

## BIBLIOGRAPHY

SABAWAY FREDERICK A. APRIL 2012. Pathogenecity Test of *Colletotrichum* Isolates on Arabica Coffee Seedlings and Berries. Benguet State University, La Trinidad, Benguet.

Adviser: Andres A. Basalong, MSc.

## ABSTRACT

The study was conducted from October 2011 to February 2012 at the Department of Plant Pathology Laboratory, Benguet State University, Plant Pathology Laboratory.

*Colletotrichum spp.* isolated from leaf(*Colletotrichum coffeanum*), stem/twig (*Colletotrichum gleosporioides*) and berry (*Colletotrichum kahawae*) of Arabica coffee were inoculated on leaves and stem of Arabica coffee seedlings and berries, to determine their infectivity.

All the three *Colletotrichum* species infected the leaf, stem/twig and berry of Arabica coffee but at varying degrees of severity. *Colletotrichum coffeanum* which was isolated from the leaves showed the highest infection on leaves and severe infection in berries. *Colletotrichum gleosporioides* isolated from stem/twig had the highest infection when inoculated in stem/twig and very severe infection in berries. Likewise, *Colletotrichum kahawae* isolated from berries had very severe infection to berries, but were likewise infectious to leaves and stem/twig.



## RESULTS AND DISCUSSIONS

### Symptoms on naturally infected plant parts

The symptoms are most visible on leaves and ripe fruits. At first, anthracnose generally appears on leaves as small and irregular yellow, brown, dark-brown or black spots. The spots can expand and merge to form larger affected area. The color of the infected part darkens as it ages.

The disease can also produce brown to black color lesions on the stem and blackening of the whole stem.

Infected fruit has small, water-soaked, sunken, circular spots that may increase in size up to 1 cm in diameter. As it ages, the center of an older spot becomes blackish and emits gelatinous pink spore masses (Figure 5).



Figure 5. *Colletotrichum spp.* on leaf, stems, and fruit

## Cultural and Morphological Characterization of the Pathogen

Table 1 summarizes the cultural and morphological characteristics of the *Colletotrichum* isolates.

Colony size. Isolates differed in the diameter of their colony growth in culture media within one week. *Colletotrichum coffeanum* attained the largest colony diameter of 40.3 mm, followed by *Colletotrichum gloesporioides* at 35.5 mm and the least was isolate *Colletotrichum kahawae* at 30.5 mm. Based on the size of colony, *Colletotrichum coffeanum* obtained from the infected leaves was fast growing compared to other isolates.

Colony color. The colony of *Colletotrichum coffeanum* and *Colletotrichum kahawae* was white to black, (Figure 11. A and C), while *Colletotrichum gloesporioides* was white colony (Figure 11. B).

Rate of growth. *Colletotrichum coffeanum* was observed to be fast growing as compared to *C. gloesporioides* and *C. kahawae* which were observed to be moderately fast. These observed growth characteristics conform to their differences in colony size in one week.

Mycelia. The mycelia of *Colletotrichum coffeanum* were hyaline branched, septated and elongated. Mycelia of *Colletotrichum gloesporioides* were observed to be hyaline, septated, and large walled, while mycelia of *Colletotrichum kahawae* were slender, septated and hyaline.

Conidia. Shape of conidia varied between isolates. Conidia of *Colletotrichum coffeanum* is oblong, *Colletotrichum gloesporioides* is elliptic, and cylindrical for *Colletotrichum kahawae*. Conidia colors were observed similar to all the isolates as hyaline. In terms of the conidia size, length and width of *Colletotrichum coffeanum* has a



size of 5.31 x 2.06 mm (Figure 12. A), *Colletotrichum gleosporioides* 4.25 mm x 1.40 mm, and *Colletotrichum kahawae* 5.25 mm x 0.50 mm (Figure 11. B and C). This characterization confirmed the study of Coyasan (2011).

Table 1. Cultural and morphological characteristics of *Colletotrichum* isolates at temperature range of 24-26 °C on PDA.

<u>COLLETOTRICHUM ISOLATES</u>				
CRITERIA		<i>C. coffeanum</i>	<i>C. gleosporioides</i>	<i>C. kahawae</i>
Colony diameter (mm)		40.3 mm	35.5 mm	30.5 mm
Colony color		White to black	White	White to black
Rate growth in one week		Fast growing	Moderately fast	Moderately fast
Presence of septa on mycelia		Present	Present	Present
Shape of conidia		Oblong	Elliptic	Cylindrical
Color of conidia		Hyaline	Hyaline	Hyaline
Size of conidia		5.31 mm x 2.06 mm	4.25 mm x 1.4 mm	5.25 mm x 0.50mm

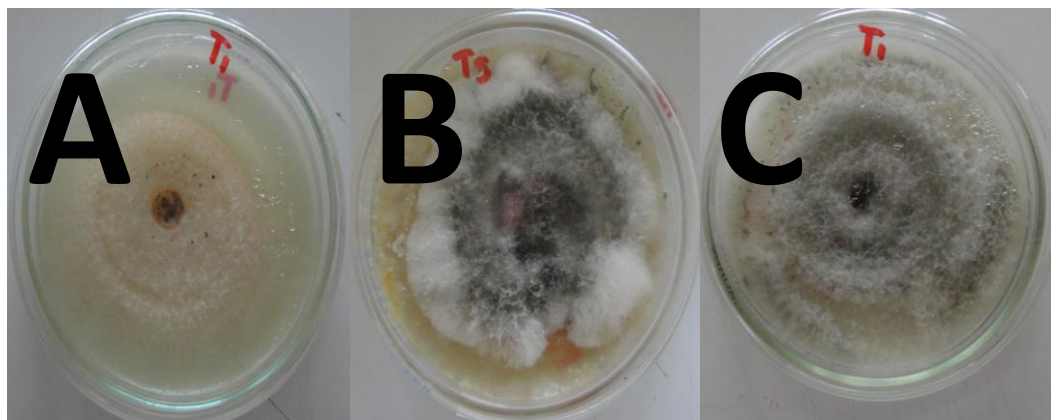


Figure 6. *Colletotrichum* spp. Isolate on A. leaf, B. twig and C. berry

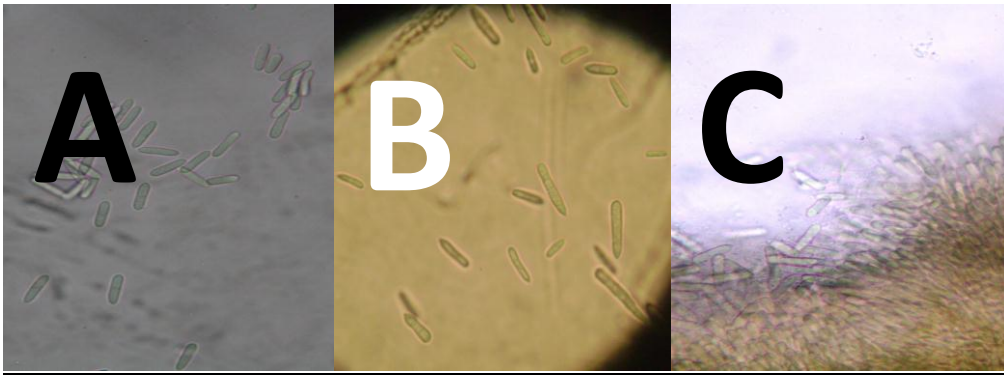


Figure 7. *Colletotrichum* spp. conidia of isolate A. leaf, B. twig and C. berry (400x)

### Pathogenicity Test and Symptom Development

Symptoms on inoculated seedlings. Symptom appearance varied between each plant parts. Irregular yellowish spots appeared on the leaves eight days after inoculation (Figure 8). The spot was observed mostly on the older leaves. The infection expands as it age (Figure 9)



Figure 8. Slight infection on leaf surface



Figure 9. Severe infection on leaf surface

Symptoms on inoculated Stem/twig. Sunken lesion with slightly raised rim was observed on the stem of the seedling twelve days after inoculation. As it ages, this causes stem and twig dieback (Figure 10).



Figure 10. Brown lesions on the stem

Symptoms on inoculated berries. Four days after inoculation, the appearance of symptom was observed on the berry. Symptom on berry isolate occurs as a small pit at the middle part of the berry that usually starts as a dark lesion on the surface of the berry (Figure 11). As it ages, the infection occupies the whole berry and produces pink spore masses and mycelia (Figure 12).



Figure 11. Dark lesion on Arabica coffee berries



Figure 12. Severe infection on Arabica coffee berries

## Disease Severity Ratings on Arabica coffee Seedlings and Berries

Table 2. Initial and final mean disease severity rating on leaves samples of inoculated seedlings

TREATMENT	Initial rating	Final rating
T <sub>1</sub> = UNTREATED	1	1 <sup>c</sup>
T <sub>2</sub> = <i>Colletotrichum coffeanaum</i>	2.5	6 <sup>a</sup>
T <sub>3</sub> = <i>Colletotrichum gleosporioides</i>	1.5	3.5 <sup>b</sup>
T <sub>4</sub> = <i>Colletotrichum kahawae</i>	2	3 <sup>b</sup>
Cv= Coefficient variance	40.75%	50.61%

\*means with the same letters are not significantly different at 0.05% level of significance

Initial disease severity rating on Leaves. Table 2 shows the initial severity rating on leaf samples of inoculated seedlings. The highest rating was obtained from *Colletotrichum coffeanaum* of 2.5 but not significantly different from the rating obtained from the other species. The lowest severity rating was observed from *Colletotrichum gleosporioides* isolate which is not significantly different from *Colletotrichum coffeanaum* and *Colletotrichum kahawae* isolates. The control had not infected with a rating of 1.0.

Final disease severity rating on leaves. The highest final disease severity rating on leaves was obtained from *Colletotrichum coffeanaum* which was significantly different from the other species (Figure 13). The result indicates that the *Colletotrichum* species either from leaves, stem/twig, and berries are pathogenic to leaves of Arabica coffee (Table 2).



Table 3. Initial and final disease severity rating on stem/ twig of Arabica coffee seedling

TREATMENT	Initial rating	Final rating*
T <sub>1</sub> = UNTREATED	1	1 <sup>b</sup>
T <sub>2</sub> = <i>Colletotrichum coffeanum</i>	1	2 <sup>b</sup>
T <sub>3</sub> = <i>Colletotrichum gleosporioides</i>	2	5.5 <sup>a</sup>
T <sub>4</sub> = <i>Colletotrichum kahawae</i>	2	2.5 <sup>b</sup>
Cv= Coefficient variance	50.77%	52.61%

\*means with the same letters are not significantly different at 0.05% level of significance

Initial disease severity rating on Stem/Twig. Initial Infection ratings on stem/twig showed no infection to very slight infection with ratings of 1 and 2, respectively. *Colletotrichum gleosporioides* and *colletotrichum kahawae* have the same infection ratings were not significantly different from *colletotrichum coffeanum* (Table 3).

Final disease severity rating on stem/twig. The highest final disease rating on stem/twig was obtained from *Colletotrichum gleosporioides* (Figure 13), which was significantly different from the other species (Table 3).

Table 4. Initial and final disease severity rating on berries

TREATMENT	Initial rating	Final rating
T <sub>1</sub> = UNTREATED	1	1 <sup>b</sup>
T <sub>2</sub> = <i>Colletotrichum coffeanum</i>	1	8.33 <sup>a</sup>
T <sub>3</sub> = <i>Colletotrichum gleosporioides</i>	1.67	9 <sup>a</sup>
T <sub>4</sub> = <i>Colletotrichum kahawae</i>	2.33	9 <sup>a</sup>
Cv= Coefficient variance	13.64%	8.41%

\*means with the same letters are not significantly different at 0.05% level of significance

Initial disease severity rating on Berries. Table 4 shows the initial infection ratings on berries. The highest infection rating was obtained from *Colletotrichum kahawae* of 2.33





but not significantly different from the ratings obtained from the other species. *Colletotrichum coffeanum* shows no infection which is not significantly different from the other species.

Final disease severity rating on berries. The highest final severity disease rating was obtained from *Colletotrichum kahawae* and *Colletotrichum gleosporioides* (Figure 13), but not significantly different from rating of *Colletotrichum coffeanum* (Table 4)

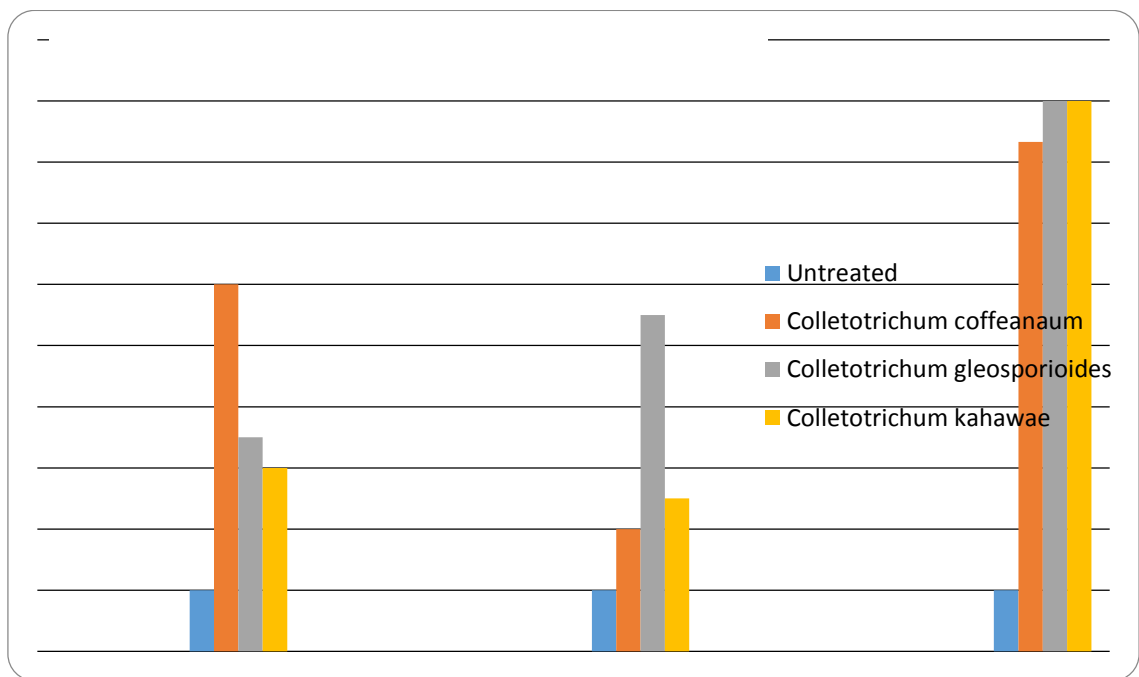


Figure 13. Graph showing the Summary of Final Ratings on leaves, stem/twig and berries



## SUMMARY, CONCLUSION AND RECOMMENDATION

### Summary

The study was conducted from October 2011 to February 2012 at the Department of Plant Pathology Laboratory, Benguet State University.

Isolates of *Colletotrichum spp.* isolated from leaf (*Colletotrichum coffeanum*), stem/twig (*Colletotrichum gleosporioides*) and berry (*Colletotrichum kahawae*) of Arabica coffee are inoculated on leaves and stem of Arabica coffee seedlings and berries to determine their pathogenicity on either leaves and stem of Arabica coffee seedling and detached berries.

The three isolates of *Colletotrichum spp.* were found to be pathogenic to Arabica coffee leaves, stem/twig and berries. Spots, and canker were the symptom developed on the Arabica coffee seedling and berries.

### Conclusion

*Colletotrichum coffeanum* was identified as the main causal pathogen in the leaves, *Colletotrichum glesporioides* in stem/twigs, and *Colletotrichum kahawae* in the berries. However all *Colletotrichum spp.* are able to infect the leaves, stem/twig, and berries.

### Recommendation

It is recommended that a verification study will be done in a greater number of test samples and in different varieties of Arabica coffee. It should also be done in the field, not on seedlings only. Likewise, trials on berries should also be done on intact berries to the mother plants.



## LITERATURE CITED

- AGRIOS, G. M. 1997. Plant Pathology. 3<sup>rd</sup> Ed. San Diego, California: Academic Press, Inc  
Pp. 116-131
- BEARMAN, P. J., BANGUIS, T., BOSWORTH, C.E., Van DONZEL, E. and  
HEINRICH, W.P. (eds) 2002. Encyclopedia of Islam (online). Brill, Leiden,  
Netherlands.
- CABI, 2005. Crop Protection Compendium 2005 Edition. CAB International, Wallingford,  
UK.
- CANNON, P. F. 2000. Linking the past, present and future of *Colletotrichum* systematic.  
In: St. Paul. MN, USA: APS press. Pp. 1-20.
- DAVIS, A. P., GOVAERTS, R., BRIDSON, D.M. and STOFFELEN, P. 2007. An  
annotated taxonomic conspectus of the genus *Coffea* L. (Rubiaceae). Botanical  
Journal of the Linnean Society {In press}.
- DODD, J. E. 1992. Epidemiology of *Colletotrichum gloesporioides* in the tropics. In:  
Wallingford, UK: CAB International . Pp. 308-325.
- FIRMAN, I. D. and J. M. WALLER. 1977. coffee berry disease and other colletotrichum  
diseases of coffee. CMI Phytopathological Paper. No. 20. Pp 53.
- GUTTIERREZ, L. H. DE 1954. Muerte descendente causado por *Colletotrichum* en las  
plantas de café en el almacigo y so comabate por medio de aspersiones en Turrialba,  
Costa Rica. Turrialba, 4,3-4, 115-24.
- HAARER, A. E. 1962. Modern Coffee Production, 2<sup>nd</sup> edu. Leonard Hill, London.
- HAKIZA, G. 1993. Characterization of the coffee, berry disease pathogen *Colletotrichum*  
*kahawae* sp. Nov. Mycological Research. Pp. 989-994
- HOLLIDAY, P. 1980. Fungus Diseases of Tropical Crops. Cambridge University Press,  
Cambridge, UK.
- HYDE, K. 2009. *Colletotrichum* names and current use. Fungal Diversity. Pp. 39, 147-  
183.
- MACANES, V. L. 2006. Assessment on the occurrence and severity of diseases infecting  
arabica coffee (*Coffea Arabica* L.) in Benguet Province/ V.1. Benguet State  
University Research Journal. Pp. 46-87.
- MUTHAPPA, B. N. 1970 Studies on the role of colletotrichum coffeanum causing stalk  
rot of leaves and berries of Arabica coffee. Indian coffee. Pp 34, 263-264.



- PHUONG, T. H. N. 2010. *Colletotrichum spp.* Associated with Anthracnose Disease on Coffee in Vietnam and in some Other Major Tropical Crops. Swedish University of Agricultural science. P. 6.
- QUIMIO, T. H. 1999. Illustrated Genera and Plant Pathogenic Fungi in the Tropics. Department of Plant Pathology, Collage of Agriculture, University of the Philippines ,  
Los Banos, Laguna, Philippines.P.62
- SACCAS, A. M. and CHARPENTIER, J. 1969. Anthracnose des cafeires robusta et excels due a *Colletotrichum coffeanum* Noak en Republique Centrafricaine. Café Cacao. Pp 1-10.
- SCREENIVASAPRASAD, S. 1993. Coffee berry disease pathogen in Africa: Genetic Structure and Relationship to the Group Species *Colletotrichum gloesporioides*, Mycological Research. Pp. 1-10.
- SILVA, M .C. 2006. Brazilian Journal of Plant Pathology. Retrieved March 26, 2011 from <http://www.Cbwinfo.Com/Biological/PlantPath/cc.html>. P. 7.
- SUTTON, B. C 1980. Coelomycetes . Fungi Imperfecti with Pycnidia, Acervuli and Stomata. Kew: commonwealth Mycological Institute. Pp. 9-10.
- WALLER, J. M. 1992. *Colletotrichum* disease of the perennial and other cash crops. In. Wallingford , UK: CAB International. Pp. 131-142.
- WELLMAN, F. L. 1961. Coffee: Botany, Cultivation and Utilization. Leonard Hill, London.
- WRIGLEY, G. 1988. Coffee. Longman, London. Bearman, P.J.,BIANGQUIS, T.,BOSWORTH, C.E.,Van DONZELD, E. and HEINRICH, W.P. (eds) (2002) Encyclopedia of Islam(online). Brill, Leiden, Netherlands.

