BIBLIOGRAPHY

RACHEL K. TUDAYAN. November 2006. Characterization of the Pathogens Associated with the Disease Complex of Mondo Grass (*Ophiopogon jaburan* (Sieb.) Lodd.). Benguet State University, La Trinidad, Benguet.

Adviser: Janet S. Luis, Ph.D.

ABSTRACT

The characterization of the pathogens infecting the mondo grass was done based on symptoms, signs and cultural and morphological characteristics.

The disease symptoms observed includes leaf spots, blights, yellowing and necrotic lesions. The pathogens appeared either singly or interacting together resulting in a disease complex.

Three organisms were identified in the disease complex which include the fungus *Fusarium* sp., the bacterium *Xanthomonas* sp. and the nematode *Aphelenchoides fragariae*.

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INTRODUCTION

Plants dominate the earth and are considered the most valuable things in the natural world, affecting all aspects of human lives either directly or indirectly. Human and animal lives depend mainly on the oxygen that these produce and recycle and the ability to produce organic matter. Plants are the major source of food and other basic necessities such as medicines, oils and fibers.

Plants contribute to the enhancement of the environment. These are used in landscaping either as cover crops, shades and decorations. These aesthetic purposes according to Rimando (2001), give warmth and color, peace and serenity to the work area.

In the past centuries, humans learned to settle down and started cultivating and domesticating plants and animals as sources of food supply. All over the world, most of the fertile lands are already converted for agricultural use. In reality, the food supply of human and animal population is determined by the growth and productivity of plants.

Plant growth and productivity are, however, affected by several factors one of which are diseases. The interaction between the plant and pathogenic organism affects the food supply. Nowadays, a constant war occurs between the farmer and these pathogens. The management of these diseases brings about financial losses in terms of the use of chemical pesticides. These affect not only the quantity but also the quality of the plant product causing considerable

economic impact. On the other hand, symptoms of the disease in certain plants may occur singly or in characteristic combinations. The causal organism may also be due to one or more agents.

Mondo grass (*Ophiopogon jaburan* (Sieb.) Lodd.) which was introduced in the Philippines during the early 1990's (Madulid,) is being used as a cover crop or as a border plant in some of the localities within La Trinidad, Benguet and Baguio City. Some are found to be planted along the roadside and or are integrated with other ornamentals in a quadrangle.

It is observed, however, that most of these plants are attacked or infected with disease. Symptoms of leaf spots, yellowing and browning of the green leaves are observed.

It is therefore apparent to verify and identify the diseases of mondo grass to determine if these pose a threat to other important crops such as vegetables, fruits and ornamentals within the locality.

To determine the range of diseases attacking the plant and to fully understand the nature and significance of these diseases, this study aimed to:

1. Describe the symptoms of the disease complex observed in mondo grass;
2. Isolate and describe the pathogens associated with the disease complex; and,

3. Identify and characterize the pathogens attacking mondo grass based on their cultural and morphological characteristics.

The study was conducted at the Plant Pathology Service Laboratory, College of Agriculture, Benguet State University, La Trinidad, Benguet from March to October 2006.

REVIEW OF LITERATURE

Host

Ophiopogon or mondo grass is a perennial crop belonging to the monocot group. It has the following plant classification (http://plants.usda.gov.java/profile):

Kingdom …….Plantae – Plants

Subkingdom ……..Tracheobionta – Vascular plants

Superdivision ……..Spermatophyta – Seed plants

Division …….Magnaliophyta – Flowering plants

Class ……Liliopsida – Moncotyledons

Subclass …….Liliidae

Order ……Liliales

Family …….Liliaceae – Lily family

Genus ……*Ophiopogon* Ker-Gawl

Species ……..*Ophiopogon jaburan* (Sieb.) Lodd.

Ophiopogon is native to the Honshu, Shikoku, and Kyushu, provinces of Japan. It grows in woods and scrub at low altitudes, flowering in July-August. This plant has thickened tuberous roots, spreading by stolons to form loose patches. The leaves are narrow at 2-3 mm width commonly planted as ground cover or as low-maintenance grass substitute (Phillip and Rix, 1996).

Mondo grass is one closely related, grass-like groundcover. The most frequently used species is *Ophiopogon japonicus*, which has several popular varieties. These are generally drought-tolerant as well as disease- and insect-resistant. Mondo grass is used for edging borders and in places where minimal maintenance is required. Variegated selections can be used as accent plants to add contrast and color (www.ctahr.hawaii.edu/oc/freepubs/pdf/of.28.pdf).

The Disease

"Since it is not known whether plants feel pain or discomfort, as plants do not speak or otherwise communicate to us, it is difficult to pinpoint exactly when a plant is diseased" (Agrios, 1997).

To cause a disease, a pathogen must find a suitable host plant, pass through the external protective layers of the host, and gain access to the nutrients that it requires for its own growth and development (Dickson, 2003).

The emerging disease is dessiminated and spread by air, water, insects and other vectors and most especially dessiminated by humans through the tools, machineries and infected planting materials (Agrios, 2005). International trade also results in all sorts of plant materials being moved from one place to place. Pathogens are often imported and become established in countries where the conditions for disease development

maybe more favorable than in the country of origin (Lucas, 1998). The movement of plant and plant products is also an important source of new diseases.

Diseases Caused by Bacteria

Bacteria are the most widely distributed, the simplest in morphology, the smallest in size and the most difficult to identify. They are prokaryotic and seldom photosynthetic (Benson, 1998).

There are a number of bacterial diseases of plants (Mount and Lacy, 1982; Circrolo, Collmer and Gillaspie, 1987; Billing, 1987). Of the 1600 known bacterial species, 100 cause diseases in plants (Dickson, 2003). Most plant pathogenic bacteria are rod-shaped except *Streptomyces* which is filamentous. Furthermore, most of the plant pathogenic bacteria have delicate thread-like flagella, considerably longer than the cells on which they are produced (Agrios, 2005).

Bacteria utilize a range of strategies for entry into plants. The leaf surface can support large populations of many bacterial species that may either be present in the air, splashed or carried by insects. These bacteria can multiply on the surface to form microcolonies or larger aggregates which enter into the plants through stomata, natural openings and wounds, with the help of water droplets formed on the leaf surfaces. In some *Xanthomonas* bacteria, they actively invade the plants through the

hydathodes, the structure containing water pores located at leaf margins. Under suitable conditions, copious amounts of fluid are exuded through the hydathodes that are collected as guttation water drops around leaf margins. The bacteria move chemotactically towards these drops, then later is drawn back into the hydathodes carrying the bacteria with them in the vascular system (Dickson, 2003.)

Bacteria can not enter plants via intact cuticles. Their entry is through wounds and natural openings such as stomata or lenticels. The presence of water is necessary to enable the pathogen to multiply and move to a point where entry is possible. Inside the plant, they first multiply in the intercellular spaces and/or the xylem and produce pectolytic enzyme which causes cell death and the cells are then invaded (Manners, 1993).

Agrios (2005) described the symptoms of bacteria such as leaf spots and blights; soft rot of fruits, roots, and storage organs; wilts; overgrowths; scabs and cankers. The most common type of disease in plants are those that appear as spots of various sizes on leaves, stems, blossoms and fruits. In some bacterial diseases, the spots continue to advance rapidly and are then called blights.

In monocotyledonous plants, bacterial spots appear as streaks or stripes. In humid weather conditions, infected tissues often exude masses

of bacteria that spread to new tissues or plants and start new infections. Almost all bacterial spots and blights of leaves, stems and fruits are caused by bacteria belonging to the genera *Pseudomonas* and *Xanthomonas.*

Diseases Caused by Fungi

Fungi are usually filamentous, eukaryotic and spore-producing organisms that lack chlorophyll. The cell walls are made up of chitin combined with other complex carbohydrates and cellulose. All species of fungi are either saprobes or symbionts. As symbionts, they may provide certain benefits to their host, or may parasitize their host (Moore, et. al, 1995). The unit structure is the hypha, a branched filament consisting of a row of cells with or without coenocytic structure. Reproduction usually involves the production of unicellular or multicellular spores by sexual or asexual processes.

Fungi may cause a very wide range of types of plant disease. More than 10,000 species of fungi can cause disease in plants (Agrios, 2005). In plant pathogenic fungi, there are sub-specific entities which attack different host genera. All plant pathogenic fungi make use of extracellular enzymes which they secrete at most stages of their attack on their hosts (Manners, 1993).

Fungi and oomycetes are generally dispersed as spores that are either deposited in the soil or transmitted through the air. Once the fungus

adheres and germinates within a suitable host, it grows into the plant and obtain its nutrients for its growth and development. It has the capacity to do this either directly through the cuticle or grow towards the natural opening or wound sites and enter through these (Dickson, 2003).

Fungi can cause local or general necrosis on plant tissues, and they often cause reduced growth of plant organs or entire plants. Some of the most common necrotic symptoms are leaf spot, blight, root-rot, damping off, anthracnose, soft rot and dry rot. In many diseases, the fungal pathogen grows or produces various structures on the surface of the host which is of particular importance including mycelia, sclerotia, sporophores, fruiting bodies and spores (Agrios, 2005).

Diseases Caused by Nematodes

Nematodes belong to the kingdom Animalia. Nematodes are cylindrical worms, with a body shape commonly described as filiform or having the shape of a thread. The name "Nematoda" is derived from the Greek word *nema,* which means thread. They are aquatic animals that inhabit oceans, seas, freshwater courses, body fluids, and in the film of water present between soil particles and in plant which is of particular importance.

Plant parasitic nematodes are present in almost every type of habitat with the limiting factors being moisture and food for survival. These are obligate feeders on plants and can only obtain nutrients for development and reproduction from the cytoplasm of living plant cells (Perry and Wright, 1998). They constitute about 20% of the described species (4,000 among 20,000) within the phylum Nematoda and are included in the classes Chromadoria and Enoplea (Ferraz and Brown, 2002).

Nematodes are considered the most successful pseudo-coelomate animals. This is due to their highly integrated body walls (cuticle, epidermis and longitudinal muscles) that permit very efficient locomotion (Lorenzen, 1985). Another contributing factor is the ability of some nematodes to enter a state of anabiosis which permits long-term survival under extremely harsh conditions. Nematodes have adapted to and are capable of surviving a variety of extreme environmental/physical stresses and even possess specialized developmental stages in order to do this (Perry and Wright, 1998).

Female nematodes lay about 300-500 eggs. Depending on the climate, the availability of hosts and the duration of each life cycle of the particular nematode species may have from two or more than a dozen generations per year.

Symptoms caused by nematodes on roots may appear as root lesions, root knots or root galls, excessive root and accompanied by plant pathogenic or saprophytic bacteria and fungi as root rots. Certain species of nematodes invade aboveground portions of plants. They cause galls, necrotic lesions and rots, twisting or distortion of leaves and stems, and abnormal development of the floral parts (Agrios, 2005).

Plant parasitic nematodes interact with many soil inhabitants (fungi, micro-arthropods and free-living nematodes) and their soil environment is significantly affected through human intervention with agricultural procedures (Ferraz and Brown, 2002). Since most of the nematodes live and operate in the soil where they are surrounded by many soil inhabitants, there is a great interrelationship between the nematodes and other plant pathogens. In many cases, an association develops between nematodes and the other pathogens. Nematodes then become a part of an etiological complex resulting in a combined pathogenic potential that sometimes appears to be greater than the sum of damages that either of the pathogens can produce individually (Agrios, 2005).

In the Philippines, published records show that nematodes are prevalent in the Philippine soils. The plant parasitic forms apparently have received much attention since they are frequent associates of plant

diseases and are thus potential threat to agriculture (Castillo and Reyes, 1972).

MATERIALS AND METHODS

A. Collection of Diseased Leaves

Sample leaves of infected mondo grass were collected, placed in transparent polyethylene bags and brought to the laboratory for further diagnosis. Samples were obtained from different locations in La Trinidad, Benguet and Baguio City.

B. Description of Symptoms and Microscopic Observation of Signs

Symptoms of the diseased plants were described. To observe the signs, thin sections and scrapings from infected fresh materials were mounted into a drop of water on a glass slide then covered with cover slip and were studied under the binocular compound microscope. All observations were recorded. Books, journals and other reference materials on fungi, bacteria and nematodes were used in characterizing the symptoms manifested by the mondo grass leaves and the signs observed.

C. Identification of the Isolated Fungus.

Isolation. The specimens were washed with running tap water to get rid of dirt, were cut into small sections about 2-5 sq. cm. then each piece of cut tissues were disinfected with 10% sodium hypochlorite for 1-2 minutes. After disinfection, the cut sections were rinsed with three changes of sterile distilled water and were then blot-dried on a sterile tissue paper. Specimens were

aseptically placed equidistantly on plated potato dextrose agar (PDA) then were incubated for 3-5 days or more at 28-30°C. The culture medium was prepared following the standard procedures. Pure culture of the fungal growth were re-isolated in PDA slants, stored at 5°C which served as stock culture.

Identification. The cultural characteristics such as color and presence of pigmentation were noted and recorded. The microorganisms were examined under the electric binocular microscope taking note of their morphological characteristics such as kind of mycelia produced, spore morphology and other structures.

D. Identification of Isolated Bacterium

Isolation. The collected infected leaves were washed with tap water to get rid of the surface dirt, disinfected with 10% sodium hypochlorite and were finally rinsed with three changes of sterile distilled water. Leaf samples were placed in a water blank and were macerated with a flamed glass rod to hasten the oozing of bacterial cells. A loopful of the resulting solution was streaked onto previously prepared nutrient agar (NA) which was prepared following the standard procedures. The plates were incubated at 28-30°C for 24-72 hours. To obtain a pure culture, a single colony of the bacterium was reisolated separately into another plated NA. Stock cultures were kept on agar slants at 5°C.

Identification. To determine the genus of the isolated bacterium, the following tests were done:

1. Growth on various media
    a. Yeast Extract Dextrose Agar (YDCA)
    b. King's Medium B Agar (KMBA)
    c. Nutrient Glucose Agar (NGA)
    d. Sucrose Peptone Agar (SPA)
2. Gram stain and potassium hydroxide (KOH) test

E. Identification of the Foliar Nematode

Isolation. Plant tissues were cut into longitudinal sections and were placed in a glass Petri dish containing distilled water. The set/up was allowed to stand for minutes to allow the nematodes to migrate and be released from the damaged tissues. With the aid of a dissecting microscope, the nematodes were then collected using an improvised hand picker (stick broom).

Identification. The mouth, stylet, tail and other distinguishing structures of the isolated nematode were examined under the binocular electric microscope. Identification was done with the aid of books, journals and other related available references.

Staining of plant tissues. In order to observe the nematodes inside the leaves, plant tissues were stained (modified technique) by cutting the leaves into small sections about 2-3 sq. mm. then were soaked in a carnoy's solution for a few days until the chlorophyll pigmentation was removed. When the leaves became clear, acid fuchsin (1 ml stock solution and 30 ml water) was added and

was left for at least a day to be enable the stain to be absorbed by the nematode. After staining, the leaves were rinsed in running tap water to remove excess stain and were soaked and covered with pure glycerin.

Data Gathered

The following data were gathered:

1. Symptoms of the disease complex. These refer to the manifested abnormalities on the diseased leaves of mondo grass.
2. Signs of pathogens involved in the disease complex. These refer to the specific structures of the fungus, bacterium and nematode that were freshly scraped or cut into thin sections from the diseased tissues and observed under the binocular compound microscope.
3. Cultural and morphological characteristics of the isolates. These refer to the color/pigmentation in PDA of the fungal isolate and gram stain, KOH reaction and colony appearance in NA and other selected differential media of the bacterial isolate.
4. Identification of pathogens involved in the disease complex. This was limited to the genus level for the fungal and bacterial components of the disease complex and species level for the nematode component.
5. Photodocumentation

RESULTS AND DISCUSSION

Fungal Component of the Disease Complex

Disease Symptoms and Signs

Sample leaves exhibited circular to oval dark round spots on the leaves surrounded by a definite yellow margin (Plate 1a). Other leaves showed blights starting from the tip of the leaf moving downward (Plate 1b) . Other symptoms observed showed association with other bacterial and nematode symptoms like yellowing and necrotic spots.

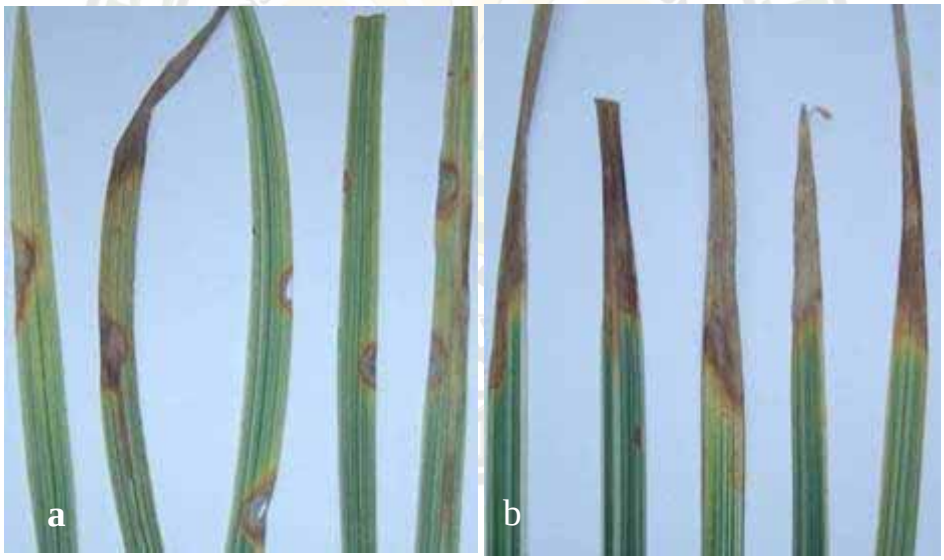

Plate 1. Symptoms manifested by the suspected fungal component of the disease complex appearing as leaf spot (a) and leaf blight (b)

Plate 2. Signs observed under the compound microscope appearing as septated boat-shaped numerous macroconidia with microconidia

The microscopic examination of slide mounts prepared from thin sections showed boat- or banana-shaped macroconidia with 4 to 6 septations. Numerous microconidia were noted which imply the pathogenicity of the isolate (Plate 2).

Cultural Characteristics

The isolated fungus produces dense and compact light pink mycelia on PDA. A pink-orange pigment is produced at the bottom of the plate but does not diffuse throughout the medium (Plate 3).

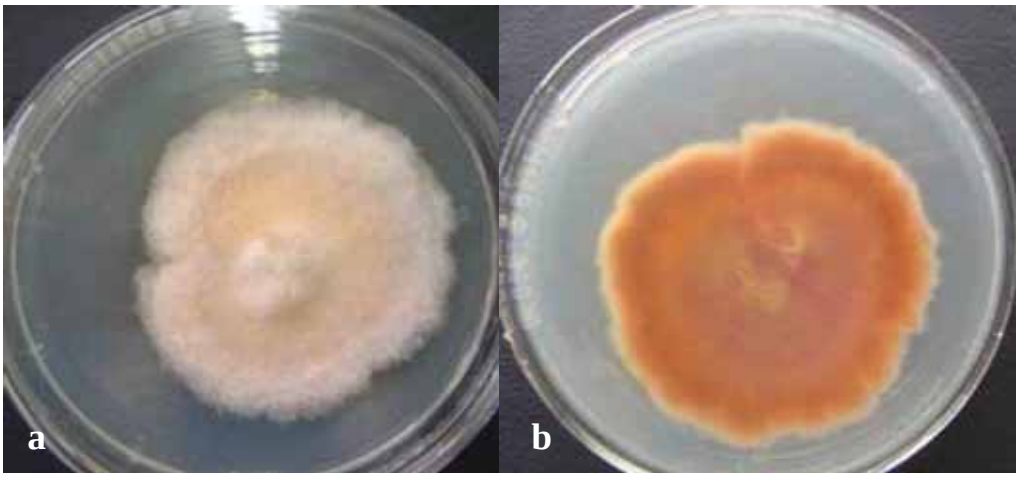

Plate 3. Pure culture of *Fusarium* sp. with light pink mycelia at the top (a) and pink-orange pigmentation at the bottom of the plate(b)

Morphological characteristics

Microscopic examination revealed slightly curved, banana-shaped macroconidia with 4 to 6 septations. The septated mycelia are thick-walled. There were numerous short and stout microconidia observed (Plate 4).

It should be noted that the type of macroconidia and microconidia observed from the pure culture in PDA are exactly similar to those observed from the fresh thin sections from diseased mondo grass leaves.

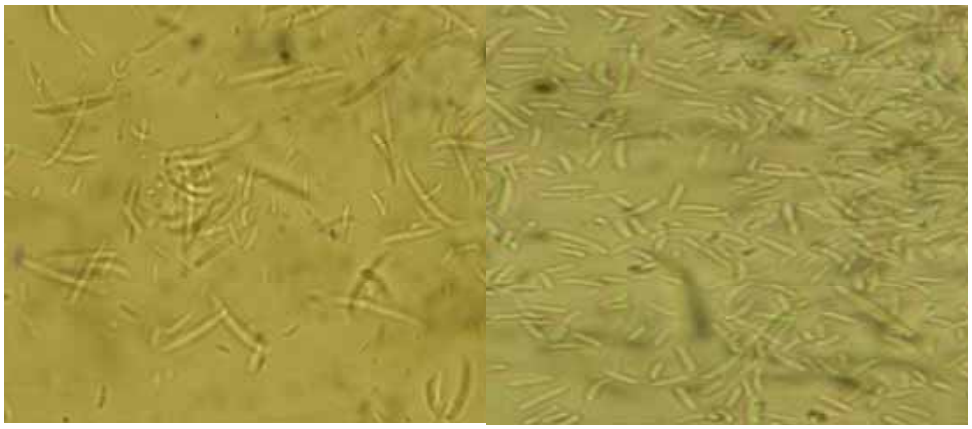

Plate 4. Macroconidia and microconidia from the PDA culture of the fungus

Bacterial Component of the Disease Complex

Disease Symptoms and Signs

Infected leaves manifested different kinds of symptoms appearing as leaf spot and blight. Some symptoms have small dark brown lesions which start from the leaf margin and moving longitudinally along the margin. Other symptoms appear as spots of various sizes on the edge of the leaves then continue to advance towards the healthy portion resulting in blights. Some symptoms first appeared at the tip of the leaves then moving downward (Plate 5a). During the rainy season, the symptoms had a yellowish and water-soaked appearance (Plate 5b).

Plate 5. Symptoms manifested by the suspected bacterial component of the disease complex appearing as small dark brown lesions (a) and yellowish water-soaked appearance (b)

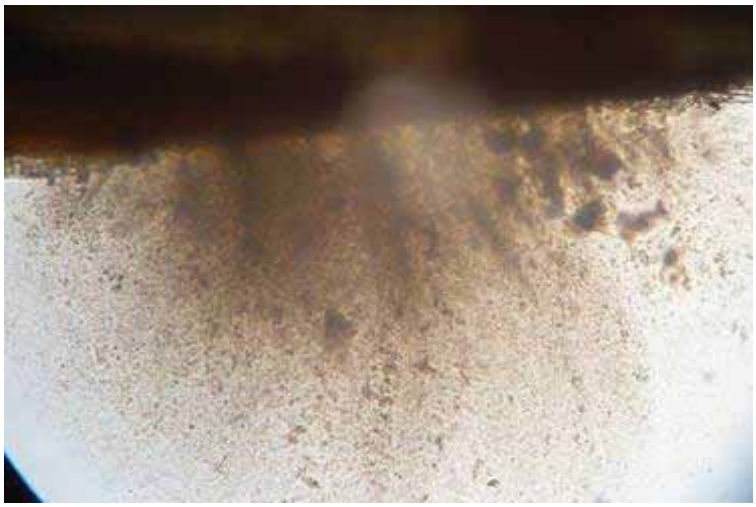

Plate 6. Bacterial streaming from cut tissues

The microscopic observation of the thin sections prepared from the diseased leaves showed massive bacterial streaming especially those taken from the lesions with dark brown discoloration (Plate 6). Bacterial streaming is one property that is synonymous to phytopathogenic bacteria which are motile due to the presence of flagella. Motility can be readily observed especially if the material is fresh (Goszczynska, et. al, 2000).

Cultural characteristics

In NA, 72-hour old cultures of the suspected bacterium had yellow colonies (Plate 7). The yellow color looked like an egg white with egg yolk at the middle when seen through light which is a typical indication that the organism involved belongs to the genus *Xanthomonas* (Lando, personal communication).

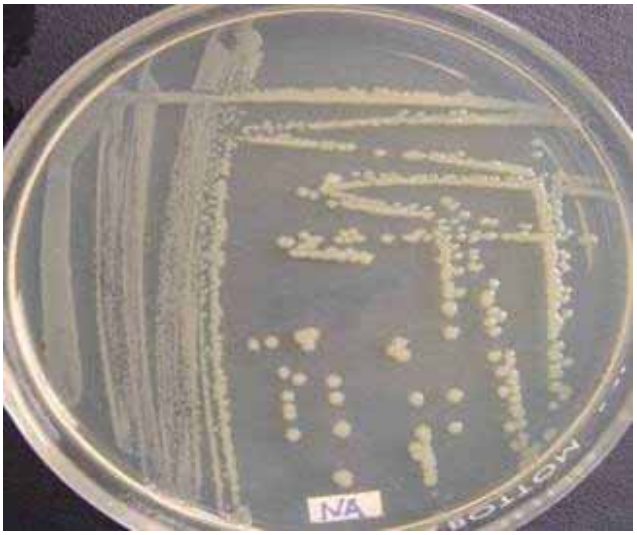

Plate 7. Bacterial isolate in NA 72 hours after isolation

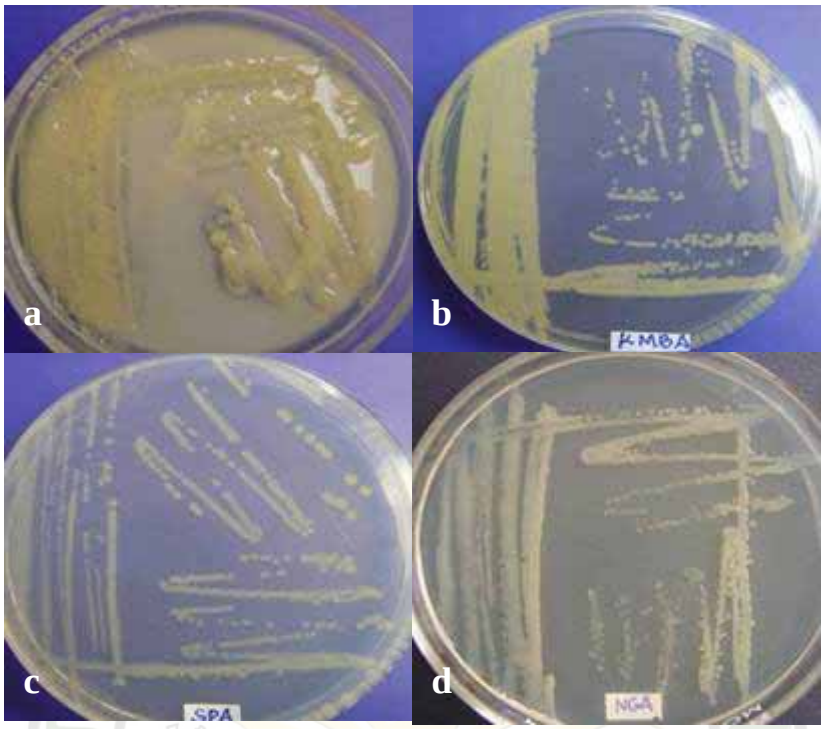

Plate 8. Bacterial isolate in differential media appearing as dark yellow in YDCA (a) and light yellow in KMBA (b) SPA (c) and NGA (d)

Confirmatory identification in differential media revealed dark yellow colonies on YDCA and light yellow color on the other media (Plate 8). These characteristics confirm the description of Schaad and Stall (1998). The colonies are mucoid, convex and shiny. The yellow membrane-bound pigments are brominated arylpolyne esters (xanthomonadins).

Gram Stain Reaction

Under the oil immersion objective (OIO), the stained bacteria are rod-shaped and appear pink in color which indicates that these absorb the color of the counterstain (safranin) thereby are classified as Gram-negative (Plate 9a.) Most phytopathogenic bacteria are Gram-negative and rod-shaped (Goszczynska, et. al, 2000). Bacteria that cause leaf spot or blight are often Gram-negative and may belong to *Pseudomonas* or *Xanthomonas* (Agrios, 2005).

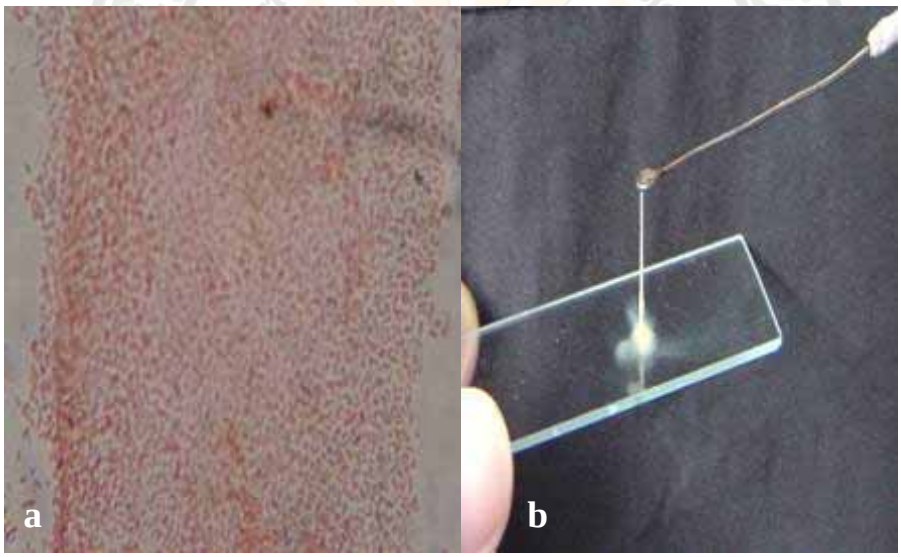

Plate 9. Rod-shaped Gram-negative bacterial cells from smear prepared from the NA culture (a) and mucoid thread produced from the KOH test done on slide suspension of the suspected bacterial isolate (b)

Potassium Hydroxide Solubility (KOH) Test

The preliminary diagnostic identification of a bacterium using potassium hydroxide (KOH - 3% aq., w/v) test showed a mucoid thread produced indicating that the bacterium is Gram-negative (Plate 9b).

Nematode Component of the Disease Complex

Disease Symptoms and Signs

The above-ground symptoms observed vary especially on the leaves. These appear as necrotic lesions then advance towards the healthy leaves where lesions coalesce. The affected tissues turn brown and sometimes discolored. Just above the ground, the leaves appear yellowish turning brown with time causing an early senescence of the leaves (Fig 10). Other symptoms manifested are leaf blotching and yellowing while others are similar to the symptoms manifested by the fungus and bacterium.

These observations indicate the presence of the three pathogens in the disease complex. The sample plants collected showed that most often, these organisms are all present at a time when examined under the microscope (Fig 11). Frequently, nematodes facilitate the entry and establishment of other plant pathogens. They may either have an "interrelationship" and "interaction" with bacteria, fungi and viruses (Lopez et. al., 2004).

In 1892, interactions of nematode with other plant pathogens had already been recorded. All plant pathogenic nematodes cause wound in plants by puncturing, rupturing or by separating cells thereby introducing or aiding the entry of other pathogens already present on the plant surface. Chen et. al. (2004)

Plate 10. Symptoms manifested by suspected nematode component of the disease complex appearing as yellow to brown discoloration starting at the base of the leaves.

cited some reports on nematode-fungi interactions like: nematode with *Fusarium oxysporum* and other *Fusarium* spp., *Verticillium* spp., *Pythium* spp., and *Cylindrocarpon* spp. Bacteria-nematode interactions are also known to occur: like nematode with *Clavibacter* spp., *Pseudomonas* spp. and *Agrobacterium* spp. For viruses, the nematode vector belongs to the genera *Xiphinema*, *Trichodorus* and *Longidorus*.

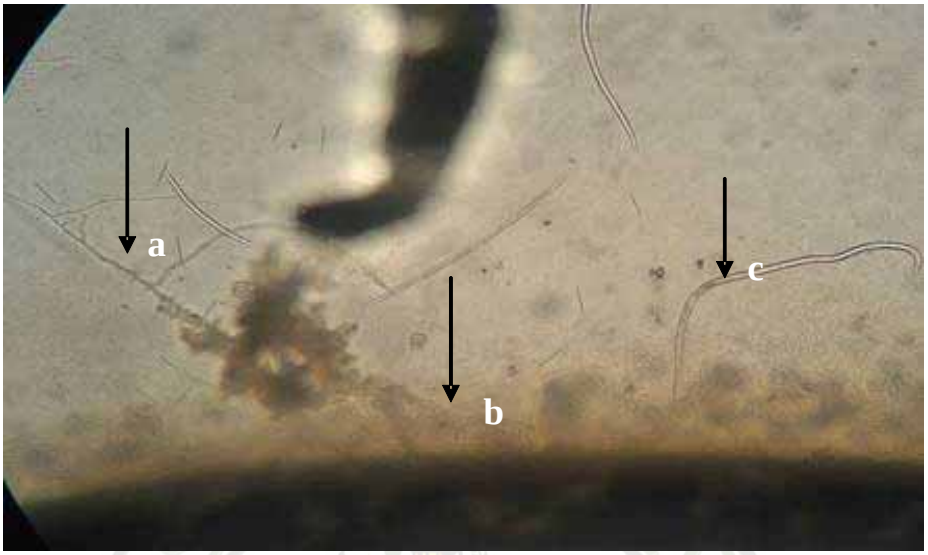

Plate 11. Disease complex in the mondo grass as indicated by the presence of hypha (a) bacterial streaming (b) and nematode (c)

Morphological Characteristics

The identifications were based mainly on the basic morphological characteristics with the aid of illustrations and descriptions from reference materials such as books and journals.

Morphological characteristics showed that the nematode are slender especially the female. They are straight when relaxed. The basal knobs are distinct. Vulva transverse and the lips are slightly protruding. The tail is elongate-conoid ending in a simple blunt-spike. There is no sexual dimorphism between the male and female. They are both essentially similar. The male tail is

Plate 12. Nematodes seen under the binocular microscope: adult (a) female tail (b) male tail (c)

arcuate when relaxed and has a simple blunt terminal spike. It has rosethorn-shaped spicules (Plate 12).

Staining Reaction

Further identification through staining of plant tissues to make nematodes showed different growth stages of the nematode such as stages such as the eggs, juveniles, and adults within the tissues clearer under the microscope (Plate 13). These were stained pink thus easily visualized under the microscope.

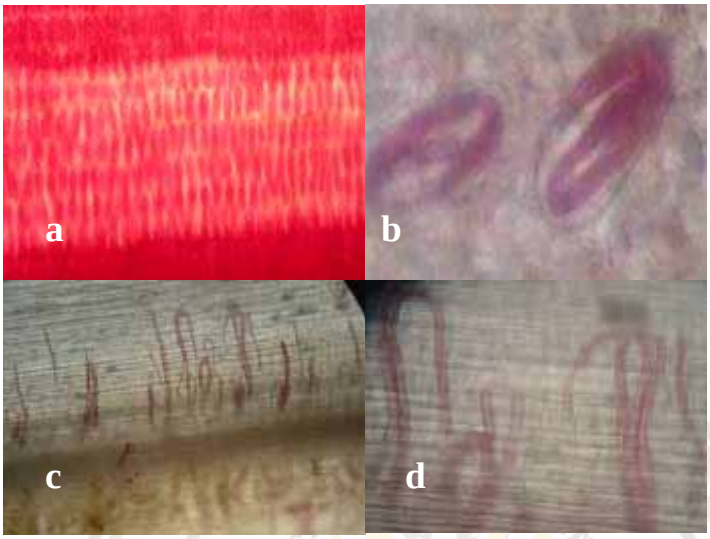

Plate 13. Stained nematodes inside the leaf tissue eggs (a and b) and adults (c and d)

Based on these initial diagnosis and identification, the nematode belongs to the Family Aphelenchoididae specifically the genus *Aphelenchoides*, a foliar nematode of important crops. Although majority of these species are free-living and fungal feeders, a number of species have evolved to animal parasitism, particularly on insects, and to plant parasitism. The nematodes *A. besseyi*, A. *fragariae* and *A. ritzemabozi* are important pests of a number of crops worldwide such as strawberry, chrysanthemum and rice. Typically, they feed on aerial parts such as buds, leaves, flowers and fruits causing serious damages to their hosts (Ferraz and Brown, 2002).

Of all these important pests, the nematodes isolated and identified based on their morphology coincide with the description of *A. fragariae*. In strawberry, they cause the "strawberry crimp" disease. As reported by Hunt (1999), it is also economically important on ornamental ferns, attacking 100 species which has an extensive host range mainly belonging to the Liliaceae, Primulaceae, Pteridophyta and Ranunculaceae. *A. fragariae* is an ecto- and endoparasite of the above-ground plant parts.

The female lays its eggs in the intercellular spaces of the leaves. The eggs hatch and produce the four juvenile stage, and the adults all inside the leaf (Agrios, 2005). They enter into the leaf via the stomata in damp conditions (Klinger, 1970), although the epidermis can also be directly penetrated (Strümpel, 1967). The life cycle is completed in about 10-18 days at 18°C. They can survive as adults in dead leaves or between scales of buds of infected tissues

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The study was conducted to characterize the pathogens associated with the leaf disease complex of mondo grass. Sample leaves were taken from selected sites in La Trinidad, Benguet and Baguio City.

The study was done by describing the symptoms manifested in diseased leaves and signs gathered from the diseased fresh leaf specimens; isolating the suspected pathogen into pure culture and describing them morphologically and culturally in addition to microscopic observation and other confirmatory tests.

Results revealed that three organisms were associated with the mondo grass disease complex. These are the fungus *Fusarium* sp., the bacterium *Xanthomonas* sp. and the nematode *Aphelenchoides fragariae*. The symptoms manifested by the leaves are either leaf spots, blights, yellowing or necrotic lesions. The pathogens appear either singly or in complex. These may be present singly or all at the same time in one symptom. However, these three pathogens are most often observed to be present in same lesion/spot/blight.

The *Fusarium* produces light pink mycelia with conidia with 4-6 septations which are similar to the conidia taken from the fresh infected leaves. It likewise produces several microconidia.

The bacterium *Xanthomonas* is Gram negative. The growth on the general and selected differential media is yellow. All species belonging to

Xanthomonas is reported to be plant pathogens and are found only in association with plants or plant materials. The Gram reaction, shape of the cells and the KOH reaction indicate that the isolate is plant pathogenic.

The nematode *Aphelenchoides* is generally slender with distinct basal knobs. Both male and female are similar having no sexual dimorphism. The tail is elongate-conoid ending in blunt terminal spike. The nematode *Aphelenchoides fragariae* is an important pest to economic crops such as the strawberry and other ornamentals. Although the nematodes can cause diseases to plants alone, since most of them are present in the soil, a disease interaction among other pathogens living in the habitat is likely to occur.

Conclusions

From the results of the study, it is therefore concluded that there is an interaction among the three pathogens resulting in a disease complex in mondo grass.

The fungus *Fusarium* sp., the bacterium *Xanthomonas* sp. and the nematode *Aphelenchoides fragariae* are pathogenic and these can pose a threat to important crops. These pathogens can serve as a ready source of inoculum to crops. In strawberry, the *Aphelenchoides fragariae* is a very important pathogen causing short, bushy-looking plants with small distorted or wrinkled leaves. Severe infection affects fruit buds leading to significant yield loss reduction

Recommendations

With these findings, the following are recommended:

1. The mondo grass should not be used as ornamental crop in the strawberry plantation areas.
2. The fungus *Fusarium* sp. should be identified further to the species level. Its pathogenicity to other crops should be evaluated.
3. The bacterium *Xanthomonas* spp. should likewise be identified further to the species level. Its pathogenicity to other crops should likewise be evaluated.
4. Further survey of the distribution of the mondo grass in other places should be done to assess the presence of these pathogens.
5. Uprooting or removal of mondo grass within the BSU campus is suggested.
6. Pathogenicity test should be appropriately done especially on the strawberry crop using the nematode *Aphelenchoides fragariae* for further confirmation of its host range.
7. More studies must be done to determine which pathogen occurs first during the interaction in relation to disease complex.

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APPENDICES

APPENDIX TABLE 1. Composition and preparation of media

A. Nutrient Agar (NA)

Component	Amount (g/li)
Beef extract	3 g
Peptone	5 g
Agar	15 g

B. King's Medium B Agar (KMBA)

Component	Amount (g/li)
$MgSO_4$ (anhydrous)	1.5 g
K_2HPO_4	1.5 g
Proteose peptone	10 g
Agar	15 g
Glycerol	15 ml

The media was dispensed in individual 250 ml Erlenmeyer flask and sterilized in an autoclave for 30 minutes at 15 pounds per square inch (psi).

C. Nutrient Glucose Agar (NGA)

Component	Amount (g/li)
Glucose	2.5 g
Agar	20 g

D. Sucrose Peptone Agar

Component	Amount (g/li)
Sucrose	20 g
K_2HPO_4	.5 g
$MgSO_4.7H_2O$	.25 g
Agar	15 g

Adjust to pH 7.2

E. Yeast Extract-Dextrose-$CaCO_3$ Agar (YDCA)

Component	Amount (g/li)
Yeast	10 g
Dextrose	20 g
Calcium carbonate, USP light powder	20 g
Agar	15 g

All the components was dissolved in 1000 ml of distilled water except for dextrose which was prepared separately into the flask with 100 ml distilled water. The media was sterilized at 15 psi for 20 minutes. After sterilization, the dextrose was mixed in a separate flask with the yeast, $CaCO_3$ and agar. It was mixed thoroughly by swirling the flask before pouring into the Petri plates. Observation was done after 24 to 72 hours.

F. Potato Dextrose Agar

Component	Amount (g/li)
Potato	250 g
Dextrose	15 g
Agar	15 g

APPENDIX TABLE 2. Composition and preparation of reagents

A. Hucker's ammonium oxalate crystal violet

Solution A

Crystal violet (90% dye content)	2.0	g
Ethyl alcohol (95%)	20.0	ml

Solution B

Ammonium oxalate	0.8	g
Distilled water	80.0	ml

Mix solutions A and B. Filter through paper into storage bottle. Store for 24 hours before use.

B. Gram's modification of Lugol's solution

Iodine	5.0	g
Potassium iodide	10.0	g
Distilled water	100	ml

Mix potassium iodide with about 30 ml distilled water and dissolve. Add iodine, bring to volume to 100 ml and dissolve the iodine several hours or overnight in a dark place.

C. Decolorizing

1. Ethyl alcohol, 95%
2. Acetone: fastest agent

3. Acetone-alcohol: intermediate (95% ethyl alcohol, 100 ml; acetone, 100 ml)

With practice, any of the three decolorizing agents will yield good results.

D. Counterstaining

Stock solution

Safranin O	2.5	g
Ethyl alcohol, 95%	100.0	ml

Working solution

Stock solution	10.0	ml
Distilled water	90.0	ml

Staining procedure:

1. Make a smear by mixing a small amount of growth with a drop of distilled water on a clean glass slide. Do not make thick smears for they may not be decolorized as rapidly as the procedure requires.
2. Air dry and fix by passing slide filmside up quickly over the flame. The slide should be hot but not burn the fingers. Do not scorch since the cell wall may rupture causing Gram-positive organisms to accept the counterstain.
3. Stain with ammonium oxalate crystal violet for 1 minute. Rinse thoroughly but gently in tap water.

4. Cover smear with Gram iodine for 1 minute. Wash with tap water. Shake off excess moisture.
5. Decolorize by dripping 95% ethyl alcohol over tilted slide for 10-15 seconds. Wash immediately with water.
6. Counterstain with safranin for 45 seconds. Wash with water.
7. Blot dry and examine under oil immersion objective.

   Results: Gram-positive bacteria, purple to blue-black; Gram-negative bacteria, red.

E. Gregersen's method of determining gram stain reaction

1. Put 1-2 drops of 3% KOH on a glass slide.
2. Pick up a colony with a sterile loop and stir into KOH. After 5-10 seconds of stirring, raise the loop from the drop.
3. If KOH solution has become viscous and thread of slime follows the loop, then organism is Gram-negative.
4. If there is no slime and a watery suspension remains, then organism is Gram-positive.

F. Selective Staining Method for Nematode (Modified Technique by Byrd et. al, 1983)

Carnoy's solution

Component	Amount
Absolute alcohol	60 ml
Chloroform	30 ml
Glacial acetic acid	10 ml

Staining solution (Stock solution)

Component	Amount
Acid fuchsin	3.5 g
Acetic acid	250 ml
Distilled water	750 ml
Pure glycerin (for preservation)	

1. Wash all debris from the leaves, cut in 2 cm segments.
2. Place cut leaves in a beaker and cover with carnoy's solution.
3. Leave in carnoy's solution for a few days until the leaves are free from chlorophyll.
5. When the leaves are free from chlorophyll, pour contents of beaker onto sieve or mesh cloth and rinse thoroughly with running tap water.
6. Put leaves in 30 ml distilled water, add 1 ml stock solution for at least a day for the stain to be absorbed by the nematodes. After staining, rinse with distilled water and cover with pure glycerine.