BIBLIOGRAPHY

REYES, MILARINE O. APRIL 2008. Characterization of *Fusarium* spp. Associated with the Crown and Roots of Strawberry (*Fragaria* x *ananassa* Duch) in La Trinidad, Benguet. Benguet State Universtiy, La Trinidad, Benguet.

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ABSTRACT

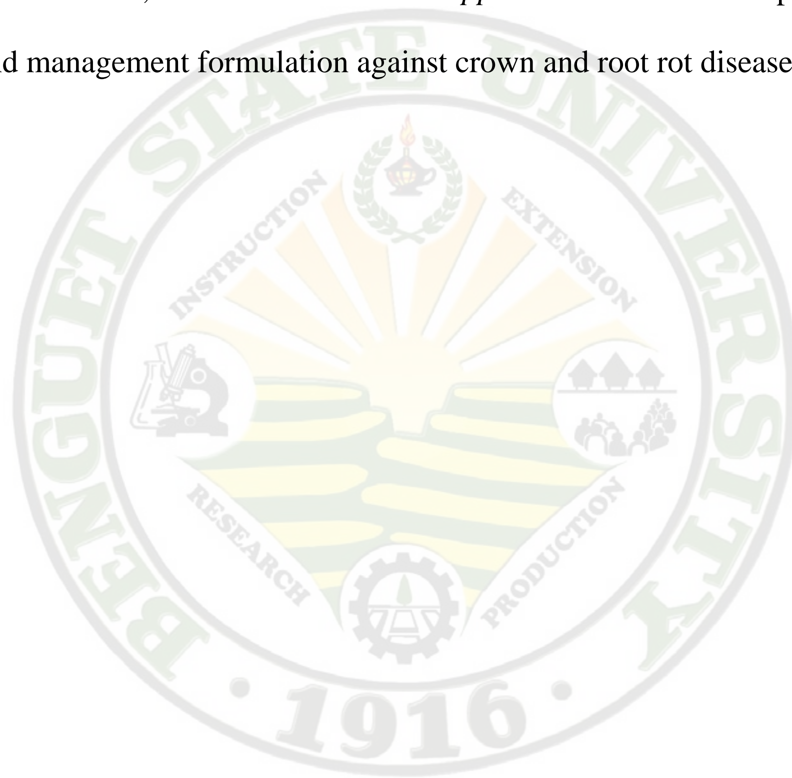
Diseased strawberry specimens were collected in the growing areas of Balili, Pomology, and Swamp area of Benguet State University, La Trinidad, Benguet on the month of November 2007. This was done to isolate and identify the *Fusarium* species associated with the crown and roots of strawberries and to determine their cultural and morphological characteristics following the criteria used in identifying *Fusarium*.

Based from the results, there are two (2) species of *Fusarium* associated with the crown and roots of strawberries grown at the collection sites. These are *Fusarium oxysporum* and *Fusarium solani*.

F. oxysporum grew fast and developed a colony of 4.1 cm in one week at 28 ^{o}C and 30 ^{o}C. It produced white and pale violate pigment in potato dextrose agar (PDA). The macroconidia is falcate in shape to “almost straight” with three (3) septations. Microconidia are abundant and are oval, ellipsoidal and kidney-shaped and are formed in false-heads from short phialides. Its chlamydospores were formed after three (3) weeks of incubation in carnation leaf agar (CLA)

F. solani on the other hand grew slowly and developed a colony of only 2.9 cm at 28 ^{o}C and 2.5 cm at 30 ^{o}C after three (3) weeks. Pigment produced in PDA ranges from cream to pale brown and white. Its macroconidia are “sausage shaped” with three (3) septations, and it produced very few microconidia that are oval and ellipsoidal in shape and are formed in false-heads with long phialides. Chlamydospores were formed after two (2) weeks of incubation in PDA.

From the results, the isolated *Fusarium spp*. should be tested for pathogenicity as basis of sound management formulation against crown and root rot disease of strawberry.



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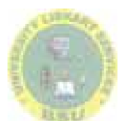
INTRODUCTION

Background of the Study

Strawberry is a sub-tropical plant grown as traditional crop in the highlands. Although some provinces in the lowlands, like Bukidnon are now cultivating strawberry, Benguet is still the main producer of this crop and because of this; the province is popularly known as the “Strawberry Region” of the Philippines. Strawberries are propagated naturally by means of runners that form on the early weeks of April. They are being grown for their edible red berries which are either eaten as fresh or processed as jams, tarts, cookies, and wines. They are planted in the latter months of the rainy season or from the month of June to August and the production period starts from the month of October until the months of April or May.

The strawberries being produced in Benguet mostly comes from the municipality of La Trinidad. About 35 percent of the farming population of the municipality is engaged in this industry. The average production per hectare in the November 2005 to April 2006 production period was 18.5 metric tons, while it declined to 14 metric tons in the 2006 to 2007 production period (Municipal Agriculturist Office, La Trinidad, Benguet, 2007). The decline in the average yield of strawberry is usually due to the improper growing practices of farmers, poor quality of planting materials and most of all, the presence of pest and diseases which is a very serious problem.

Some of the diseases that attack strawberry are anthracnose, *Botrytis* rot, red stele, and diseases caused by *Fusarium* sp. such as *Fusarium* wilt. Most of the pathogens that caused these diseases are soil borne and are difficult to manage. In this study, the pathogen *Fusarium* was given importance.



<u>Importance of the Study</u>

Fusarium causes *Fusarium* wilt and was also found as one of the pathogen that shares in the black root rot disease complex on strawberries together with *Pythium* spp., *Rhizoctonia* spp., and the lesion nematode *Pratylenchus penetrans*. *Fusarium* wilt (*Fusarium oxysporum* f. sp. *fragariae*) is a very serious disease for cultivated strawberries and recently, the damage from this disease has spread to open-culture farming in cooler regions such as Akita Prefecture (40˚N, 139˚E, approximately 200 meters elevation) (Takashaki et al., 2006). On the other hand, Pecknold (2001) stated that black root rot is the most common of all root diseases on strawberries in Indiana, United States. Since temperature in Benguet is closely similar to weather conditions of these countries, the presence of *Fusarium* in the strawberry growing areas of the province can not be discounted. It is therefore imperative to conduct studies on the *Fusarium* spp. attacking strawberry so that proper management of the disease will be formulated.

This will further help strawberry growers to improve their strawberry production thereby increasing their yield.

<u>Objectives of the Study</u>

This study aimed to:

1. isolate and determine the *Fusarium* spp. associated with the crown and roots of strawberries in La Trinidad, Benguet using carnation leaf agar (CLA) and potato dextrose agar (PDA) medium.
2. characterize the *Fusarium* isolates based on their cultural and morphological attributes.

Time and Place of the Study

This study was conducted at the Plant Pathology Service Laboratory, College of Agriculture of Benguet State University, La Trinidad, Benguet from October 2007 to March 2008.

REVIEW OF LITERATURE

The Crop

Strawberry is a common name for low, perennial herbs of the genus *Fragaria* of the rose family, and also for the edible fruit of these herbs. Strawberries, which are native to temperate regions throughout the world, were first cultivated in the United States about 1835 and have since became an important and widely distributed crop in farms and in home gardens. The white flowers, which are borne in cymes, have a five-cleft calyx, five rounded petals, many stamens, and numerous nut lets distributed on enlarge, pulpy, scarlet receptacle (Redmond, 2007).

According to Hermano (1999), strawberry is a sub-tropical plant grown for about a century as traditional crop in the high lands. Because of this crop, the Baguio-Benguet area has been noted and popularly known as "Strawberry Region" of the country. Strawberry production started in early years of the present century. This crop was introduced and was found to be adaptable to the region. The introduction and evaluation were done probably by the Americans who established an Agricultural School in 1916 at La Trinidad, Benguet.

Strawberry is a semi-temperate and photoperiodic crop. Its sweet, juicy fruit is one of the highly-priced commodities in Baguio City despite its abundance during the months of December to April. The crop is grown in the Baguio-Benguet area (4,500 ft asl) of the Northern Philippines where the temperature ranges from 14.7 to 23.3 ^{o}C with a recorded day length of 11.06 hours and a maximum of 13.06 hours in June, the period suited for strawberry production (Balaki, 1992).

At present, strawberry is the number 1 and the most popular fruit produced in Baguio City and Benguet Province. It is a lucrative source of income for farmers and revenue of the province of Benguet and the City of Baguio (HARRDEC, 1996).

The Pathogen

Fusarium is a filamentous fungus widely distributed on plants and in the soil. It is found in normal mycoflora of commodities, such as rice, bean, soybean, and other crops. While most species are more common at tropical and subtropical areas, some inhabit in soil in cold climates (Anonymous, 2007).

The genus *Fusarium* currently contains over 20 species with *Fusarium solani*, *oxysporum*, and *chlamydosporum* the most common. Some are plant pathogens causing root and stem rot, vascular wilt or fruit rot. Other species cause storage rot and are important mycotoxin producers. Several species, notably *F. oxysporum*, *F. solani* and *F. moniliforme*, are recognized as being pathogenic to man and animals causing mycotic keratitis, onychomycosis and hyalohyphomycosis, especially in burn victims and bone marrow transplant patients (Ellis, 2006).

General Characteristics of *Fusarium* sp.

According to Ellis (2006), colonies of *Fusarium* are usually fast growing, pale or brightly colored (depending on the species) and may or may not have a cottony aerial mycelium. The color of the thallus varies from whitish to yellow, brownish, pink, reddish or lilac shades. Species of *Fusarium* typically produce both macro- and microconidia from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform- to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell.

Microconidia are 1- to 2-celled, hyaline, pyriform, fusiform to ovoid, straight or curved. Chlamydoconidia may be present or absent.

Diseases Caused by *Fusarim* spp.

Fusarium causes vascular wilts primarily of annual vegetables and flowers, herbaceous perennial ornamentals, plantation crops, weeds and of the mimosa tree (silk tree). Most of the vascular wilt-causing *Fusaria* belong to the species *Fusarium oxysporum*. Different host plants are attacked by special forms or races of the fungus (Agrios, 1997).

On the other hand, Los and Schroeder (2007) stated that black root rot is caused by a complex interaction of environmental factors, fungi, and nematodes such as *Pratylenchus penetrans*. Several fungi are implicated in the disease including *Rhizoctonia* spp., *Pythium* spp., and *Fusarium* spp.

Furthermore, Pscheidt (2007) stated that a research in Oregon in the 1930's and 1940's implicated *Rhizoctonia* sp., *Fusarium* spp., and *Ramularia* spp. with root rot of strawberry. He also added that winter injury to roots encourages infection by *Fusarium* spp.

Symptoms Caused by *Fusarium oxysporum* and *Fusarium solani*

Agrios (2005) described the symptoms caused by *Fusarium oxysporum* and *Fusarium solani* on the infected plants as follows:

Fusarium oxysporum

Fusarium oxysporum causes vascular wilt and rotting of crown and roots. The leaves of infected plants or of parts of infected plants lose turgidity, become flaccid and lighter green to greenish yellow, droop and finally wilt, turn yellow then brown and die. Wilted leaves maybe flat or curled. Young, tender shoots also wilt and die. In cross sections of infected stems and twigs, discolored, brown areas appear as a complete or interrupted ring consisting of discolored vascular tissues. In the xylem vessels of infected stems and roots, mycelium and spores of the causal fungus may be present. Some of the vessels may clogged with mycelium, spores, or polysaccharides produced by the fungus. Clogging is increased further by gels and gums formed by the accumulation and oxidation of breakdown of products of plant cells attacked by fungal enzyme. The oxidation and translocation of such breakdown of product seem to be responsible for the brown discoloration of affected vascular tissue.

Fusarium solani

Fusarium solani caused rotting of seeds and seedlings (damping-off), rotting of roots, lower stems, and crowns, and rots of corms, bulbs, and tubers. In root rots, tap roots of young plants show a reddish discoloration that later becomes darker and larger. The discoloration may cover the tap root and the stem below the soil line without a definite margin or it may appear as streaks extending up to the soil line. Longitudinal cracks appear along the main root, whereas small, lateral roots are killed. Plant growth is retarded, and in dry weather, the leaves may turn yellow and even fall-off. Sometimes, infected plants develop secondary roots and rootlets just below the soil line that maybe sufficient to carry the plant to maturity and to production of a fairly good crop.

MATERIALS AND METHODS

A. Field Activity

Collection of Diseased Samples

Diseased strawberry plants exhibiting symptoms suspected to be caused by *Fusarium* sp. were collected from the strawberry growing areas of La Trinidad, Benguet particularly at the Balili Experimental Station, BSU Pomology area and Swamp area in the month of November, 2007. The collected samples were wrapped with newspapers and were brought to the laboratory for isolation and for further examination.

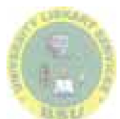
B. Laboratory Activities

Description of Symptoms

Before isolation, the symptoms of the diseased strawberry specimens were described based on how they appeared on the crown and roots of the collected samples. Vascular discoloration was observed by cutting the crown to reveal the inside part and sectioning the roots longitudinally. Above ground symptom was also noted on the leaves of the specimens. The observed symptoms were documented.

Isolation

Crown and roots of the infected strawberries were washed thoroughly and were cut into small sizes. The cut tissues were disinfected by soaking them in a 10 % chlorox (NaCl) for 10 minutes, after which they were washed three (3) times with sterile distilled water. The disinfected tissues were isolated equidistantly in the previously plated carnation leaf agar (CLA). Ten (10) plates were prepared having five (5) tissues isolated



in each plate. This makes a total of 50 tissues isolated: 25 crown tissues and another 25 root tissues. After isolation, the plates were sealed with parafilm and incubated until growth was observed.

Observation of the *Fusarium* Isolates

After 10 days of incubation, growth of the organism from the CLA plates was observed. The plates were examined directly under the 10x magnification field of the light microscope to confirm the growth of *Fusarium* sp.

To be able to characterize the organism, single sporing was done in CLA and PDA plates. Single sporing was initiated to ensure the purity of the cultures and to avoid possible mutation, thereby retaining the original wild type colony of the *Fusarium*.

Single Spore Initiation

Single sporing was done by pouring 15 ml of 2 % water agar into petri dishes and allowing them to solidify. A suspension of spores was prepared in 5 ml sterile distilled water blank. The spore suspension was poured over the solidified agar so as to cover the entire surface, and then the excess was drained off. The seeded dishes were incubated in an inclined position at room temperature for 18-20 hours. At the end of this time, the dishes were opened and then shaken to remove the accumulated moisture, after which they were examined under a dissecting microscope. Small squares of the agar containing single spore were cut out with a dissecting needle and were transferred to PDA and CLA media. The spore was inoculated at the center of the agar for the PDA plates, while near the leaf pieces for the CLA plates.

Characterization of the *Fusarium* Isolates

Characterization was done by examining the cultures directly under the light microscope. Slide preparations were made to ensure correct characterization. The book "Laboratory Manual for *Fusarium* Research" by Burgess and Liddell (1983) was used as reference guide for the identification. The important criteria used as basis for characterization includes:

A. Morphological characteristics

a. Presence or absence of microconidia and chlamydospores.

b. Shape of the macroconidia as well as the microconidia if present.

c. Mode of formation of the microconidia. In this criterion, the microconidia were examined on the CLA plates under the light microscope to determine whether they are formed in chains or in false-heads.

d. Nature of the conidiogenous cell bearing the microconidia. This determined whether the microconidia are formed from monophialidic or polyphialidic conidiogenous cell.

B. Cultural characteristics

a. Colony diameters on PDA plates after dark incubation for 3 days at 28°C and 30°C.

b. Colony morphology on PDA plates. This criterion determined the growth of the organism, color of mycelia, and the colony pigmentation produced by *Fusarium* isolates.

Measuring the Conidia

Conidial measurement was done by subjecting the conidia of each *Fusarium* sp. in a calibrated microscope with the use of an ocular and stage micrometer. The length and the width of the conidia were measured then multiplied with the calibration factor of the microscope to get the exact dimension of the conidia. Forty (40) samples of conidia were measured.

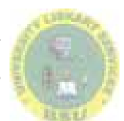
Calibration of the Microscope

Calibration of the microscope was done by inserting the ocular micrometer on the eyepiece of the microscope while the stage micrometer was placed on the stage of the microscope. Focusing was done on the stage micrometer scale using the Low Power Objective (LPO) and then on the High Power Objective (HPO) of the microscope. Zero (0) point of the stage micrometer was set to coincide with that of the ocular micrometer. The ocular divisions that cover the space between the zero and coincident lines were counted. Calibration factor (CF) or calibration constant (CC) was calculated using this formula:

$$CF = \frac{\text{n divisions of stage micrometer}}{\text{n divisions of ocular micrometer}} \times 10 \text{ units/division}$$

Data Gathered:

1. Symptoms manifested by the collected diseased strawberry specimens.
2. Species of the *Fusarium* that were isolated.
3. Cultural and morphological characteristics of the *Fusarium* isolates.
4. Frequency of occurrence of *Fusarium* per isolate that was determined by examining the growth of the organism in each tissue on the CLA plates.
5. Comparison of conidia of the *Fusarium* isolates grown in CLA and PDA.



RESULTS AND DISCUSSION

Collection Sites of Diseased Strawberry Specimens

Diseased strawberry plants were collected at the Balili Experimental Station, BSU Pomology area and Swamp area on the month of November, 2007. The collected specimens were of Sweet Charlie variety

Symptoms of the Collected Diseased Specimens

Table 1 shows the above ground symptom manifested by the collected diseased strawberry plants which was browning of the outer leaves (Fig. 1). On the other hand, below ground symptoms observed were vascular discoloration of the crown which ranges from orange, brown and black discoloration (Fig. 2a & b). Rotting was observed in most of the roots (Fig. 3a & b), and this conforms to the reports of Los and Schroeder (2007) and Pscheidt (2007) that *Fusarium sp*. is one of the fungi that causes root rotting of strawberries. Aside from rotting, brown discoloration was observed in some of the roots.

Table 1. Above and below ground symptoms manifested by the collected specimens

Plant Part Affected	Symptoms
Leaves	Browning of the outer leaves
Crown	Orange, brown and black vascular discoloration
Root	Rotting in most of the roots and brown discoloration in some

Figure 1. Browning on the leaves of the collected specimens

Figure 2a. Symptoms on the crown of the collected specimens showing orange (pointed by arrow) and black discoloration (encircled)

Figure 2b. Brown discolorations on the crown of the collected specimens

Figure 3a. Roots of the collected strawberry showing rotting in most of the roots

Figure 3b. Infected roots of the collected strawberry showing mycelia of *Fusarium*

Result of Isolation

Result of isolation revealed that there are two species of *Fusarium* associated with the crown and roots of strawberries in La Trinidad, Benguet particularly at the collection sites. They were temporarily assigned as Isolate 1 and Isolate 2 while characterization is being done.

Frequency of Occurrence of *Fusarium* on CLA Plates

Table 2 shows that among the 50 tissues isolated; only 18 tissues or 36 % were infected with *Fusarium.* Six of the 18 infected tissues or 33 % were crown tissues while 12 or 67 % were root tissues. Isolate 1 was more abundant in the crown tissues while Isolate 2 in the root tissues. This result implies that Isolate 1 is more associated with the crown tissues while Isolate 2 with the root tissues.

Table 2. Frequency of occurrence of *Fusarium* from crown and root tissues isolated in carnation leaf agar (CLA)

Plate Number	Tissue Number					Number of times isolated	*Fusarium* Isolates
	T1	T2	T3	T 4	T 5		
Crown tissues							
Plate 1	-	-	-	-	-	-	none
Plate 2	-	1	-	-	-	1	Isolate 1
Plate 3	1	1	1	-	-	3	Isolate 1
Plate 4	-	-	-	1	-	1	Isolate 2
Plate 5	1	-	-	-	-	1	Isolate 1
Total						**6**	
Root tissues							
Plate 6	1	1	1	1	1	5	Isolate 2
Plate 7	-	-	-	-	-	-	none
Plate 8	1	-	-	1	-	2	Isolate 1
Plate 9	1	1	1	1	1	5	Isolate 2
Plate 10	-	-	-	-	-	-	none
Total						**12**	
Grand total						**18**	

Legend: - = not infected with *Fusarium*

Morphological Characteristics of the *Fusarium* Isolates

The morphological characteristics of the *Fusarium* isolates from cultures germinated from single spore (conidia) are summarized in Table 3. The different structures of the *Fusarium* were observed directly on the plates under the 10x

magnification field of the light microscope. Slide preparations were also made using methylene blue lactophenol as mounting medium.

Conidia. Isolate 1 has falcate to "almost straight", three (3) septated, macroconidia with hooked apex and notched base (Fig. 4a). It produced abundant, non-septated microconidia that are oval, elliptical and reniform (kidney-shaped) in shape (Fig. 5). On the other hand, Isolate 2 has "sausage-shaped", three (3) septated macroconidia with round apex and notched base (Fig. 4b). It produces very few, oval to ellipsoidal microconidia (Fig. 6) that are mostly two (2) celled. Macroconidia of both isolates are mostly formed from phialides on the hyphae but some are formed from phialides on branched conidiophores (Fig. 7 & 8). They also both form microconidia in false-heads, but Isolate 1 produced them from short monophialidic conidiogenous cell (Fig. 9), while Isolate 2 produced them from long monophialidic conidiogenous cell on the hyphae (Fig. 10).

Chlamydospores. Isolate 1 produced chlamydospores that are located terminally and intercalary on the hyphae (Fig. 11), while Isolate 2 produced them terminally and intercalary on a macroconidia (Fig. 12). Chlamydospores of Isolate 1 are produced on a three (3) week old culture in CLA while Isolate 2 on a two (2) week old culture in PDA.

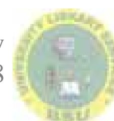


Table 3. Morphological characteristics of the *Fusarium* isolates

CRITERIA	*FUSARIUM* ISOLATES	
	Isolate 1	Isolate 2
a. Microconidia	Present, produced abundantly	Present but very few
b. Number of Septa		
Macroconidia	3 septations	3 septations
Microconidia	Not-septated	Septated (1)
c. Mode of formation of microconidia	False-heads	False-heads
d. Nature of conidiogenous cell bearing microconidia	They are formed from short monophialidic conidiogenous cell.	They are formed from long monophialidic conidiogenous cell.
e. Formation of macroconidia	They are formed from phialides on the hyphae or on branched conidiophores.	They are formed from phialides on the hyphae or on branched conidiophores.
f. Chlamydospores	Present and are formed terminally and intercalary on the hyphae on a 2 week old culture in CLA.	Present and are formend terminally and intercalary on the macroconidia on a 3 week old culture in PDA.

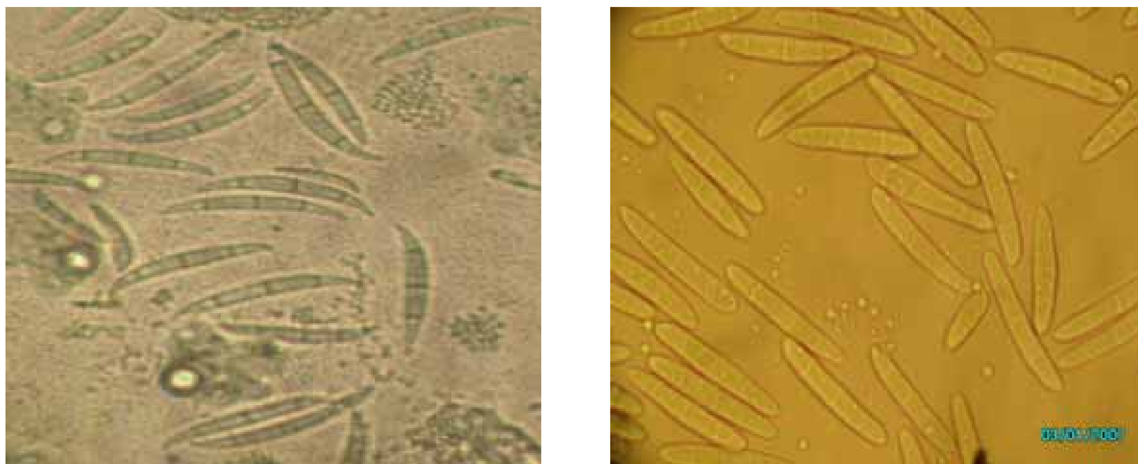


Figure 4. Macroconidia of (a) Isolate 1 in CLA (*in-situ*) and (b) Isolate 2 (in slide mount) 400x

Figure 5. Microconidia of Isolate 1 showing (a) oval-shaped (b) elliptical-shaped and (c) kidney-shaped (400x)

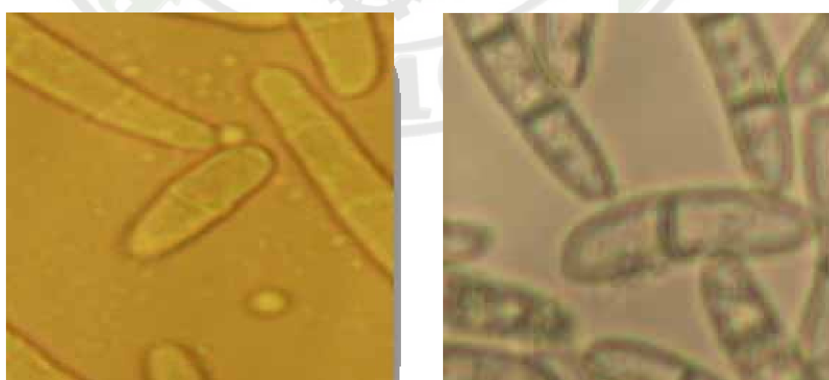


Figure 6. Microconidia of Isolate 2: (a) elliptical shaped and (b) kidney-shaped (400x)

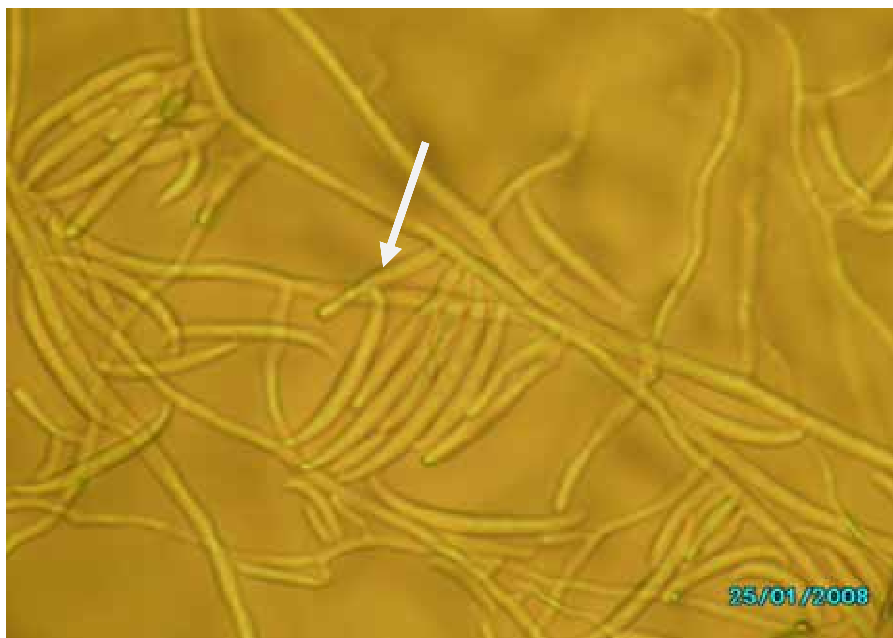


Figure 7. Macroconidia of Isolate 1 from phialides on hyphae and from branched conidiophores (with pointer), *in situ*:CLA (400x)

Figure 8. Macroconidia of Isolate 2 from (a) phialides on hyphae and from (b) phialides on branched conidiophores, *in situ*: PDA (400x)

Figure 9. Microconidia of Isolate 1 in false-head from short monophialides *in situ*: CLA (400x)

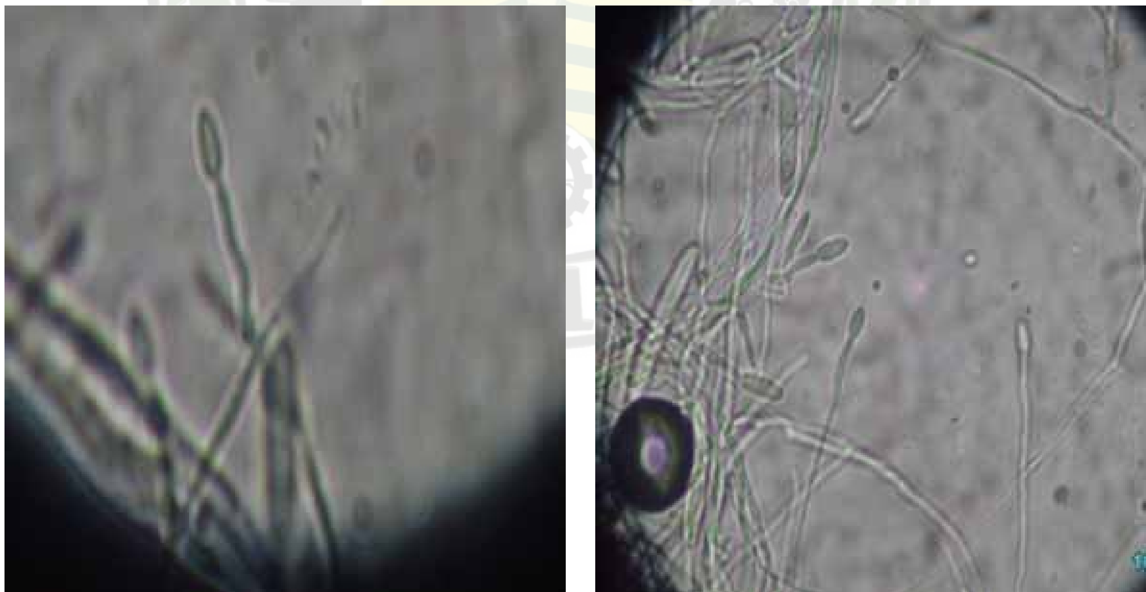


Figure 10. Microconidia of Isolate 2 (a) in false-head and (b) from long monophialides *in situ*: PDA (400x)

Figure 11. Chlamydospores of Isolate 1 formed terminally (encircled) and intercalary (with pointer) on hyphae in three week old culture in CLA (400x)

Figure 12. Chlamydospores of Isolate 2 formed intercalary on macroconidia in two week old culture in PDA (400x)

Cultural Characteristics of the *Fusarium* Isolates

Cultural characteristics of the *Fusarium* Isolates are shown in Table 4.

Isolate 1 is fast-growing and produces abundant, floccose, white mycelium which growth is compact. Pale violet macroconidia are produced in a central spore mass in PDA plates (Fig. 13). Reverse of cultures appeared yellow with pale violet at the center (Fig. 15b). Black sclerotial bodies were formed after two weeks. Colony diameters after three (3) days was 2.5 cm at 28 °C and 2.3 cm at 30 °C and increased to 4.1 cm at both temperatures after one (1) week. The PDA plates were filled with the mycelia of Isolate 1 after two (2) weeks (Fig. 15).

On the other hand, Isolate 2 is slow-growing and produced slightly thick, mycelium that is not cottony. Three (3) day old cultures are cream colored which turns into pale brown after a week (Fig. 14). After two (2) weeks, the pale brown color was surrounded with white color (Fig. 16a). Reverse of cultures appeared dark brown (Fig. 16b). Colony diameters measured 0.27 cm at 28 °C and 0.53 cm at 30 °C after three (3) days, and expand to 0.5 cm at 28 °C and 0.97 cm at 30 °C after one (1) week. At two (2) weeks, the colony enlarges to 2.9 cm at 28 °C and 2.5 at 30 °C in diameter. From the results, Isolate 1 prefers a growth temperature of 28 °C than 30 °C, while Isolate 2 prefers a higher temperature of 30 °C than 28 °C for colony growth.

Table 4. Cultural characteristics of the *Fusarium* isolates

CRITERIA	*FUSARIUM* ISOLATES	
	Isolate 1	Isolate 2
a. Average colony diameter		
Three days	2.5 cm at 28 °C 2.3 cm at 30 °C	0.27 cm at 28 °C 0.53 cm at 30 °C
One week	4.1 cm at 28 °C and 30 °C	0.5 cm at 28 °C 0.97 cm at 30 °C
Two weeks	Plate is full at 28 °C and 30 °C	2.9 cm at 28 °C 2.5 cm at 30 °C
b. Pigmentation	White and pale violet. Reverse of cultures appear as yellow with pale violet at the center. Black sclerotial bodies were produced at the center of the cultures after two weeks.	The isolate produced cream color after three days which turns into pale brown after a week. After two weeks the pale brown color becomes surrounded with white and its reverse appear as dark brown.
c. Colony growth	Fast-growing and produced abundant, floccose and compact mycelium.	Slow-growing and produced slightly thick mycelium that is not cottony.

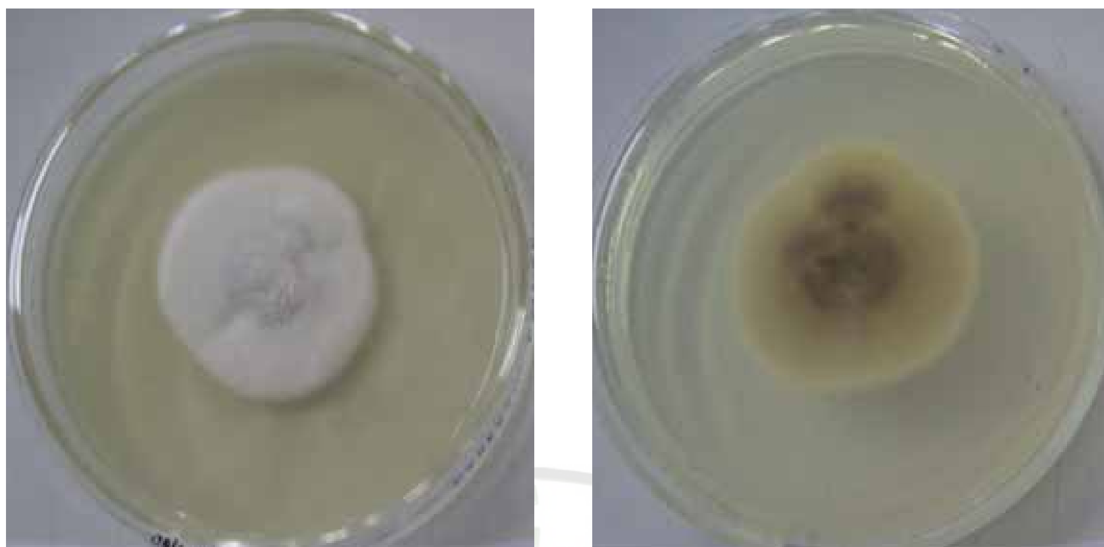


Figure 13. One week old culture of Isolate 1 in PDA (a) front view and (b) reverse view

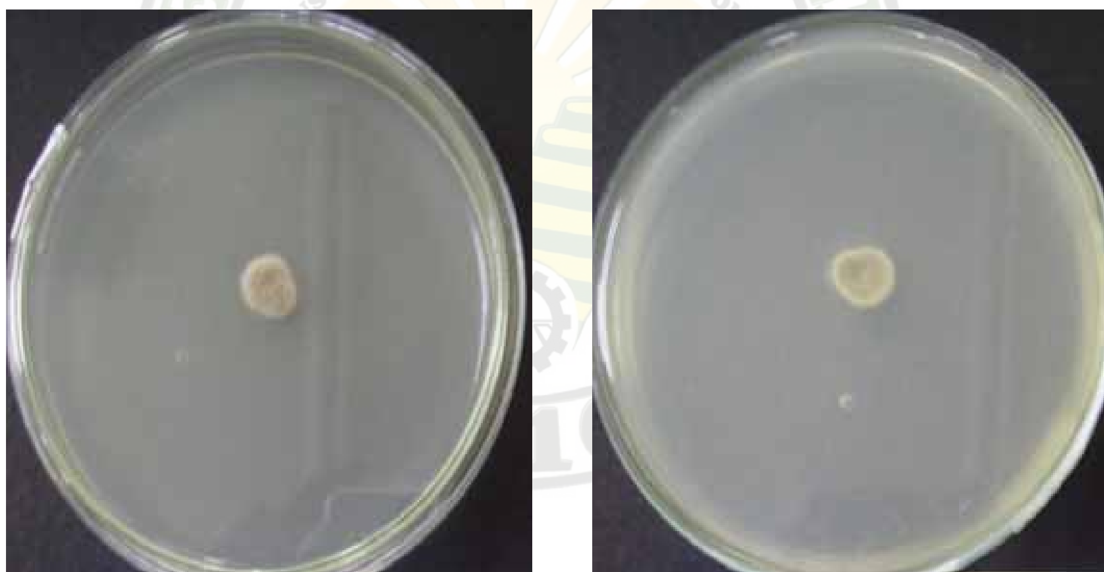


Figure 14. One week old culture of Isolate 2 in PDA (a) front view (b) reverse view

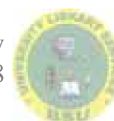


Figure 15. Two week old culture of Isolate 1in PDA (a) front view (b) reverse view

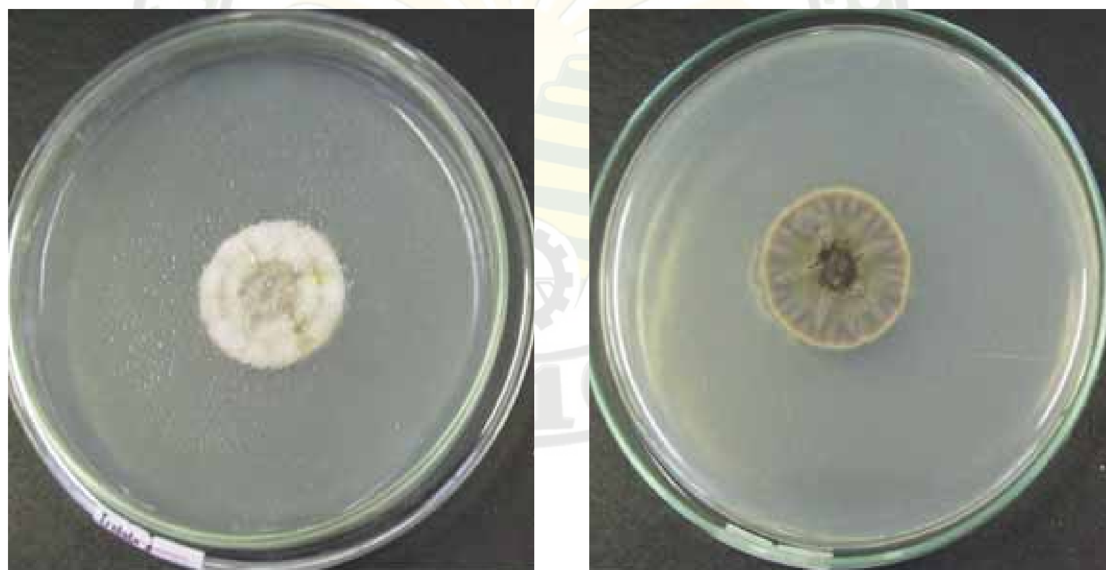


Figure 16. Two week old culture of Isolate 2 in PDA (a) front view (b) reverse view

Comparison of Conidia of the *Fusarium* Isolates Grown in CLA and PDA

Table 5 shows the difference between the shape of the conidia of the *Fusarium* isolates grown in CLA and PDA. Macroconidia and microconidia of Isolate 1 and Isolate 2 in CLA are mostly uniform in shape (Fig. 17a & 18a) while in PDA they are mostly irregular in shape and some were even distorted (Fig. 17b & 18b).

Table 5. Comparison of shape of conidia of the *Fusarium* isolates grown in CLA and PDA

CRITERIA	Isolate 1		Isolate 2	
	CLA	PDA	CLA	PDA
a. Macroconidia	Mostly uniform	Mostly irregular	Mostly uniform	Mostly irregular
b. Microconidia	Mostly uniform	Mostly irregular	Mostly uniform	Mostly irregular

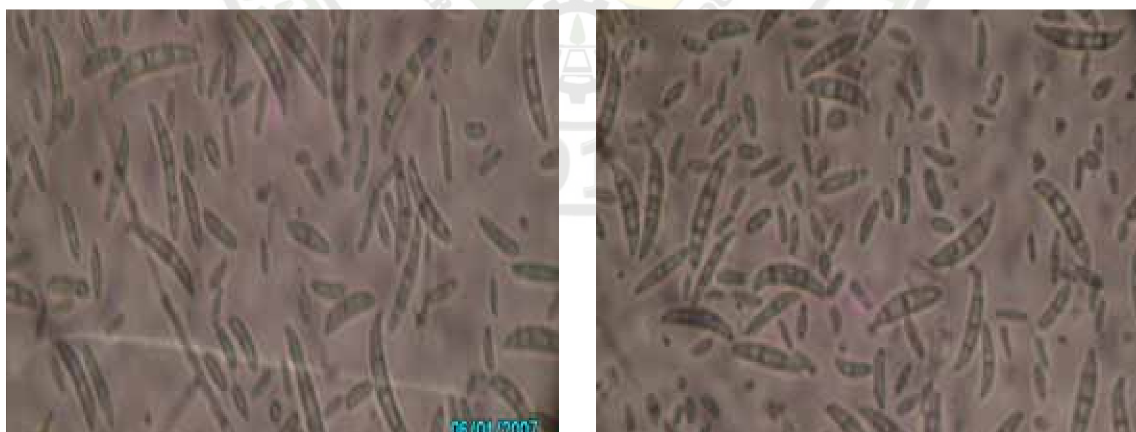


Figure 17. Macro and microconidia of Isolate 1 grown in (a) CLA and in (b) PDA (400x)

Figure 18. Macro and microconidia of Isolate 2 grown in (a) CLA and in (b) PDA (400x)

Identified Species of *Fusarium*

On the basis of the criteria outlined by Burgess and Liddell in the reference titled "Laboratory Manual for *Fusarium* Research" (1983), the morphological and cultural characteristics of Isolate 1 matches the characteristics described for *Fusarium oxysporum* *w*hile Isolate 2 is close to the reported characteristics of *Fusarium solani* in the same book.

The description of *Fusarium oxysporum* in the reference that was used conforms to the cultural and morphological characteristics of Isolate 1 in this study. These include falcate to "almost straight", three (3) septated macroconidia that are tapered or pointed at each end, have hooked apex and notched base; and are formed from phialides on branched condidiophores and from phialides on hyphae; presence of abundant, one (1) celled microconidia that are formed in false-heads on short phialides, and are oval, elliptical or reniform (kidney-shaped) in shape; chlamydospores that are formed in a three (3) week old culture; mycelium that are floccose and abundant, ranging in color from white and pale violet; and the production of black sclerotial bodies. Colony diameter of

Fusarium oxysporum after three (3) days is from 2.5 to 4.0 cm at both 25 °C and 30 °C which is not far from the colony diameter of Isolate 1 as shown in Table 4.

On the other hand, description of *Fusarium solani* matches with the cultural and morphological characteristics of Isolate 2 which include three (3) septated macroconidia that are "sausage-shaped", with a round apex and notched base and are formed from phialides on branched conidiophores and from phialides on hyphae; two (2) celled microconidia that are oval to elliptical in shape and are formed in false-heads on long phialides; chlamydospores that are formed in a two (2) week old culture and are formed from the macroconidia; and last is the production of cream to pale brown mycelium. The colony diameter of Isolate 2 does not conform to that of *Fusarium solani* which is 2.1 to 2.9 cm at 25 °C and 2.6 to 3.6 at 30 °C. This could be attributed to the temperature of 28 °C where the isolate was incubated instead of 25 °C. This needs further investigation.

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

The study was conducted to isolate and determine the *Fusarium* species associated with the crown and roots of strawberries in Benguet particularly at the Balili Experimental Station, BSU Pomology area and Swamp area; and to characterize them based on their cultural and morphological attributes.

Results revealed that that there are two (2) species of *Fusarium* associated with the crown and roots of strawberries in the collection sites. The symptoms they caused on the collected specimens were browning of the outer leaves, rotting in most of the roots and brown discoloration in some, while orange, brown and black discoloration were observed on the crown.

The identified species of *Fusarium* were *Fusarium oxysporum* and *Fusarium solani*. However, *Fusarium solani* does not conform to the colony growth description of *Fusarium solani* in the reference book used in this study, thus further investigation is needed.

Fusarium oxysporum is fast-growing (4.1 cm colony diameter in 1 week at 28 °C and 30 °C) and produced white and pale violet pigment in PDA. The macroconidia has 3 septations and falcate to "almost straight" in shape. It produced abundant microconidia that are oval, ellipsoidal and kidney-shaped and were formed in false-heads from short phialides. Chlamydospores were formed after three (3) weeks of incubation in CLA.

Fusarium solani on the other hand is slow-growing (2.9 cm at 28 °C and 2.05 at 30 °C colony diameter after three weeks) and produced cream to pale brown and white pigment in PDA. Its macroconidia has 3 septations , and "sausage-shaped". It produced

very few microconidia that are oval and ellipsoidal in shape and were formed in false-heads from long phialides. Chlamydospores were formed after two (2) weeks of incubation in PDA.

Fusarium oxysporum is more associated with the crown of the collected strawberry specimens while *Fusarium solani* is more associated with the roots. Both species are present in the collection sites.

Conclusion

On the basis of the criteria outlined by Burgess and Liddell in their book "Laboratory Manual for Fusarium Research" (1983) Isolate 1 matches the morphological and cultural characteristics of *Fusarium oxysporum*, while Isolate 2 conforms to some of the morphological and cultural characteristics of *Fusarium solani*.

Fusarium oxysporum and *Fusarium solani* are the species of *Fusarium* found in the strawberry growing areas of La Trinidad, Benguet particulary at Balili Experimental Station, BSU Pomology area and Swamp area.

Recommendations

1. The identified *Fusarium* species will have to be verified thru molecular analysis especially the *Fusarium solani* to make sure that the identification is correct.
2. The *Fusarium* isolates must be tested for pathogenicity to verify if they are pathogenic and to confirm the symptoms they caused on strawberries.

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