paved the way” for industrial production of the pigment.

There is plenty of pharmaceutical potential of rubrofusarin and derivatives to be unlocked, and some of it has already been demonstrated. Rubrofusarin has antymycobacterial, antiallergic and phytotoxic properties to herbs Amaranthus hypochondriacus and Echinochloa crus-galli [1,38,40,55]. According to Alqahtani, et al. [64], rubrofusarin can enhance the cytotoxicity of paclitaxel (PTX) against the adriamycin-resistant breast cancer cell line MCF-7_{adr}. Still regarding cancer research, rubrofusarin B was cytotoxic for the colon cancer cell line SW1116 [55], and rubrofusarin presented complete inhibitory ability against human DNA topoisomerase II-α meaning that it can be used to develop potential drugs anticancer and antiviral [52]. Jing, et al. [58] found rubrofusarin glycosides to have antioxidant properties, one even stronger than vitamin C. The pigment was also found to exhibit estrogenic activity [65]. Rubrofusarin bioactivity has led Moreira, et al. [51] to effectively demonstrate its fitness to be applied as a fluorescent probe.

3.3. Culmorin

Culmorin (CAS: 18374-83-9) is a natural colorless metabolite found in various Fusarium species [8,20,66,67] with C_{15}H_{26}O_{2} as molecular structure (Figure 5), first isolated by Ashley, et al. [8] and later Barton, et al. [11] establish its structure. Culmorin is defined as a longifolene sesquiterpene diol with
a tricyclo-[6.3.0.0] undecane skeleton [68], and its molecular weight is 238.3 g/mol [69]. It seems to be biosynthesized from trans-farnesyl pyrophosphate [66] and compounds closely related include related compounds include hydroxyculmorins, culmorone and hydroxyculmorone [70-72]. Technically, it is not a pigment in the sense that it has no color, as already mentioned, but it was initially isolated during pigment studies together with aurofusarin and rubrofusarin [12], possibly because they share some chemical properties.

Figure 5. Structure of culmorin. Made on ChemDoodle [73], based on Nara Institute of Science and Technology [74].

So far, the culmorin producing Fusarium species mentioned in the literature include F. graminearum, F. culmorum, F. crookwellense (F. cerealis), F. venenatum [75], and more recently F. praegraminearum, a basal species of the F. graminearum complex [76]. Laraba, et al. stated that 77% of F. culmorum genotypes were capable of producing culmorin in Algeria, and most were around the subtropical areas. Besides Fusarium, the marine fungi Leptosphaeria oreamaris [77] and Kallichroma tethys also synthesize culmorin [75].

Biosynthesis of culmorin requires the gene CLM1 (GenBank: GU123140.1), responsible for encoding a longiborneol synthase for the compound’s pathway [78,79]. The gene CLM2 encodes a cytochrome P450 and this is responsible for the subsequent hydroxylation of longiborneol. Culmorin was also synthesized in vitro using tetrahydroeucarvone [77] (Figure 6). Citric and lactic acids (5%) seem to attenuate the synthesis in feed, and this also happens to other Fusarium metabolites including DON [80].

Figure 6. Reaction scheme for total synthesis of culmorin. Courtesy of Hans J. Reich [81].

F. graminearum grown in durum wheat was found to produce culmorin at concentrations exceedingly high (2,500,000–14,000,000 μg/kg) in central Italy, and there were considerable variations between strains [34]. It was also detected in Norway at median concentrations of 100 μg/kg (wheat), 292 μg/kg (barley) and 2000 μg/kg (oats) [82]. Similar results were found in an ensemble study from Austria, Denmark and Hungary [83]. In Cameroon, Abia, et al. [84] detected culmorin in cereals, nuts...
and derivatives at a median concentration of 100 μg/kg. Generotti, et al. [85] demonstrated that culmorin from contaminated wheat flour can endure an entire biscuit baking process, with final levels up to 92 μg/kg in baked biscuits, corresponding to a percentage of degradation between 25% and 80%. Their final products also had 15-hydroxi-culmorin. Culmorin and derivatives also seem to resist through brewing process, even after the treatment of the substrates with the fungicide Prosaro® 250 [86].

Culmorin has mild [75] antifungal activity against several molds, particularly wheat and corn parasites [77]. It is also phytotoxic [68]. Regarding fungal-plant interaction, culmorin was presented correlation with amount of lutein in durum wheat contaminated with Fusarium [87], and this could be a way to empirically estimate the extent of contamination. Furthermore, it is frequently detected with DON [66,72,85], usually in a quantity 3-fold higher though some variables can influence this ratio [68]. Culmorin and other F. graminearum secondary metabolites seem to enhance DON toxicity in caterpillars [88] and pigs [72,89]. Yet, culmorin itself is weakly toxic and it is negative to Ames test of mutagenicity [75].

3.4. Black perithecial pigment

*Fusarium* is among the deuteromycota now known for having a teleomorphic ascomycota called Gibberella, and as such it develops a reproductive fruiting body called perithecium. The black perithecial pigment, sometimes described as dark blue, violet or purple [28,90], is almost restrict to the fruiting body [91], thought the producing gene is present even in *Fusarium* species with unknown teleomorph [92]. The pigment’s nature is still under study and only recently it has been related to fusorubins [93] and described as "5-deoxybostrycoidin-based melanin" [28] with C_{15}H_{11}NO_4 as molecular formula [94] (Figure 7).

![Figure 7. Structure of 5-deoxybostrycoidin. Made on ChemDoodle [73], based on Frandsen, et al. [28].](image)

Several *Fusarium* species, including *F. graminearum*, *F. verticillioides* and *F. fujikuroi* [28,93], produce the blackish perithecial pigments, and it appears to be related to and ancestral highly conserved gene cluster [95]. The fungus Nectria haematococca also showed the ability to produce 5-deoxybostrycoidin [94]. In *F. graminearum*, the six-gene PGL cluster, particularly the gene PGL1 or PKS5, seems associated with the production of the polyketide synthase responsible for biosynthesis of the black perithecial pigment [37,96]. PGL1 is related to a transcription-associated protein (TAP) cluster called TC3 [91]. Studt, et al. [93] demonstrated association between the dark pigments and fusarubin under the intervention of the so-called *fsr* gene cluster.

Evidences suggest that this pigment is important for the UV or desiccation protection during the differentiation of perithecia and ascospores [37], and this role is a function of its chemical composition, free radical quenching and spherical spatial arrangement [97].

3.6. Carotenoids

Natural carotenoids comprise a family of more than 750 natural lipophilic terpenoids, several of which produced by fungi, though they are not essential for these organisms [15]. Carotenoids are common in molds and they contribute to the yellow, orange and reddish coloration [98]. They have been produced industrially and widely used as food and feed additives [99].

An important characteristic of carotenoids worth mentioning is their sensitivity to light, as Avalos, et al. [15] said illumination induces synthesis of carotenoids through transcriptional inducional of structural genes in *Fusarium*. Jin, et al. [21] identified neurosporaxanthin and torulene
among as the most relevant but other carotenoids likely to play a minor role in *F. graminearum* color pattern are torularhodin, β-carotene, γ-carotene, ζ-carotene and β-zeacarotene [100-102].

### 3.6.1. Torulene

Torulene (CAS: 547-23-9) is a natural carotenoid of importance for industrial application [103,104] with C_{40}H_{54} as molecular formula and 534.9 g/mol as molecular weight [104]. As Figure 8 shows, it has 13 double bonds, a β-ionone and a long polyene chain [103]. In petroleum ether solution, Torulene has a pinkish-red color depending on the concentration [102].

![Figure 8. Structure of torulene. Source: Royal Society of Chemistry [105].](image)

*Neurospora crassa* is the most well-known producer of torulene, but it is also synthesized by *F. fujikuroi* [106], *F. graminearum* [21], the red yeasts *Sporidiobolus pararoseus* and *Rhodotorula glutinis* [107] and related organisms, and the genera *Cystofilobasidium*, *Dioszegia*, *Rhodosporium* and *Sporobolomyces* [102]. These organisms probably produce it as a protection against photo-oxidation and free radicals [108].

Geranyl-geranyl diphosphate is the precursor of torulene, and it is transformed under the intervention of two enzymes called AL-2 and AL-1 produced by eponymous genes [106]. The biosynthesis is mostly influenced by nutrients, especially the sources of carbon and nitrogen, but it also responds to other factors such as aeration, temperature, acidity, exposure to radiation and the presence of chemicals such as alcohols [102].

There is very little information on torulene’s bioactivity and nutritional value, perhaps because it is rare in food [109], but its nature, structure and few evidences provide some hints. Animal studies demonstrated its safety to be used as food additive [103], and Kot, *et al.* [102] added that they can also be used as feedstock and cosmetic additives. The presence of a non-substituted β-ionone ring makes torulene pro-vitamin A, it is likely to present higher antioxidant or free radical scavenging activity than β-carotene, higher reactivity in aqueous solutions, more efficient electron transfer-reactions than lycopene [107], and there is evidence of anti-prostate cancer activity [103].

### 3.6.2. Neurosporaxanthin

Neurosporaxanthin (CAS: 2468-88-4), or β-apo-4′-carotenoic acid, is carboxilic apocarotenoid [98,110] xanthophyll with C_{35}H_{46}O_{2} as molecular formula (Figure 9) and 498.8 g/mol as molecular weight [106,111]. Neurosporaxanthin carries the name of *Neurospora crassa*, from where it was originally isolated, but *Fusarium*, *Verticillium* and *Podospora* species also synthesize it [98,110].

![Figure 9. Structure of neurosporaxanthin. Source: Royal Society of Chemistry [112].](image)

*Prado-Cabrero, et al.* [113] described neurosporaxanthin as a “cleavage product of torulene”. The biosynthesis initiates from the condensation of two geranylgeranyl pyrophosphate into phytoene and following desaturations [98,99]. The cleavage of torulene into neurosporaxanthin requires the carotenoid oxygenase oxygenase CAO-2 resulting first in β-apo-4′-carotenal, the aldehyde of neurosporaxanthin [106]. The major genes involved in the entire process are carRA, carB, carT and carD [98]. The latter gene is in a different cluster.
4. Conclusions

*F. graminearum* pigmentation is very complex but most pigments have similar colors, ranging from yellow and orange to red. Thus, it is perhaps difficult to know how much is each pigment contributing to its color. Yet, the literature points towards aurofusarin and neurosporaxanthin, possibly also rubrofusarin, as the ones impacting *F. graminearum* surface color the most. No source covered simultaneously non-carotenoid and carotenoid pigments and the ones describing each of these classes showed the tendency to state the respective compounds as the main source of coloration, maybe because the pigments have similar colors. It would be a good idea to find out which in fact contributes the most for the coloration, perhaps by experimentation based on distinctive properties of the pigments. For instance, carotenoids are expected to be reactive to light but as far as the literature showed, polyketides such as aurofusarin and rubrofusarin are not likely to change considerably in the presence and absence of illumination. By simple observation, aurofusarin appears to be the predominant because *F. graminearum* specimens grown in dark and illuminated settings do not seem to present different coloration when maintained at the same temperature.

In any case, the color change has previously been demonstrated to follow a predictable trend, disregarding the pigments involved. Thus, whichever the dominant pigments, they follow a consistent pattern over time. It is still difficult to advocate if the changes are mostly due to variations in the proportion of different pigments or chemical reactions leading to changes of the same compound into its derivatives, just like the case of aurofusarin at different pH settings. It can even be simply the breakdown of aurofusarin into rubrofusarin molecules.

Regarding the relationship between pigmentation and mycotoxin contamination, there is solid yet scarce literature relating the genetic and biosynthetic origins of aurofusarin and both DON and ZEA. These studies are still in the preliminary stages but they demonstrate that some genetic factors are common for aurofusarin and these mycotoxins. If such factors have similar influence over aurofusarin and the toxins, variations on the quantity of aurofusarins might theoretically be used to predict the quantity of DON or ZEA. Thus, there is a possibility that *F. graminearum* surface color can be a good predictor of toxicity. Furthermore, aurofusarin is a toxin itself. It can surely be quantifiable through the mold’s surface color. Most of the logic mentioned above also applies to rubrofusarin because its biosynthesis is a step in aurofusarin synthesis. It is also maybe worth mentioning that culmorin is even more related to DON but it is colorless and theoretically cannot be directly measured through *F. graminearum* surface color. In summary, it is reasonable to assume the possibility of using *F. graminearum* surface color to estimate how much toxin the mold is producing and possibly estimate its potential bioactivity.

There is still a lot to be investigated, not only in relation to *F. graminearum*. It is necessary to catalog the pigments produced by each species and clarify their biosynthetic relationships to prevent information “gaps”. For instance, a trichothecene-producing mold is perhaps likely to also produce culmorin, and there are also parallelisms between aurofusarin and rubrofusarin or torulene and neurosporaxanthin. It is true that commercial demand is frequently a major driving force for research and sometimes is not mere choice or curiosity leading investigators, but it is important to always try to build a very cohesive body of knowledge from which other researchers can craft their own contributions.

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