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Fusarium graminearum growth and its fitness to the commonly used models

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Abstract

Fusarium graminearum causes head blight in wheat and corn, and produces chemicals harmful for humans and other animals. It is important to know how it grows in order to prevent outbreaks. There are three well-known growth models for microorganisms and they seem applicable to molds: linear, Gompertz and Baranyi. This study aimed to see which could better represent *F. graminearum's* growth. Three replicates were grown in yeast extract agar (YEA) for 20 days, the Feret's radius was measured in ImageJ software, and then related to the models. Baranyi's model was only acceptable according to a Wilcoxon test ($p = 0.280$). Thus, this shall be the one used, even in routine analyses.tional properties of some wild plants, and the results may be useful for the evaluation of dietary information.

Keywords: *Fusarium graminearum,* Mold growth, Linear model, Gompertz, Baranyi

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Introduction

Fusarium graminearum is a fungal plant parasite responsible for the disease known as FHB (*Fusarium* head blight) in crops such as wheat and corn, and mycotoxin contamination in humans (Weidenbörner, 2001; Yoshizawa, 2013). The toxins include deoxynivalenol, nivalenol and zearalenone (Hussein & Brasel, 2001; Pestka, 2007; Y. Sugiura, 1996). The former two are emetic and the latter has estrogenic properties.

There is a body of studies on fungal growth (Berger, Le Meur, Dutykh, Nguyen, & Grillet, 2018; Garcia, Ramos, Sanchis, & Marin, 2009; Møller, Andersen, Rode, & Peuhkuri, 2017; Sadovský & Koronthályová, 2017). Most are done for practical purposes such as brewing or drug production rather than mere scientific curiosity. Thus, the knowledge is either focused on substrate or environmental settings, very superficial or speculative based on bacterial studies. Thus, there is a need to properly describe how in fact fungi grow, especially molds.

Three major models used to describe fungal growth: one linear and two sigmoidal (Gompertz and Baranyi) (Garcia et al., 2009). Authors have been arguing about which is the best for practical purposes. While some prefer the linear's simplicity, others claim the sigmoidal to be more accurate representing the irregular biological nature of the phenomenon(Buchanan, Whiting, & Damert, 1997).

The choice of an appropriate growth model for *F. graminearum* will allow scientists to more accurately predict the propagation of FHB and prevent outbreaks. This study aimed to find out which major model explains *F. growth* in a system with limited nutrient supply, in minimal medium and at room temperature.

Material and Methods

Isolate

This study used *F. graminearum* from the JCM Catalogue. It is registered as the teleomorph *Giberella zeae* (Schwabe) Petch isolated by Y. Sugiura (1996) from rice stubble in Hirosaki, Aomori Prefecture, Japan. It is a known producer of deoxynivalenol, 15-acetyldeoxinivalenol and zearalenone (Yoshitsugu Sugiura, Watanabe, Tanaka, Yamamoto, & Ueno, 1990).

Incubation and Growth Analysis

Three *F. graminearum* replicates were grown in yeast extract agar (YEA) inside a black box inside a chamber, at room temperature during 20 days. Daily photos were taken using Nikon D3200. The shots were performed vertically at 25 cm above the specimens after opening the Petri dishes.

The radii were measured in ImageJ software. After setting the scale, taking the 90 mm of the plate's diameter as reference, the fungal area was isolated through RGB color threshold. Then, the Feret's diameter was determined and converted in radius. The growth models were determined using reference values such as the duration of lag phase (λ), end of the exponential growth (t_{max}), maximum growth rate (μ_{max}) and the maximum radius (y_{max}). The models were compared to the actual growth using Wilcoxon's paired test at $\alpha = 0.05$.

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Results and Discussion

Observed growth

The mold grew in a logistic pattern with its typical sigmoidal curve (Figure 1**).**

The lag phase took 1 day, followed by 10 days of exponential growth. The maximum growth rate was 33 mm/d and the maximum radius was 45 mm. The Figure 2 shows the mold's growth rate during the exponential phase. It seems consistent with a 4th degree polynomial.

The growth was at its peak in the 2nd day, between the 4th and 5th, and also around the 9th and 10th days. There is a notable valley between the 6th and 7th days.

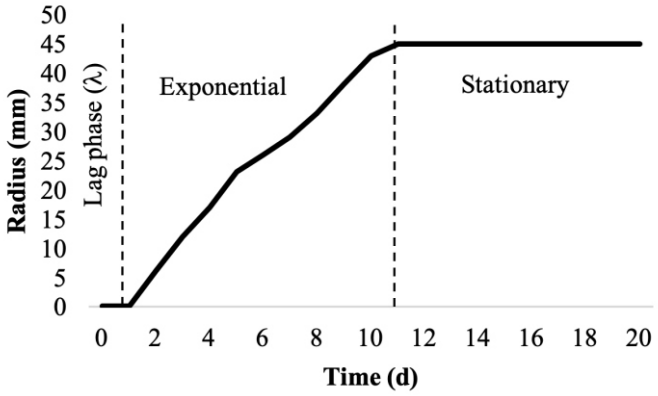

Figure1. *F.graminearum*'s growth during 20 days.

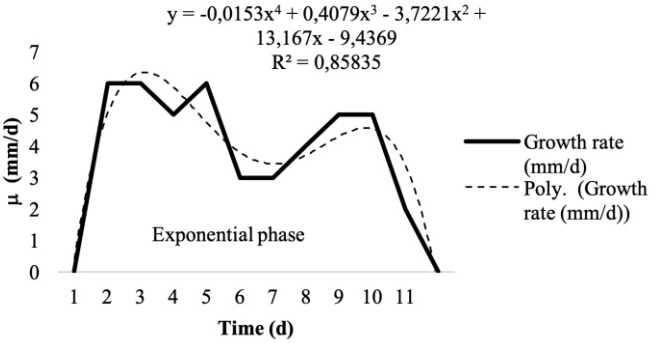

Figure 2. *F. graminearum*'s growth from the first day up to the end of the exponential phase.

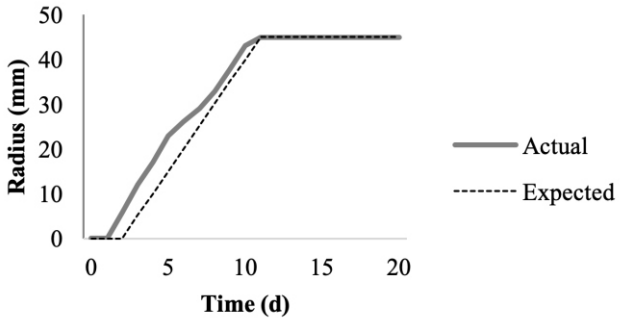

Figure 3. Comparison between the linear growth model and the actual growth.

Comparison with the models

First, the linear model was compared to the actual growth. A paired test suggests significant differences between them (p = 0.002). The Figure 3 below shows how the linear model would represent *F. graminearum*'s growth for 20 days.

The differences were noticeable, especially during the log phase. The lag phase also showed some discrepancy, as the actual growth started at least one day before what the linear model shows. On the other hand, the curves seemed to get closer as the time passes and finally connected in the beginning of the stationary phase. According to these observations, the linear model was not fit to represent *F. graminearum*'s growth.

Then, the Gompertz model was analyzed (Figure 4). A paired test also showed considerable differences between it and the actual growth ($p < 0.001$).

It seemedless representative if compared with the linear model. The Gompertz model seemed very smooth, almost without a lag phase and showing very harmonious transitions between the phases. The stationary phase's onset also took days longer than expected. Thus, this model was not the most adequate to represent *F. graminearum*'s growth.

The last model compared was Baranyi's (Figure 5).

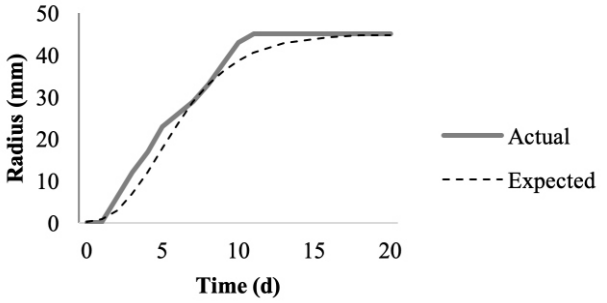

Figure 4. Comparison between the Gompertz model and the actual growth.

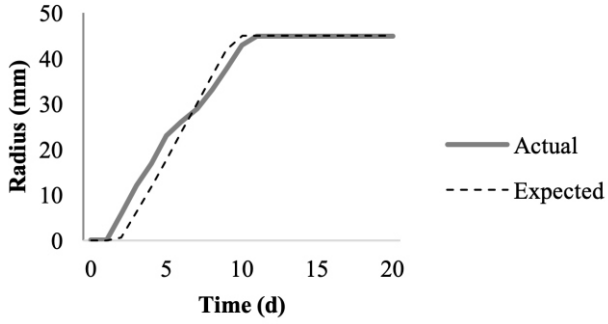

Figure 5. Comparison between the Baranyi's model and the actual growth.

This model did not show enough statistical differences to be regarded as substantially distinct from the observed values (p = 0.28), according to a paired test. They had noticeable discrepancies, especially in the slope variations in the log phase. Baranyi's model also seemed smooth but not as much as Gompertz. Its shapewas somewhere between the two previously analyzed. Yet, besides the considerably irregular shape of the actual growth curve during the log phase, Baranyi's model seemed fit enough to represent *F. graminearum*'s growth.

Discussion

The logistic pattern was expected, as it is common for biological systems, especially if subjected to confined spaces rich in nutrients (Deacon, 2006). In this case, the mold stopped growing in size due to the surface area available, rather than nutrient shortage. Once it happens, a color change is noticeable and it is probably a result of nutrient shortage (Cambaza, Koseki, & Kawamura, 2018).

Neagu and Borda (2013) also studied *F. graminearum*'s growth. Their maximum growth rate was higher (13.5 mm/d) and the fungus attained its full size at the 8th day. The difference might have been due to their media, enriched with barley and wheat extract, while here the medium is minimal, with yeast extract. Even between different yeast extracts the same molds present different behaviors (Sorensen & Sondergaard, 2014). The strain might have been another reason for the differences in growth rates, particularly for the case of *F. graminearum* comprises a polyphyletic group with the distinction between the strains and species still under scrutiny (Goswami & Kistler, 2004). Unfortunately, Neagu and Borda (2013) did not use the current models and ignored details such as phase distinction, probably because they just wanted to find out how long the mold takes to occupy the entire plate's surface.

The growth rate might be explained by some biological interactions (Deacon, 2006; Madigan, Martinko, & Parker, 2017). The lag phase consists of adaptation, followed by rapid growth. The following reduction is probably due to signals sent by the first hyphae reaching the plate's borders and facing the first signs of nutrient exhaustion. But it is not a major problem because there are more nutrients underneath the surface. After a re-adaptation to the new situation, they grew some millimeters and finally stopped growing as most reached the borders.

The linear model is certainly the easiest to work with and it is the most widely used (Garcia et al., 2009), but it should not be applied for *F. graminearum*, even for screening purposes. Gompertz should not also be used for the same purpose. Baranyi seems to be the only representative model for *F. graminearum*'s growth among the analyzed. This result agrees with Garcia's opinion on fungal growth (Garcia et al., 2009) based on Buchanan's meta-analysis on bacterial growth (Buchanan et al., 1997).

Conclusion

F. graminearum grows exhibiting a sigmoidal shape. A 4th degree polynomial regression is fit to predict its growth rate. Further studies may provide more insight at the current observations but as far as this experiment was carried, one shall regard, among size-based models originally developed for bacteria, the Baranyi as the best to represent the growth of *F. graminearum* and certainly many related fungi.

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