PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.1295

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Influence of nutrient sources and environmental conditions on vegetative growth of fungus, *Erythricium salmonicolor* (Berk. & Broome) Burdsall in cocoa

Ishmael Amoako-Attah*, **Eric Kumi-Asare & Yahaya Bukari**

Plant Pathology Division, Cocoa Research Institute of Ghana, Akim-Tafo, Ghana

*Email: amoaksi@yahoo.com

ARTICLE HISTORY

Received: 05 June 2021
Accepted: 15 September 2021

Available online
Version 1.0 : 05 September 2022

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS etc.
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

CITE THIS ARTICLE

Amoako-Attah I, Asare E K, Bukari Y. Influence of nutrient sources and environmental conditions on vegetative growth of fungus, *Erythricium salmonicolor* (Berk. & Broome) Burdsall in cocoa. Plant Science Today (Early Access). https://doi.org/10.14719/pst.1295

Abstract

Erythricium salmonicolor is the cause of pink disease in cocoa in the humid tropics. The growth of 4 isolates (ES-WR006, ES-WR008, ES-ER009 and ES-ER010) of the fungus on different sources of nutrient (carbohydrate, amino acid and vitamin) and environmental conditions (temperature, pH and light) were evaluated in the laboratory. The pink disease isolates utilised the nutrients to different extent, with the best radial mycelial growth rate achieved with isolate ES-WR008 followed by ES-WR006. None of the isolates produced spores in the laboratory. The isolates utilised polysaccharides as best as disaccharides and monosaccharaides. Among 7 amino acids assayed, aspartic acid was the most promising for vegetative growth. The best radial mycelial growth on vitamins was attained on folic acid and riboflavin. The minimum, optimum and maximum temperatures for growing the fungus were 18°C, 28°C and 34°C respectively. Survival of *E. salmonicolor* isolates within the temperature range of 18-34°C is consistent with temperatures for growing cocoa in Ghana. The isolates also grew well within a pH range of 4-8, with the best growth at pH 6. Light quality and duration influenced the vegetative growth of the isolates. Pink disease isolates maintained in 24 hr darkness or 24 hr light performed better than in alternating 12 hr darkness and 12 hr light. There was a significant ($p < 0.05$) repressive effect of the alternating dark and light exposures on vegetative growth of the fungus. The implications of the results for efficient management of the cocoa disease are discussed.

Keywords

Corticiaceae, fungal nutrition, growth condition, pink disease

Introduction

Erythricium salmonicolor (Berk. & Broome) Burdsall, is the cause of stem girdling cankerous infection on tree crops. It causes the disease commonly called 'pink disease' because of a distinctive salmon to pink coloured mycelial growth of the fungus on affected plants. Although the pathogen is globally distributed, it is commonly found in the humid tropics (1, 2). It occurs on a wide range of important tropical crops including *Theobroma cacao* L., (cocoa). An early sign of the pathogen on cocoa is a superficial white, cobweb-like mycelial growth on twigs and branches mostly away from direct sunlight (3). The fungus penetrates the branch resulting in rotting of the bark tissues which dry-up quickly to become a dry rot. Usually, the fungus enters the plant through the natural openings of lenticels or cracks and forms white pustules. As the disease progress, pink-coloured mycelium co-

vers (incrustation) consisting of hyphae and fruiting bodies (4). At this point, the fungus enters the cambium layer, kills the cambium tissues with an accompanying yellowing and subsequent browning of leaves on the affected branches. The most characteristic feature of pink disease infection in cocoa is a die-back symptom often seen as wilting of leaves (Fig. 1) and there is complete defoliation under severe infection. At the advanced stages of the disease, there are formations of orange-red pustules containing spores as the previous white pustules fade away (5). These pustules are formed on portions of the affected branches exposed to direct sunlight.

Fig. 1. Pink disease affected cocoa plant showing the pinkish colouration on the branches with symptoms of die-back and severe wilting of the leaves.

Effective control of pink disease incidence in cocoa has always been problematic. This is because the initial cobweb-like mycelial growth of the fungus is difficult to detect until the pink incrustation stage when the pink disease is most visible to farmers (6). This means that farmers are unable to detect the disease at the early stages until the cocoa plant has succumbed to the pathogen with the accompanying die-back symptom (Fig. 1). In Ghana, the pink disease of cocoa was once categorised as a minor fungal infection but it is now an emerging threat to production of the crop. The causal pathogen is a canopy-dwelling fungus and a decomposer of living woody plants (4, 7). It was reported that in some disease-affected areas, the pathogen could cause cocoa yield losses ranging between 60-100% (6). Akrofi and his team (3) carried out an epidemiological survey in some cocoa growing regions in Ghana and reported that the disease is still spreading widely and reducing cocoa yield. The continuous spread of pink disease is due to a lack of appropriate information to produce potent antagonists for effective pathogen control. There is little information available in the literature on the basic growth requirements of this pathogen, particularly on nutritional and environmental conditions (3, 8).

The primary aim of a pathogen is to access nutrients of the host cell (9). The role of nutrients in fungi growth and development is complex but the pathogens utilise different nutrients such as amino acids, vitamins, carbohydrates and mineral elements for colonisation, growth and reproduction (10-13). Amino acids form the building blocks of the fungus and also affect the infection pathway of the pathogen by influencing the deployment of virulence factors (14). The main function of vitamins is in the enzymatic reactions of the fungus, serving as either coenzymes or cofactors. Carbohydrates are substrate for the production of energy and may be involved in the cellular signaling pathways of the fungus. According to Bani and others (15) successful fungal invasion of a host cell is dependent on a lot of factors which also vary according to nutrient consideration and environmental conditions. Understanding the nutritional needs of the pink disease fungus may provide useful insight into conditions conducive for infection, survival and host range. The influence of environmental conditions such as light, temperature, water activity and pH preferences on growth and development of fungi is well known (11, 16, 17). However, the data on these environmental influences cannot be inferred from one region of the world to the other. It is therefore important to know the requirements for environmental conditions for the fungus causing pink disease in cocoa in Ghana. Such information will help to understand better the population dynamics, adaptation and host-pathogen interrelationships for efficient management of the pink disease. Therefore in this study, the vegetative growth of four isolates of *E. salmonicolor* was evaluated on carbohydrates, amino acids and vitamins media. The effects of temperature, pH and light exposure on the growth of the pathogens were also assessed in the laboratory.

Materials and Methods

Pink disease isolates

Four isolates of the pink disease obtained from mycelium of the fungus growing on bark cankers of infected cocoa trees were used for the study. Isolates ES-ER009 and ES-ER010 were sampled from cocoa farms at Saamang (6° 20′ 84.4′′ N, 0° 29′ 64′′ W) and Asamanma (6 19′ 0″ N, 0 35′ 0″ W) respectively, in the Eastern Region of Ghana. Isolates ES-WR006 and ES-WR008 were from farms at Wiawso (6 12′ 57″ N, 2° 29′ 6″ W) and Elubo (5° 17′ 0″ N, 2° 46′ 0″ W) respectively, in the Western Region of Ghana. Single strand isolations were made successively (twice) from the growing tip of an isolate on water agar and finally onto malt extract agar (MEA). Live cultures of the pure isolates are maintained at a fungal library at Cocoa Research Institute of Ghana.

Inoculum preparation, inoculation and mycelial growth measurement

Discs of the mycelial plug were removed from actively growing margins of each isolate growing on MEA at 25°C for 5 days in the dark and used as inoculum. An inoculum plug (6 mm diameter) was placed with the top-side down at the centres of 4 replicate agar plates per medium. Inoculated plates were sealed with Parafilm (Bemis flexible packaging, Neenah, Wisconsin) and arranged in a completely randomised design (CRD) inside a dark incubator (GenlabPrime®, Genlab Ltd, Cheshire) at 25°C. The colony diameter of each plate was measured along two perpendicular axes at 24 hr. intervals for 96 hr. The 2 measurements for each day were averaged and converted to the

final daily radial growth rate after subtracting the original diameter of the agar plug and dividing by 2. A similar procedure was used to study the effects of temperatures, pH and light on the mycelial growth of the fungus. All the experiments were repeated twice.

Preparation of media

Oxoid MEA media was prepared according to label instructions. Vegetable-8 juice agar (V8JA) was prepared by adding 200 ml each of clarified V-8 juice (Campbell Soup Co., Camden, New Jersey) to 800 ml SDW. Agar powder was added to the media (except MEA) at 2% (w/v) over a water bath at 65 °C and stirred continuously to dissolve. Media were sterilised at 121°C and 1.1kg cm^{-3} pressure for 15 min. in an autoclave machine (Priorclave® Biocote Ltd, Wolverhampton, UK). Preliminary experiments showed that media prepared from V8JA had a low pH value affecting media solidification after heat sterilisation. Hence, 3 g $CaCO_3$ was added to V8JA before sterilisation. All media were dispensed into 90-mm diameter Petri plates (20 ml/plate) and kept for 2 days on a laboratory bench before use.

Assessing the effect of carbohydrate, amino acid and vitamins sources on mycelial growth

Ingredients required to formulate a base medium similar to (18) were glucose (10 gm), KNO_3 (3.5gm), KH_2PO_4 (1.75 gm), MgSO4.7H2O (0.75 gm), NaCl (0.2gm), thiamine hydrochloride (0.5mg) and agar (12gm). These were dissolved in one liter of SDW and chloramphenicol was added at 1% to inhibit bacteria growth. The medium was sterilised by autoclaving for 10 min. Vitamins used in the medium were sterilised through Millipore filtration (0.22 µm filter) and added after heat sterilisation. The different nutrient media were formulated as follows:

1. Glucose as the carbohydrate in the base medium was substituted during the assessments by equivalent amounts of starch, lactose, maltose, sucrose, mannose and fructose. These were calculated using their molecular weights.
2. Similarly, KNO_3 as the amino acid source of the base medium was substituted with equivalent amounts of alanine, aspartic acid, cysteine, glutamic acid, tryptophan and arginine.
3. Moreover, thiamine in the base medium was substituted by the vitamins pyridoxine, folic acid, riboflavin, ascorbic acid, biotin or niacin for assessment.

Each medium was poured into 90 mm Petri plates, kept on a laboratory bench to cool before use. The media were inoculated with the 4 isolates and radial mycelial growth rates calculated as previously described.

Determination of the effect of temperature on mycelial growth

The effect of incubation temperatures on the mycelial growth of the fungal isolates was assessed on V8JA media. The isolates were placed on agar plates as described previously and incubated in the dark at temperatures ranging from 20-36°C at 2°C intervals. At each temperature, 4 replicated plates per isolate were arranged in CRD and assessed at 24 hr. intervals for growth up to 96 hr. It was observed that inoculum plugs of isolates at 36°C did not grow after 7 days. The plugs were transferred onto fresh V8JA plates, incubated at room temperature (23±2°C) and observed for another 7 days to determine their viability.

Determination of the effect of pH on mycelial growth

Due to the challenge of V8JA solidification which needed to be adjusted with $CaCO_3$ as described previously, MEA media was used for the pH studies. The media were mixed with equal volumes of buffer to give hydrogen ion concentrations of 4, 5, 6, 7 or 8. Citrate phosphate (50 mM) buffer was used for pH 4 - 6 while 50 mM Tris-HCl was used to attain pH 7 and 8 according to the method described in (19). The buffer and the media were autoclaved separately and mixed. The media pH was confirmed by measuring with corning pH meter (Corning Inc., NY). Plates were inoculated with the isolates and the radial mycelial growth rates recorded as previously.

Determination of the effect of light on mycelial growth

To determine how light affects the mycelial growth rate, the four isolates were grown on V8JA plates under 3 different light exposures with 4 replications. The fungal isolates were placed under 24 hr. light (2 cool fluorescent lamps, 1.8 kilolux per lamp, SunLite, China), 12 hr. light/12 hr. dark and 24 hr. dark inside GenlabPrime® incubators. The experiments on 24 hr.dark and alternate 12 hr. light/12 hr. dark were performed simultaneously in the same incubator. Plates placed under 24 hr. dark were wrapped in aluminium foil (Caterers foil) and kept inside paper bags in the incubator. Daily radial growth rates were calculated for the isolates as described previously.

Statistical analysis

Initial analysis of each experiment was conducted to check for homogeneity of variances across experiments. Following results that showed similar variances, the averages of treatments in each experiment was used to pool all the three separate experiments together for further analyses. Data were analysed as factorial in CRD based on a nested design structure where the fungal isolates were considered as nested within the respective factors used. All analyses were performed with GenStat 11.1 (2008) and differences in the means were compared at $p < 0.05$ significance.

Results and Discussion

The pink disease isolates grew to different extent on the carbohydrate sources. The radial mycelial growth rates were significantly ($p < 0.05$) influenced by the interactive effects of isolate and the carbohydrate used (Fig. 2). The isolates utilisation of glucose (monosaccharide), fructose (monosaccharide), mannose (monosaccharide), lactose (disaccharide sugar), maltose (disaccharide sugar), sucrose (disaccharide sugar) and starch (polysaccharide) indicates that the fungus is capable of degrading mono-, di- and poly-saccharides for growth and development. This is an advantage to the pathogen in competition with other fungi for nutrients. The result agrees with the earlier reports (20-22) that fungus in the Phylum: Basidiomycota utilises different carbohydrate sources as energy. The best

growth rate was obtained with isolate ES-WR008 attaining 7.1 mm day^{-1} on starch, 7.0 mm day^{-1} on lactose and 6.9 mm day^{-1} on glucose in 4 days in the dark at 25°C. The isolate (ES-WR008) utilised starch as best as glucose and lactose without any significant (p>0.05) differences. However, the rest of the isolates, particularly ES-ER010 and ES-ER009, grew poorly on starch attaining radial mycelial growth rates ranging from 3.1 mm day^{-1} to 3.3 mm day^{-1} in 4 days at 25°C (Fig. 2). Curiously, the isolates grew poorly on fructose (fruit sugar) attaining growth rates ranging from 3.1-4.3 mm day^{-1}. The only exception was ES-ER010 which reached a growth rate of 6.2 mm day^{-1} on fructose. In most instances, isolate ES-ER009 gave the poorest mycelial growth rate on the different carbohydrate media (Fig. 2). Efficient utilisation of a nutrient is dependent on the nature of enzymes produced by the fungus (23); therefore, the poor utilisation of some of the nutrients may be attributed to the low production of the degrading enzymes. For instance, the absence of α- and β-glycosidase in some fungi which are required to break the α-1,4 and β-1,6-glycosidic linkages in the starch molecule has been implicated in poor utilisation of starch (24). Also, repressive effect of fructose on vegetative growth of fungi when used as a sole carbon has been reported (25) and as confirmed here will be useful in the provision of potent antagonists to the pink disease pathogen.

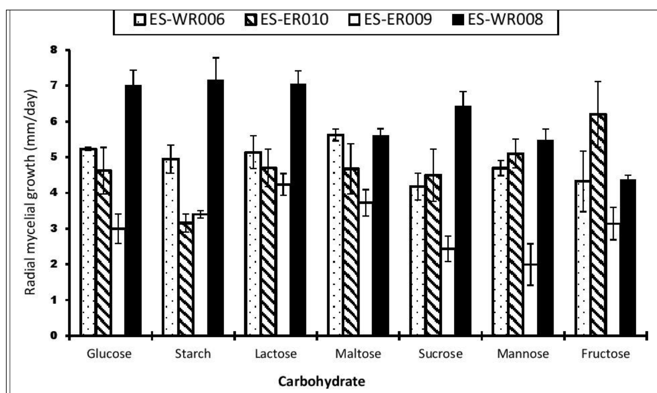

Fig. 2. Effect of different carbohydrate sources on the radial mycelial growth rate of 4 *E. salmonicolor* isolates in the dark at 25 °C. Values are means of pooled data from 3 runs of experiments (4 replications per medium for each isolate). Bars represent the standard error of means.

The fungal pathogen utilised potassium nitrate, alanine, aspartic acid, cysteine, glutamic acid and arginine successfully for growth. There were significant (p = 0.001) differences in the mycelial growth of the isolates on amino acid agar media (Fig. 3). Isolate ES-WR008 and ES-WR006 gave the best radial mycelial growth rates on the media in 4 days in the dark at 25°C. Generally, aspartic acid supported rapid mycelial growth of the pink disease isolates. The growth rates observed ranged from 4.8-6.6 mm day^{-1} in 4 days at 25°C. Thus, aspartic acid proved one of the most important amino acids for vegetative growth of *E. salmonicolor*. Similarly, report is available on aspartic acid and asparagine as the most important amino acids enhancing biomass production in Agaricales (Phylum: Basidiomycota) (26). Additionally, the potassium nitrate which was included in the assay as an inorganic source of amino acid supported the growth of the pink disease isolates comparable to the organic ones. This shows that the pink disease isolates can utilise both organic and inorganic amino acid resources for growth albeit with differences in the metabolic rates (27). The least growth rates of the isolates were on medium amended with tryptophan. On the tryptophan medium, there were no significant (p >0.05) differences in the mycelial growth rates of the 4 isolates (Fig. 3). The poor growth of the isolates on tryptophan may be due to inadequate production or the absence of enzymes to break down the substrate.

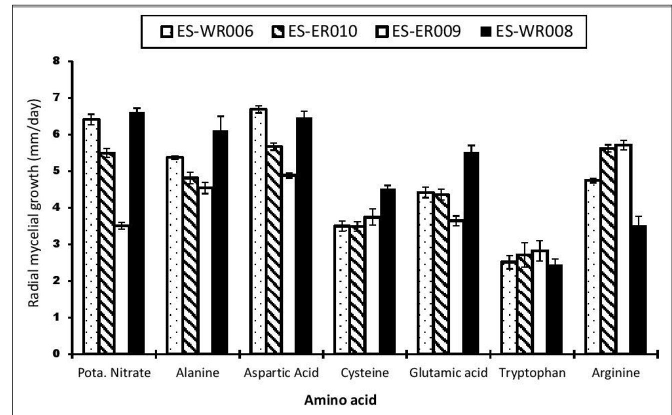

Fig. 3. Effect of different amino acids sources on the radial mycelial growth rate of 4 *E. salmonicolor* isolates in the dark at 25 °C. Values are means of pooled data from 3 runs of experiments (4 replications per medium for each isolate). Bars represent the standard error of means.

Vitamins are involved in regulating the pH of host tissues which directly influences activities of the infecting fungus (13, 28). The 7 test vitamins of thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), biotin (B7), folic acid (B9) and ascorbic acid (vitamin C) variably supported vegetative growth of the four isolates of pink disease (Fig. 4). It has been shown (10) that only vitamins at the right pH could facilitate abundant mycelial growth and sporulation of fungi. The variable support of the vitamins for vegetative growth of pink disease-causing fungus is therefore not surprising. Significant differences (p < 0.05) were observed within isolates on the same vitamin (Fig. 4) emphasising physiological variability of the isolates. The best radial mycelial growth rates of 5.5 mm day^{-1} (folic acid) and 5.4 mm day^{-1} (riboflavin) were attained with isolate ES-WR008.

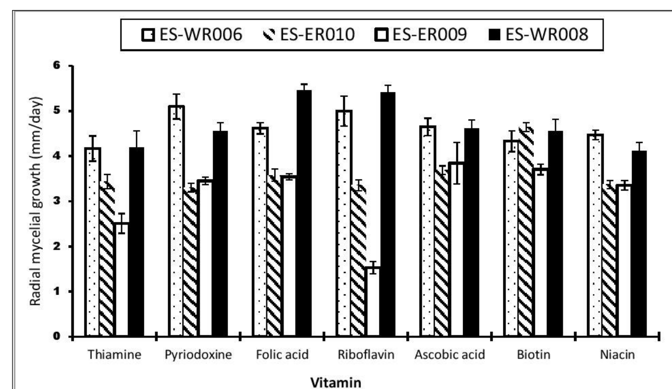

Fig. 4. Effect of different vitamin sources on the radial mycelial growth rate of 4 *E. salmonicolor* isolates in the dark at 25 °C. Values are means of pooled data from 3 runs of experiments (4 replications per medium for each isolate). Bars represent the standard error of means.

This was followed by isolate ES-WR006 attaining radial mycelial growth rates of 5.1 mm day^{-1} on pyridoxine and

5.0 mm day^{-1} on riboflavin media (Fig. 4). The least radial growth rate of 1.5 mm day^{-1} was obtained with isolate ES-ER009 on riboflavin. Coincidentally, the performance of the isolates on vitamins followed a trend similar to the instances on carbohydrates and amino acids. Thus, a one-stop approach to the control of pink disease using a single chemical or biological agent may not achieve the desired results because of variability of the isolates.

There were variations in radial mycelial growth rate of the isolates on V8JA after 4 days at temperatures (18-34°C) representative of ambient conditions in cocoa farms. The optimum mycelial growth rates of the isolates were attained at 28°C (Fig. 5). The optimum growth rates attained with isolates ES-WR008 and ES-WR006 at 28°C were 8.5 mm day^{-1} and 8.3 mm day^{-1} respectively. Mycelial growth rates of the remaining two isolates (ES-ER009 and ES-ER009) were lower ($p < 0.05$) but followed a similar temperature-growth curve (Fig. 5). Generally, the fungal growth rate was least at 18°C but best at 28 C. After that the growth rate declined with increasing temperature and almost stopping at 34°C. This possibly suggests that autolysis sets in at 34°C when growth of the mycelium was significantly depressed in all test isolates (Fig. 5). At 36°C, mycelial growth of the isolates stopped completely and in most cases the inoculum plugs were not viable. The data were therefore excluded for further analysis. Therefore, one can state that the minimum temperature for growing *E. salmonicolor* isolates from Ghana is 18 °C and the opti-

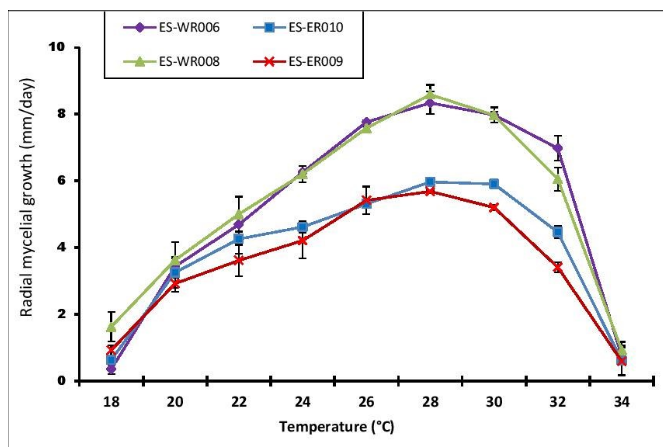

Fig. 5. Effect of temperature on the radial mycelial growth rate of four *E. salmonicolor* isolates on V8JA in the dark at 25 °C. Values are means of pooled data from 3 runs of experiments (4 replications per medium for each isolate). Bars represent the standard error of means.

mum is 28°C whereas the maximum temperature is 34°C. This result is supported by a similar report on the fungus from Brazil (29), India (30) and Malaysia (31). In Brazil, it was reported that the optimum temperature for *E. salmonicolor* on cocoa is within a range of 23-26 °C and at the temperature of 35°C, mycelial growth was completely inhibited (29). In Malaysia, it was stated that the minimum, optimum and maximum temperatures for the fungus is 10°C, 28°C and 35°C respectively (31). There was complete inhibition of the mycelial growth of the fungus at 5°C and 40°C. In the current study, the survival of the isolates within a temperature range of 18°C and 34°C agrees with the minimum and optimum temperatures for cultivating cocoa trees in Ghana (i.e. minimum 18-21°C and optimum 30-32°C) as (32) reported. This explains the adaptation of the pink disease fungus to the cocoa ecosystem and thus improving our understanding of how environmental condition impacts host-pathogen interaction.

Changes in the pH of the medium affected the rate of radial mycelial growth of the pink disease isolates. The isolates grew well within a pH range of 4-8 with an optimum growth at pH 6 (Fig. 6). At pH 6, the growth rates ranged from 8.9-9.7 mm day^{-1} in 4 days in the dark at 25°C. Significant ($p < 0.05$) interaction in the mycelial growth rates was observed between the fungal isolates and the different pH of the medium. However, ES-ER009 gave the least growth rates ranging from 5.9-8.9 mm day^{-1} at all pH assayed (Fig. 6). It is well established that most fungi grow best in acidic conditions (33, 34) and this is confirmed in the current study. Medium with pH value lower or above the optimum was poorly utilised. This indicates that in the event of using chemical or biological eradicant for the control of pink disease in the field, pH of the formulation above or below 6 will assist in achieving the best results.

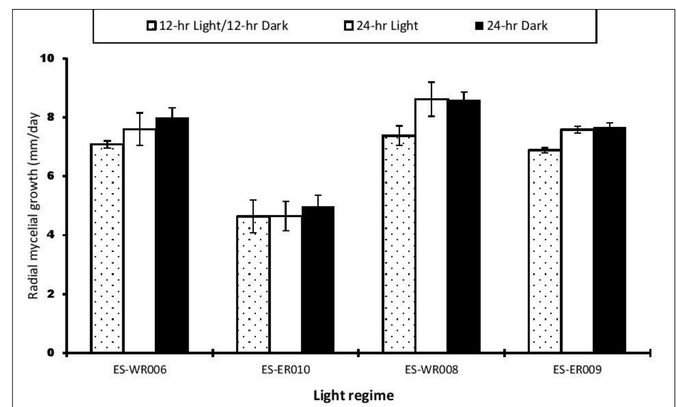

Fig. 6. Effect of light exposure on the radial mycelial growth rate of 4 *E. salmonicolor* isolates on V8JA in the dark at 25 °C. Values are means of pooled data from 3 runs of experiments (4 replications per medium for each isolate). Bars represent the standard error of means.

The various pink disease isolates responded differently ($p < 0.05$) to the different light exposures (24 hr light, 24 hr dark; 12 hr dark/12 hr light). Performance of mycelium of the isolates placed under 24 hr continuous light was similar to total darkness for the same period. This finding contradicts the report of (35) that fungi exposure to light decrease their mycelial growth. The best radial mycelial growth rate in darkness was obtained with isolate ES-WR008 attaining 8.6 mm day^{-1} in 4 days at 25 °C and the least was in isolate ES-ER010 (5 mm day^{-1}). Radial mycelial growth of isolates maintained in alternating 12 hr darkness and 12 hr light performed poorly when compared to those in either total darkness or continuous light for 24 hr except isolate ES-ER010 (Fig. 7). According to one report, rapid mycelial growth under continuous darkness or light may imply a photo-receptor system in the fungus that responds to the light or the darkness (36). It is therefore presumed that alternating between light and darkness conditions interrupted and slowed down the mycelial growth of the pink disease fungi. Future research should be directed at confirming such a photoreceptor system in Corticiaceae (Basidiomycota) among the pink disease fungi. The least

radial mycelial growth rate recorded on isolate ES-ER010 showed no significant differences ($p>0.05$) in the growth rates under 24 hr darkness, 24 hr continuous light or alternating 12 hr darkness/12 hr light (Fig. 7). Essentially, the isolate (ES-ER010) was behaving as a day neutral pathogen. These findings means that shade manipulation in cocoa farms should be further studied for its incorporation into pink disease management.

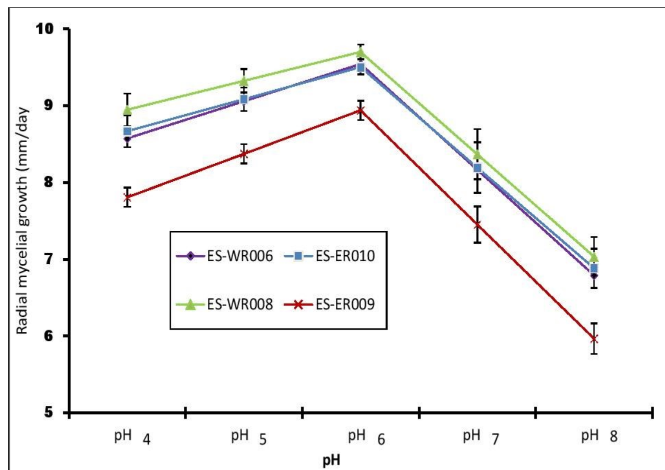

Fig. 7. Effect of pH on the radial mycelial growth rate of 4 *E. salmonicolor* isolates on MEA in the dark at 25 °C. Values are means of pooled data from 3 runs of experiments (4 replications per medium for each isolate). Bars represent the standard error.

Conclusion

The pink disease isolates grew well in the carbohydrates, amino acids and vitamins media but to different extent suggesting possible variable physiological differences within the fungal isolates studied. The isolates grew well over a range of pH from 4 to 8 with best growth at pH 6 and this wide range of pH (acid-neutral-alkaline) indicates physiological versatility of the isolates which may also adapt them to infect many different tree crops. Hence, a further study on the role of alternative hosts as agents of spread of pink disease in cocoa farms is important. It is established that the minimum, optimum and maximum temperatures for vegetative growth of the fungus are 18˚C, 28˚C and 34 C respectively. These temperatures are consistent with cocoa cultivation in Ghana and support the adaptation of the fungus to the cocoa ecosystem. Therefore, for an effective control of pink disease on cocoa, a potent antagonist or eradicant is always required. In terms of light exposure, the isolates grew successfully in either continuous light or continuous darkness.

Acknowledgements

This is a contribution from Plant Disease Management Thrust (project CRIG/CC/6/12) of Cocoa Research Institute of Ghana (CRIG). The assistance of the technical staff of the Plant Pathology Division of CRIG in the data collection is gratefully acknowledged. This paper CRIG/04/2021/041/004 is published with the kind permission of the Executive Director, CRIG, Akim-Tafo.

Authors contributions

IAA conceived the idea of the study and participated in its design and coordination. EKA assisted in the design of the study, data collection and analysis. YB participated in drafting the manuscript. All the authors jointly supported the correction and approval of the final manuscript for submission.

Compliance with ethical standards

Conflict of interest: The authors do not have any competing interest to declare.

Ethical issues: None.

References

1. Old KM, Davison EM. Canker diseases of *Eucalyptus*. In: Keane PJ, Kile GA, Podger FD, Brown BN, Editors. Diseases and Pathogens of Eucalypts. CSIRO Publishing, Collingwood, Australia. 2000; p. 245-46.
2. Kwarteng FG, Cornelius E, Acquah KK, Asare-Kumi E. Morphological and Molecular Identification of the fungus associated with Pink Disease of cocoa (*Theobroma cacao* L.) in the Eastern Region of Ghana. International Journal of Pathogen Research. 2018;1(1):1-8. https://doi.org/10.9734/ijpr/2018/v1i11161
3. Akrofi AY, Amoako-Attah I, Assuah MK, Kumi-Asare E. Pink Disease caused by *Erythricium salmonicolor* (Berk. & Broome) Burdsall: an epidemiological assessment of its potential effect on cocoa production in Ghana. Journal of Plant Pathology and Microbiology. 2014; 5:215. https://doi.org/10.4172/2157-7471.1000215.
4. Roux J, Coetzee MPA. First report of pink disease on native trees in South Africa and phylogenetic placement of *Erythricium salmonicolor* in the Homobasidiomycetes. Plant Disease. 2005;89:1158-63. https://doi.org/10.1094/PD-89-1158.
5. Moraes SRG, Furtado GQ, Scaloppi ÉAG, Barreto M, Júnior M, Sidnei N. Sporulation of *Erythricium salmonicolor* and its anamorphic stage *Necator decretus*, causal agent of citrus pink disease in Brazil. Fitopatologia Brasileira. 2006; 31(5):519. https://doi.org/10.1590/S0100-41582006000500016.
6. Opoku IY, Akrofi AY, Osei-Bonsu K, Acheampong K. An outbreak of pink disease in Ghana. In: Proc. of the 13th Int. Cocoa Res. Conf, Kota Kinabalu, Sabah, Malaysia. 2001; p. 761-67.
7. Akrofi AY, Amoako-Attah I, Acheampong K, Assuah MK, Melnick RL. Fruit and canopy pathogens of unknown potential risk. In: (Bailey B, Meinhardt L editors. Cacao diseases: a history of old enemies and new encounters. Springer International Publishing, Switzerland. 2016; p. 361-82. https://doi.org/10.1007/978-3-319-24789-2_11.
8. Schneider-Christians J, Fliege F, Schlosser E. On the release, survival and importance of basidiospores of *Corticium salmonicolor*, the pathogen causing pink disease. J Plant Dis Prot. 1986;93:397-403.
9. Castro-Moretti FR, Gentzel IN, Mackey D, Alonso AP. Metabolomics as an emerging tool for the study of plant-pathogen interactions. Metabolites. 2020;10(2):52. doi: 10.3390/metabo10020052. PMID: 32013104; PMCID: PMC7074241. https://doi.org/10.3390/metabo10020052
10. Gao L. Optimization of culture medium for sporulation and biomass production of a nematophagous fungus: consideration of nutritional and environmental conditions, Journal of Phytopathology. 2015;163:536-42. https://doi.org/10.1111/jph.12351

11. Kavanagh K. Fungi: Biology and Applications, 3rd Edition. John Wiley & Sons, Inc. 2018. 35p.

12. Formela-Luboinska M., Remlein-Starosta D, Wa´skiewicz A, Karolewski Z, Bocianowski J, Stepie´n Ł *et al*. The role of saccharides in the mechanisms of pathogenicity of *Fusarium oxysporum* f. sp. *lupini* in Yellow Lupine (*Lupinus luteus* L.). Int J Mol Sci. 2020; 21:7258. https://doi.org/10.3390/ijms21197258

13. Li Y, Yang C, Ahmad H, Maher M, Fang C, Luo J. Benefiting others and self: Production of vitamins in plants. J Integr Plant Biol. 2021;63:210-27. https://doi.org/10.1111/jipb.13047

14. Sun Y, Wang M, Mur LAJ, Shen Q, Guo S. Unravelling the roles of nitrogen nutrition in plant disease defences. Int J Mol Sci. 2020;21(2):572. doi: 10.3390/ijms21020572. PMID: 31963138; PMCID: PMC7014335. https://doi.org/10.3390/ijms21020572

15. Bani M; Pérez-De-Luque A; Rubiales D; Rispail N. Physical and chemical barriers in root tissues contribute to quantitative resistance to *Fusarium oxysporum* f. sp. *pisi* in pea. Front. Plant Sci. 2018; 9:199. https://doi.org/10.3389/fpls.2018.00199

16. García-Guzmán G. Environmental factors associated with disease incidence in plant species from a Mexican seasonal tropical dry forest. J Torrey Bot Soc. 2016; 143(3):254-64. https://doi.org/10.3159/TORREY-D-15-00055.1

17. Pokhrel B. Effects of environmental factors on crop diseases. J Plant Pathol Microbiol. 2021; 12:553.

18. Adebayo-Tayo BC, Ugwu EE, Musa H. Physiological requirement for growth and extracellular polysaccharides (EPS) production by *Marasmius* sp. and *Fomes* sp. (A comparative study). J Microbiol Biotech Res. 2013;3(5):1-11.

19. Gomori G. Preparation of buffers for use in enzyme studies. 2008. Available from: http://2008.igem.org/wiki/images/8/84/Protein_Buffers.pdf (cited: 2018 July 3)

20. Manjunathan J. and Kaviyarasan V. Studies on the growth requirements of *Lentinus tuberregium* (Fr.), an edible mushroom. Middle-East Journal of Scientific Research. 2010;5(2):81-85.

21. Gbolagade JS, Fasidi IO, Ajayi EJ, Sobowale AA. Effect of physico -chemical factors and semi-synthetic media on vegetative growth of *Lentinus subnudus* (Berk.), an edible mushroom from Nigeria. Food Chemistry. 2016; 99:742-47. (Britain) https://doi.org/10.1016/j.foodchem.2005.08.052

22. Sánchez ÓJ and. Montoya S. Assessment of polysaccharide and biomass production from three white-rot fungi by solid-state fermentation using wood and agro-industrial residues: A Kinetic Approach. Forests. 2020;11:1055. https://doi.org/10.3390/f11101055

23. Aneja KR, Mehrotra RS. Fungal Diversity and Biotechnology. New Age international Publishers, India. 2011; p. 93-100.

24. Lu WY, GQ Lin, Hi Yu, AM Tong, JH Xu. Practical Methods for Biocatalysis and Biotransformations. Whittall J, Sutton PW. Editors. John Wiley & Sons. 2009; p. 236-39. ISBN 978-0-470-74859-6.

25. Lilly VG, Barnett HL. The utilisation of sugars by fungi. West Virginia. University Agricultural Experimental Station Bulletin. 1953;262:1-58. https://doi.org/10.33915/agnic.362T

26. Adebayo-Tayo BC, Ugwu EE. Influence of different nutrient sources on exopolysaccharide production and biomass yield by submerged culture of *Trametes versicolor* and *Coprinus* sp. Assumption University Journal of Technology. 2011;15(2):63-69.

27. Lazarević J, Stojičić D, Keča N. Effects of temperature, pH and carbon and nitrogen sources on growth of *in vitro* cultures of ectomycorrhizal isolates from *Pinus heldreichii* forest. Forest Systems. 2016;25(1):48-58. https://doi.org/10.5424/fs/2016251-07036.

28. Kemmitt SJ, Wright D, Jones DL. Soil acidification used as a management strategy to reduce nitrate losses from Agricultural lands. Soil biology and Biochemistry. 2005;37:867-75 https://doi.org/10.1016/j.soilbio.2004.10.001

29. Luz EDMN. Comparacao Morfologica e Patologica de Isolados de *Corticium salmonicolor* Berk and Br. de Cacaueiro na Bahia, Brasil (morphological and pathological comparison of *corticium salmonicolor* Berk and Br, isolates from cacao in Bahia, Brazil). Revista-Theobroma. 1983;13(3):165.

30. Verma KS, Munjal RL. Studies on the toxicity of various fungicidal sprays and paints on the control of pink disease *(Corticium salmonicolor* Ber. and Br.) of apple in Rajgarh area of Himachal Pradesh. Pesticides*.* 1980;14:25.

31. Shamsuri MH, Omar M, Napi D. Studies on epidemiology of pink disease and the effect of temperature on mycelial growth of *Corticium salmonicolor* of Hevea Rubber. Journal of Natural Rubber Research. 1997;12(1):58-66.

32. Anim-Kwapong GJ, Frimpong EB. Vulnerability of agriculture to climate change: Impact of climate change on cocoa production. Vulnerability and Adaptation Assessment under the Netherlands Climate Change Studies Assistance Programme Phase 2 (*NCCSAP*). New-Tafo Akim: Cocoa Research Institute of Ghana. 2004.

33. Sanchez F, Honrubia M, Torres P. Effects of pH, water stress and temperature on *in vitro* growth of ectomycorrhizal fungi from Mediterranean forests. Cryptog Mycol. 2001;22(4):243-58. https://doi.org/10.1016/S0181-1584(01)01076-4

34. Richie BJ. Mycological media and methods In: Waller, IM, Lenne JM, Waller SJ, Editors. Plant Pathologist Pocketbook, 3rd Edition, CABI Publishing, Wallingford. 2002; p. 516.

35. Bermudes D, Gerlach VL, Nealson KH. Effects of culture conditions on mycelial growth and luminescence in *Panellus styp-ticus*. Mycologia. 1990;82:295-305. https://doi.org/10.1080/00275514.1990.12025884

36. Corrochano LM. Fungal photobiology: A synopsis. IMA Fungus. 2011;2:25-28. https://doi.org/10.5598/imafungus.2011.02.01.04

§§§