Competitive Saprophytic Ability of Three Major Fusarium Pathogens *Fusarium pseudograminearum, F. culmorum and F. graminearum* on Wheat

Mohammed Waleed Khudhair
Ministry of Science and Technology/ Directorate Agricultural Research / Baghdad-Iraq
E-mail: mohammedwaleed 74 @yahoo.com

Abstract

Fusarium pseudograminearum, F. culmorum and F. graminearum are the main causative pathogens of the diseases Fusarium head blight (FHB), crown rot (CR), and brown foot rot on cereals, causing remarkable yield losses. Climate factors such as CO_2 has a direct impact on plant diseases development and pathogencity whereas positive, increasing the infection or negative, decreasing disease development. Fusarium species spend part of their life cycle as saprophytes on wheat straw, providing inoculum for the next season. This paper has studied the impact of two CO_2 treatments (ambient and elevated) on these three Fusarium species in particular on their saprophytic growth fitness. The results showed that *F. pseudograminearum* has the highest saprophytic ability; however, there are no significant differences between species in relation to both CO_2 treatments; while, there were significant differences between isolates response to CO_2.

Key words: Competitive, Saprophytic Ability and Fusarium and Wheat.

قابلية التنافس الرمية لثلاث انواع من فطر الفيوزاريوم *Fusarium pseudograminearum F. culmorum and F. graminearum* على الحنطة .

محمد وليد خضير
وزارة العلوم والتكنلوجيا / دائرة البحوث الزراعية / بغداد- العراق

الخلاصة

Fusarium pseudograminearum ,F. culmorum and F. graminearum , هي المسببات الفطرية الرئيسية للامراض الاتية: شحوب الرأس الفيوزارمي، التعفن التاجي و التعفن البني على الحبوب مسببة خسائر اقتصادية ملحوظة خاصة في الحاصل. العوامل المناخية مثل CO_2 تمتلك تاثير مباشر على امراضية وتطور المسبب المرضي بصورة متباينة من حيث كونه ايجابيا يؤدي الى زيادة الاصابة او سلبيا يؤدي الى خفض تطور المرض.بما ان انواع المسببات المرضية العائدة للفطر فيوزاريم تقضي جزء من دورة حياتها رمية على بقايا الحنطة في الحقل موفرة لقاح للموسم القادم مما يؤدي الى اعادة دورة الحياة، لذلك لايمكن اهمال تاثير عوامل المناخ خاصة CO_2 على هذا الجزء من دورة حياة المرض. محور هذا البحث الرئيسي كان دراسة تاثير مستويين من CO_2 (المحيط،مضاعف) على ثلاثة انواع من الفيوزاريم لقياس قابلية ترممها. اظهرت النتائج بان الفطر *F. pseudograminearum* يمتلك اعلى قابلية رمية مع عدم وجود فروقات معنوية بينه وبين النوعين الاخريين من الفيوزاريوم من حيث الاستجابة لتركيز CO_2 في كلا المعاملتين(مضاعف ومحيط) في حين كان هناك فروقات معنوية من حيث استجابة العزلات في الاستجابة لثاني اوكسيد الكاربون.

الكلمات المفتاحية: تنافس، القابلية الرمية، فطر الفيوزاريوم و الحنطة.

Introduction

These three *Fusarium* species are the major causes of *Fusarium* head blight disease (FHB), crown rot disease (CR), and brown foot rot on cereals especially wheat. *F. graminearum* and *F. culmorum* cause the disease FHB which is re-emerging especially with the use of conservative agricultural practices (*Luck et al*., 2011). Chakraborty and Newton (2011) reported that this disease is considered as an important disease worldwide. There is significant losses cause by the disease FHB, exemplified by reducing yield production and price due to the effects on grain quality, and the estimated cost of these losses in USA were about $2.7 billion between 1998 and 2000 as reviewed by (Chakraborty and Newton, 2011). CR is also considered as a vital disease in many countries and regions including Australia, causing high crop losses from the overall yield (Akinsanm*i et al.*, 2004). This disease is a main problem in Australia especially on wheat and it is wide-spread in many Australian regions and states, producing enormous damage (*Mudge et al*., 2006). The disease brown foot rot is also recorded as the most important disease of cereal especially wheat in UK, causing high losses (Bateman and Murray, 2001).

Fusarium species spend their overwinter period as saprophytes in the infected wheat or maize or other types of cereals and grasses, producing ascospores or macroconidia that disseminate by wind, rain and insect, repeating the disease life cycle (Chakraborty and Newton,(2011). *Fusarium* Climate elements such as temperature, moisture, and CO_2 are the most effective factors that can influence disease distribution, growth and competitive ability; however, those essentials are changing in the last four decades, so their effects on plant diseases also might be changed as reviewed by (*Brosi et al.*, 2011, Chakraborty, 2011, Chakraborty and Newton, 2011, *Luck et al*., 2011, *Magan et al*., 2011, *Pangga et al*., 2011, Pritchard, 2011, Shaw and Osborne, 2011 and *Sturrock et al*., 2011). Climate changes have a direct impact on agriculture system and ecosystem; therefore, it is extremely feasible for plant diseases to be affected by these changes. Eastburn, *et al.*, (2011) mention that the alteration of air gases may have a direct influence on plant diseases severity and outbreak by affecting host, pathogen, or their interaction. For instance, the disease severity and epidemiology might be decreased by increasing CO_2 level which may increase crop health (Carter *et al*., 2002).

The main objectives of this study: is to determine the fast and the highest growth rate among the three Fusarium species in plant straw under two CO_2 treatments ambient and elevated with almost stable temperature, and humidity. This study is going to test whether the three species, *F. graminearum, F. culmorum and F. pseudograminaerum* differ in their competitive ability to colonize wheat straw. This is an indication of their ecological fitness.

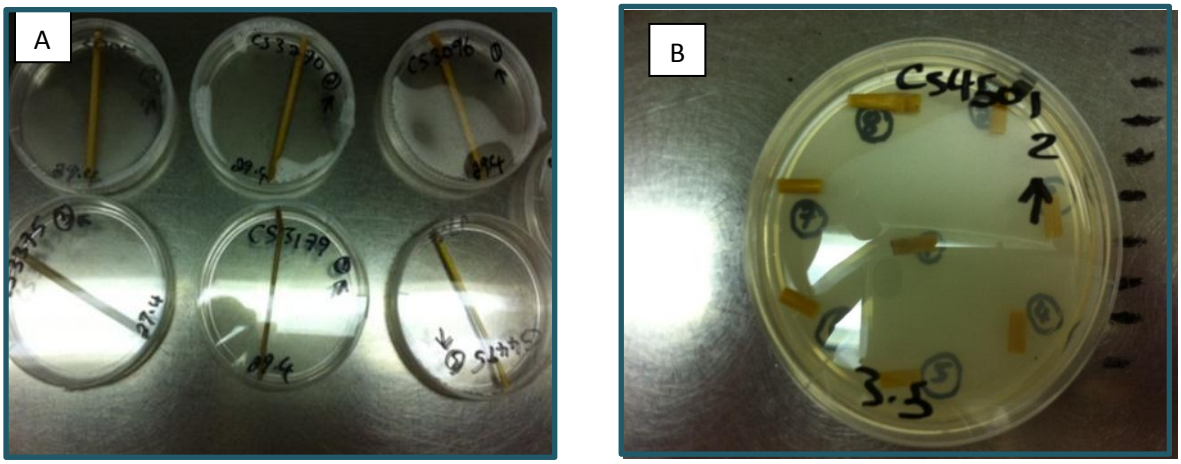
Materials and methods

The study process was done in the CSIRO Brisbane laboratories in Australia. Thirty isolates from the three *Fusarium* species Table(1), *F. graminearum, F. culmorum and F. pseudograminaerum* were cultured onto quarter strength PDA media to enhance fungal sporelation for three weeks and they were incubated at the room temperature. Then the thirty isolates were sub-cultured in order to get pure pathogen cultures, at the same growth level, at the time of fitness assessment onto three quarter strength PDA media which were prepared previously, to motivate fungi growth. The isolates distributions were not equal with thirteen isolates from *F. pseudograminaerum,* ten isolates from *F. graminearum,* and seven isolates from *F. culmorum* see table(1).

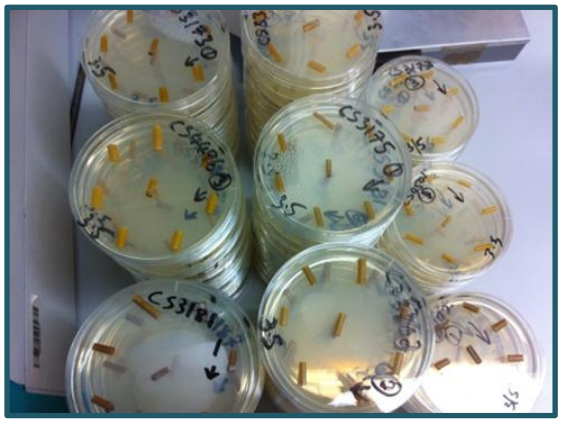
Table (1) Details of Fungal Isolates that were Used for this Study

Isolate	Species	Host	Plant part
CS3716	*F. culmorum*	*T. aestivum*	Crown
CS4494	*F. culmorum*	Wheat	Crown
CS4495	*F. culmorum*	Wheat	Crown
CS4496	*F. culmorum*	Wheat	Crown
CS4497	*F. culmorum*	Wheat	Crown
CS4498	*F. culmorum*	Wheat	Crown
CS4501	*F. culmorum*	Wheat	Crown
CS3005	*F. graminearum*	*H. vulgare*	Head
CS3179	*F. graminearum*	*T. aestivum*	Head
CS3187	*F. graminearum*	*T. aestivum*	Head
CS3192	*F. graminearum*	*T. aestivum*	Head
CS3196	*F. graminearum*	*T. aestivum*	Stubble
CS3200	*F. graminearum*	*T. aestivum*	Head
CS3259	*F. graminearum*	*T. aestivum*	Head
CS3375	*F. graminearum*	*T. aestivum*	Head
CS3386	*F. graminearum*	*T. aestivum*	Stubble
CS3407	*F. graminearum*	*T. aestivum*	Flag leaf node
CS3096	*F. pseudograminearum*	*T. aestivum*	Crown
CS3173	*F. pseudograminearum*	*T. aestivum*	Crown
CS3181	*F. pseudograminearum*	*T. aestivum*	Crown
CS3220	*F. pseudograminearum*	*T. aestivum*	Head
CS3270	*F. pseudograminearum*	*T. aestivum*	Crown
CS3321	*F. pseudograminearum*	*T. aestivum*	Crown
CS3361	*F. pseudograminearum*	*T. aestivum*	Crown
CS3427	*F. pseudograminearum*	*T. aestivum*	Crown
CS3438	*F. pseudograminearum*	*T. aestivum*	Crown
CS3442	*F. pseudograminearum*	*T. aestivum*	Crown
CS3175	*F. pseudograminearum*	*T. aestivum*	Crown
CS3342	*F. pseudograminearum*	*T. aestivum*	Crown
CS3350	*F. pseudograminearum*	*T. aestivum*	Crown

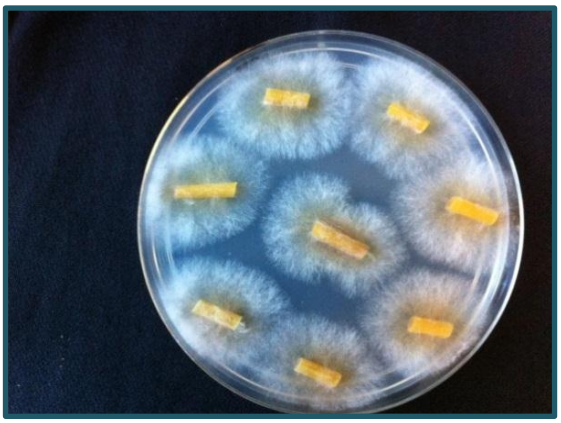
After that, 180 free disease straw stems were cut into small pieces with around 8cm without nods(Melloy *et al.*, 2010), a length which is suitable for fitting in the Petri dishes. The stems were soaked in water for 24hr and autoclaved for two successive days. For each *Fusarium* isolates six wheat sterile straws were inoculated via pressing one end onto the prepared pure isolates cultures, marking those ends with leaving part of the media to get inside the stems. The inoculated stems were placed onto Petri dishes. The Petri dishes that contain the inoculated straws were incubated in the glass house in the CSIRO building rooftop, with incubating 3 replicate plates in the ambient CO_2 from each isolate and other 3 replicates in elevated CO_2 glasshouse. The Petri dishes were incubated in two glass houses without sealing them with parafilm for 2-3 hours to ensure that the atmosphere within the Petri dishes was at equilibrium the atmospheric CO_2. Then the Petri dishes were sealed by parafilm to conserve moist after the initial period in the glass houses environments. After four days incubation, the straws were cut into 8 pieces each piece 1cm. The straw pieces were numbered consecutively and placed into fresh full PDA media plates that prepared previously; followed by incubation at the room conditions at 25˚C for three days (Melloy *et al.*, 2010). Saprophytic fitness was measured after that via measuring the length of straw that have been colonized by *Fusarium* species. The experiment designed with completely randomized design isolates nested within species.



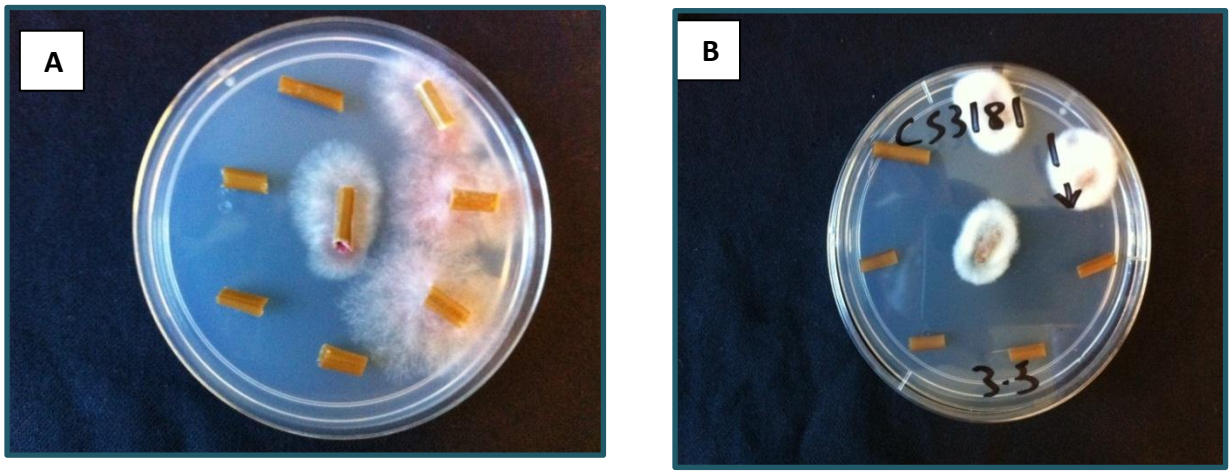
Picture(1): These Pictures Illustrate Inoculation Process, A) Straws before Cutting Them; B)Straws after Cutting Them in the Press Media with Numbering System



Picture (2) The Incubation of Plates in the Room Conditions



Picture (3) Full Growth of *Fusarium* Isolate



Picture (4) (A) and (B) Number of the Wheat Straw Pieces Which were Infected.

Results and Discussion

As previously mentioned the experiment designed with completely randomized design isolates nested within species. Data

Table (2) The Relationship Between Analyzed Data. Analyses show that the results were reliable with p <.0001 which reveals very low error in relation to species

Source	Degrees of Freedom	Type III SS	Mean Square	F Value	P > F
Species	2	185.6805250	92.8402625	60.55	<.0001
CO_2	1	3.5844172	3.5844172	2.34	0.1289
Isolate (species)	27	317.8972527	11.7739723	7.68	<.0001
Isolate*CO_2 (species)	27	49.3551282	1.8279677	1.19	0.2561
Species*CO_2	2	1.6448718	0.8224359	0.54	0.5863

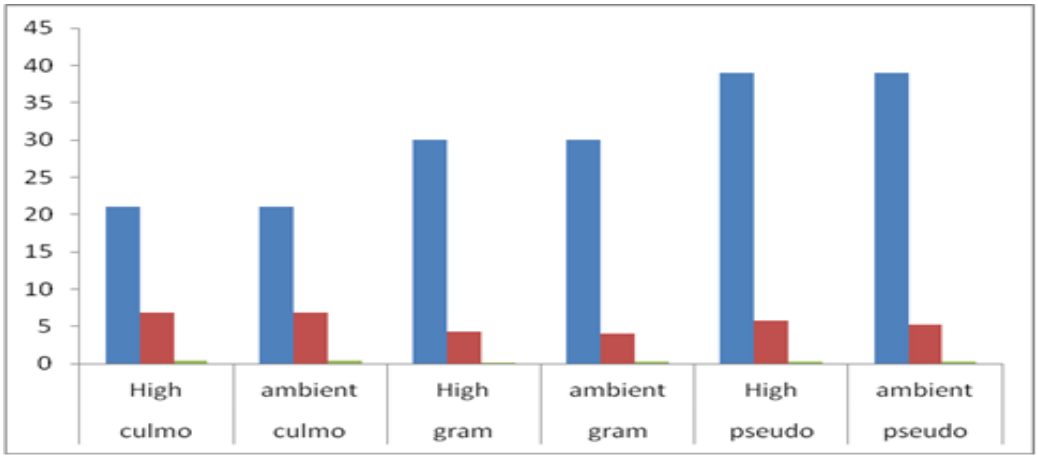


Figure (1) Growth Frequency Among Species (Red), Mean Growth (Blue), and Standard Error (Green) Within the Two Treatments High and Ambient CO_2.

The results show that there is no significant differences between the treatments ambient and elevated CO_2 within species. However, the results show that there are significant difference within species in relation to pathogen growth and fitness. Some of the isolates within species also showed differences in terms of growth rate and colonization as a response to the increase of CO_2 level. *F. pseudograminearum* showed the highest growth in both CO_2 levels without any difference in both CO_2 levels in comparison with other species with around 40%; while, *F. graminearum* was the second last in terms of growth rate without any differences in both treatments with about 30%. However, *F. culmorum* revealed the lowest growth rat with around 20%.

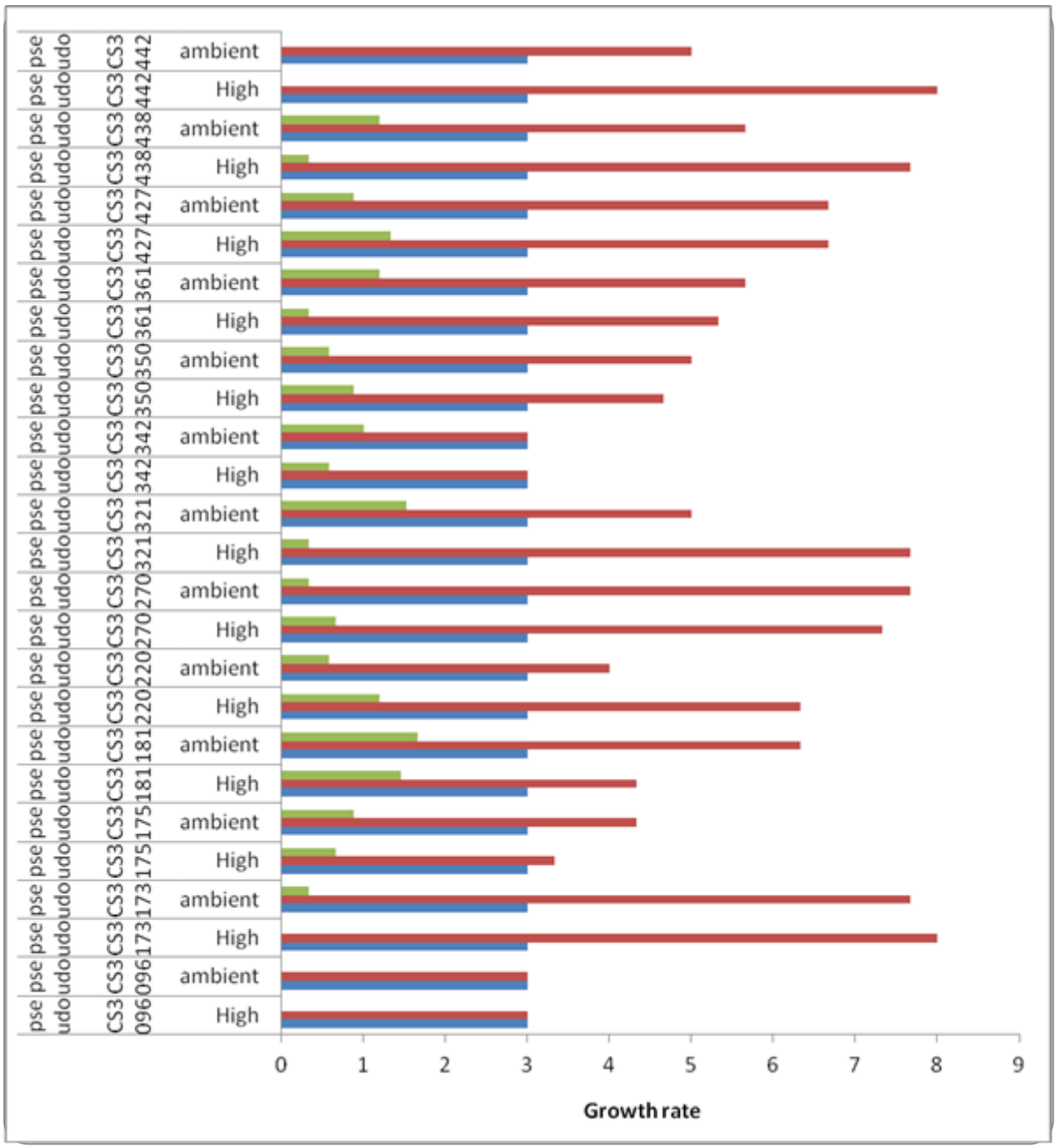
Figure1 illustrates the differences among species within the two treatments high and ambient CO_2 via revealing the relationship between growth frequency, mean growth, and standard error. It can be seen from this study that the standard error is very low among all treatments which give reasonable indication about the experiment reliability.

Table (3) illustrates the main tree group or species and means of growth, revealing that Means with the same letter are not significantly different. Group A has the highest mean with 6.9 which is *F. culmorum*, followed by group B with 5.5 for *F. pseudograminearum ;* however, group C got 4.1 in relation to *F. graminearum* growth means.

Table (3) Group Means Nested in Species and N Scores.

REGWQ Grouping	Mean	N	species
A	6.9048	42	culmo
B	5.5513	78	pseudo
C	4.1833	60	gram

The results showed that there is no significant difference between ambient CO_2 and elevated CO_2 on the three *Fusarium* species fitness in terms of growth rate; however, it was noticeable deference between isolates. This result indicts that CO_2 level does not have impact on saprophytic pathogen fitness in relation to those three species which is the same as Melloy *et al*. (2010) outcome. However, Melloy *et al*. (2010) examined the effect of CO_2 level on *F. pseudograminearum* only; while, this experiment studied the effects of other two *Fusarium* specie fitness and growth under two CO_2 levels. Some isolates that are belong to *Fusarium* species showed different response to CO_2 treatments; for instance, the isolate CS3442 from the species *F. pseudograminearum* revealed significant differences in relation to growth rate average under elevated CO_2 with 8cm; while, it scored 5cm growth rate average under ambient CO_2 (Figur 2). The isolate CS3438 from the same species also expressed significant variation between the two CO_2 treatments with 7.6 growth rate average for elevated CO_2 and 5.6 for ambient ones. The isolate CS3321 from the species *F. pseudograminearum* showed the same trend with high growth level under high CO_2 with 7.6 in comparison with 5 cm under ambient CO_2. The trend in all those three isolates was high level of growth under high CO_2 which might indicate that high CO_2 enhanced the fungus growth fitness. Nonetheless, some isolates that belong to the species *F. pseudograminearum* did not showany growth differences in both CO_2 treatments (e.g. CS3270, CS3427, CS3361….) which indicate that CO_2 level did not affect fungus growth fitness(Figure 2). Overall growth *F. pseudograminearum* showed the highest saprophytic fitness among all other Fusarium species with 40% (Figure 1), revealing that this pathogen species has the highest growth fitness in comparison with other species. However, *F. culmorum* isolates did not reveal any significant growth differences between the two CO_2 levels, showing that CO_2 concentration do not have any impact on this fungus species growth fitness (Figure 3). The isolate CS3716 illustrates the lowest level of growth rate in both CO_2treatements with slightly over 3 cm, revealing that the trend of this isolate is originally low. Overall growth rate of F. *culmorum* was the lowest among the three species with 20% which indicates that the growth fitness of this pathogen is low. *F. graminearum* showed second highest growth rate with almost 30% which is quite high in comparison with *F. culmorum* with 20%. Some of the pathogen isolates within the species *F. graminearum* expressed significant differences in response to CO_2 concentrations; for example, the isolates CS3407, CS3259, CS3200, and CS3187 showed high level of growth average under high CO_2 in comparison with ambient treatment (figure 4). This trend of growth indicates that those isolates have affected by the level of CO_2 which increase their growth fitness. However, some isolates showed the opposite trend of growth exemplified by higher growth under ambient CO_2 comparing with high CO_2 such as CS3179, CS3192, and CS3196 from the species *F. graminearum* which reveals that these isolates have affected negatively by the increase of CO_2 level (Figure 4), reducing their growth fitness.



Figure(2) *F. Pseudograminearum* Isolates Growth Rate: Mean (Red), Replicates (Blue), and Standard Error (Green).

While the effects of CO_2 on overall *Fusarium* species were not significant, there were significant impact on isolates within species, revealing various effects on the isolates either positive or negative particularly growth fitness. *Fusarium* species spend part of their life cycle as saprophytes on plant debris; therefore, this study focused on measuring the pathogen growth fitness under two CO_2 levels in order to identify if there is any impact of CO_2 on the pathogen.

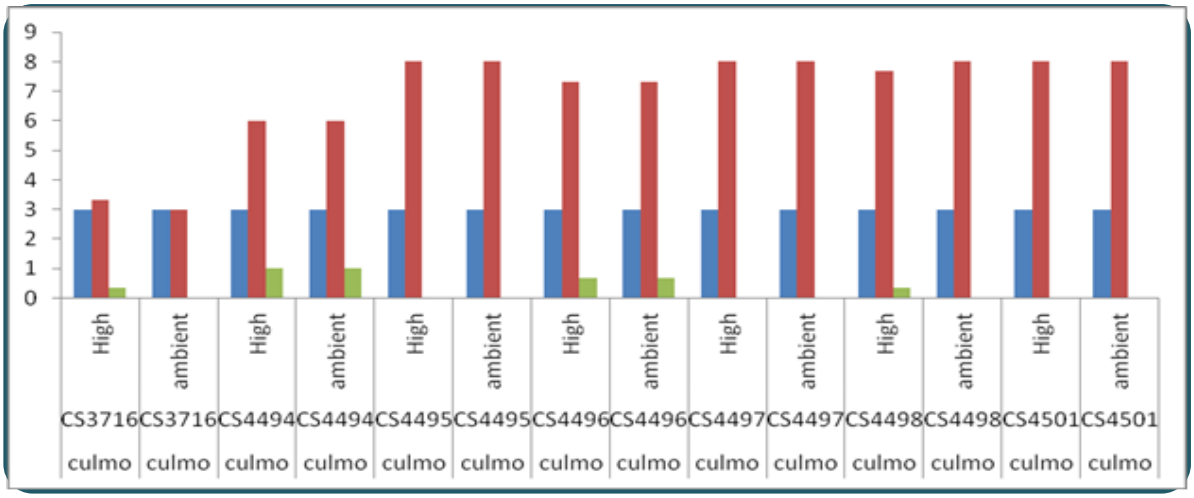


Figure (3) *F. Culmorum* Isolates Growth Rate: Mean (Red), Replicates (Blue), and Standard Error (Green).

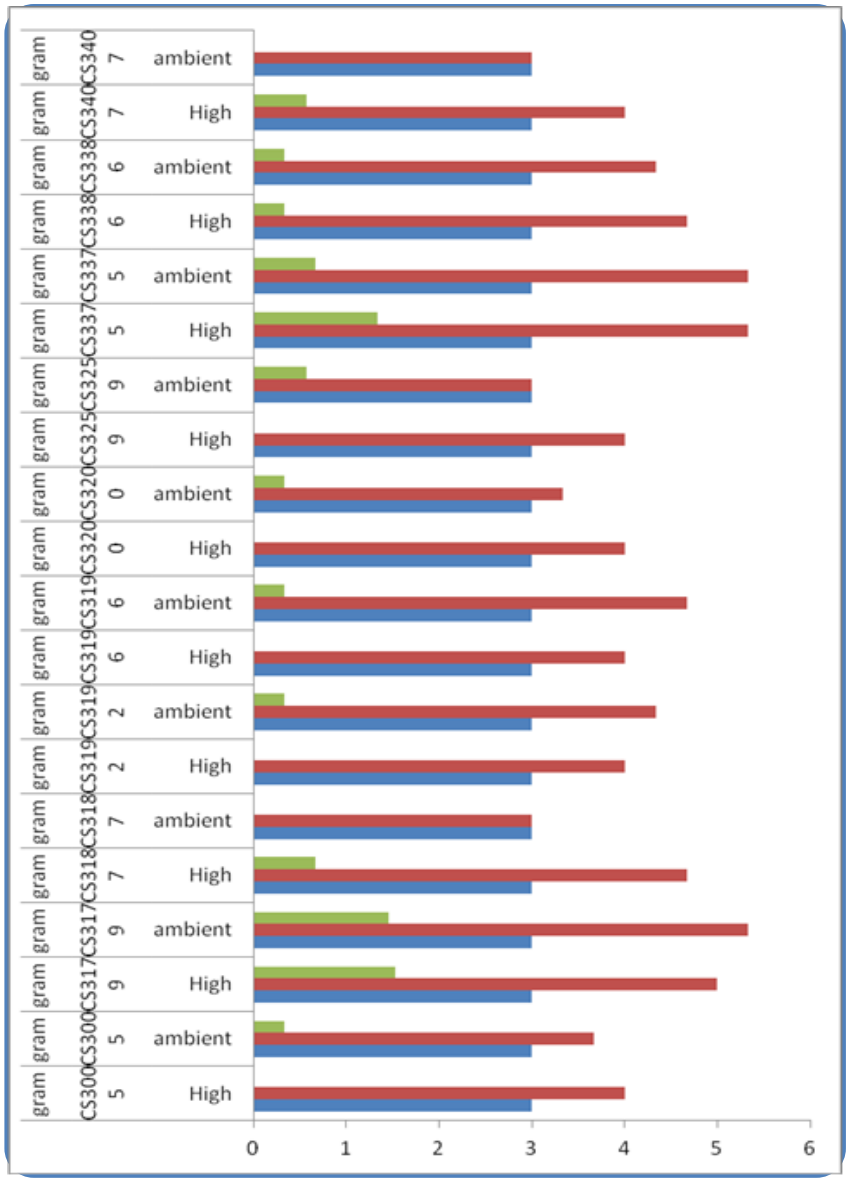


Figure (4) *F. Pseudograminearum* Isolates Growth Rate: Mean (Red), Replicates (Blue), and Standard Error (Green).

Melloy *et al.*(2010) used RT-PCR to calculate the effects of CO_2 on *F.pseudograminearum* pathogencity in wheat, identifying the fungus biomass in the plant stems which was increased extremely under elevated CO_2 (825

p.p.m) in contrast with infected once under ambient CO_2 through studying the correlation between fungal DNA and wheat DNA. However, this experiment is not about saprophytic fitness because they got the same result in relation to *F. pseudograminearum* saprophytic fitness but it was about actual pathogen colonization during crop infection. CO_2 level has a direct impact on plant pathogens growth and development weather positive, increasing their progression or negative, decreasing their pathogency (Eastburn *et al.,* 2011, Chakraborty, 2011). However, these effects might not be the same in relation to the pathogen saprophytic fitness. Another suggestion is that, the expressing of inoculated wheat straw to high CO_2 during the experiment was not sufficient in comparison with the expressing to the ambient which was all the time during the experiment preparation.

Conclusion

The outcomes of this experiment shows that the three Fusarium species, *F. graminearum, F. culmorum and F. pseudograminaerum* are different in their growth fitness as ranked in figur1. *F. pseudograminaerum* got the highest growth fitness among other *Fusarium* species, revealing that it is the most competitive one among other species especially as a saprophyte on wheat straw. The impact of CO_2 level was variable on the isolates within species; while, there were no significant differences between species. These results can give sufficient indication about the most competitive *Fusarium* species on wheat straw with highly growth during saprophytic stage which might be the causative pathogen of next season disease via providing inoculums. Therefore, disease management practices should target those pathogens during their saprophytic phase especially *F. pseudograminaerum.*

Acknowledgement

This paper acknowledges CSIRO Brisbane Australia, many thanks to the Professor Sukumar Chakraborty for all the help producing this study, Dr. Friday Obanor, and Mr. Ross Perrott for the technical supports and consultation. This study also gives the great thanks to professor Elizabeth Aitken University of Queensland for the direction and support.

References

Akinsanmi, O. A.; Mitter, V.; Simpfendorfer, S.;Backhouse, D. and Chakraborty, S.,(2004) Identity and Pathogenicity of Fusarium spp. Isolated from Wheat Fields in Queensland and Northern New South Wales. Australian Journal of Agricultural Research, 55**,** 97-107.

Bateman, G. L. and Murray, G. ,(2001) Seasonal Variations in Populations of Fusarium Species inWheat-Field Soil. Applied Soil Ecology, 18**,** 117-128.

Brosi, G. B.; Mcculley, R. L.; Bush, L. P.; Nelson, J. A.; Classen, A. T. and Norby, R. J. ,(2011). Effects of MultipleClimate Change Factors on the Tall Fescue-Fungal Endophyte Symbiosis:Infection Frequency and Tissue Chemistry. New Phytologist, 189**,** 797-805.

Carter, J. P.; Rezanoor, H. N.; Holden, D.; Desjardins, A. E.; Plattner, R. D. and Nicholson, P. ,(2002) Variation in Pathogenicity Associated with the Genetic Diversity of and lt;i and gt;Fusarium Graminearum and lt;/i and gt. European Journal of Plant Pathology, 108**,** 573-583.

Chakraborty, S.,(2011) Climate Change and Plant Diseases. Plant Pathology, 60**,** 1.

Chakraborty, S. and Newton, A. C. ,(2011) Climate Change, Plant Diseases and Food Security: An Overview. Plant Pathology, 60**,** 2-14.

Eastburn, D. M.; Mcelrone, A. J. and Bilgin, D. D.,(2011) Influence of Atmospheric and

Climatic Change on Plant-Pathogen Interactions. Plant Pathology, 60**,** 54-69.

Luck, J.; Spackman, M.; Freeman, A.; Trebickl, P.; Griffiths, W.; Finlay, K. and Chakraborty, S. ,(2011) d Crops. Plant Pathology, 60**,** 113-121.

Magan, N.; Medina, A. and Aldred, D. ,(2011) Possible Climate-Change Effects on Mycotoxin Contamination of Food Crops pre- and Postharvest. Plant Pathology, 60**,** 150-163.

Melloy, P.; Hollaway, G.; LUCK, J.; Norton, R.; AITKEN, E. and Chakraborty, S.,(2010) Production and Fitness of Fusarium Pseudograminearum Inoculum at Elevated Carbon Dioxide in FACE. Global Change Biology, 16**,** 3363-3373.

Mudge, A. M.; Dill-Macky, R.; Dong, Y.; Gardiner, D. M.; White, R. G. and Manners, J. M.,(2006). A Role for the Mycotoxin Deoxynivalenol in Stem Colonisation During Crown Rot Disease of Wheat Caused by Fusarium Graminearum and Fusarium Pseudograminearum. Physiological and Molecular Plant Pathology, 69**,** 73-85.

Pangga, I. B.; Hanan, J. and Chakraborty, S.(2011). Pathogen Dynamics in a Crop Canopy and Their Evolution Under Changing Climate. Plant Pathology, 60**,** 70-81.

Pritchard, S. G.,(2011) Soil Organisms and Global Climate Change. Plant Pathology, 60**,** 82-99.

Shaw, M. W. and Osborne, T. M. (2011). Geographic Distribution of Plant Pathogens in Response to Climate Change. Plant Pathology, 60**,** 31-43.

Sturrock, R. N.; Frankel, S. J.; Brown, A. V.; Hennon, P. E.; Kliejunas, J. T.; Lewis, K. J.; Worrall, J. J. and Woods, A. J. (2011). Climate Change and Forest Diseases. Plant Pathology, 60**,** 133-149.