BIBLIOGRAPHY

BASALONG, JOVYLLE L. APRIL 2011. Etiology of Stem Rot of Chayote (*Sechium edule*). Benguet State University, La Trinidad Benguet.

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ABSTRACT

The fungus causing stem rot was isolated from infected chayote stem and was able to produce similar symptoms on artificially inoculated chayote stems in about four days.

The pathogen is *F.oxysporum* based on symptom and microscopic properties and is supported by pathogenicity tests. A pure culture of the fungus produces fast growing and white to orange color on the media. The fungus has a conidiospore in a sporodochium with an aggregate and branching formation. Moreover the conidia of the fungus are phialides, slightly curved, boat-shaped which produces three to five cells and is produced in large number, often in clumps known as sporodochia.

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INTRODUCTION

Chayote (*Sechium edule*) is a trailing vine-bearing edible fleshy vegetable. It is a member of the gourd family Cucurbitaceae which may grow to 100 feet (30 m) in length and each vine may produce up to several hundred fruits (Encyclopedia Americana, 1829).

Chayote is commonly grown in Atok, Kapangan, Kibungan, La Trinidad and Tublay of Benguet and is a major source of income for farmers. Chayote is also the only sustainable vegetable in the market because of its availability throughout the year. Hence, earning the description "green gold" (Fialen, 2004).

Chayote has many uses. The roots, leaves and young shoots are edible for human and livestock consumption. The fruits can be prepared as pies, pudding, and salads. It is also a source of food, energy, protein, fats, carbohydrates, vitamins and minerals (Elstrom, 1990). Chayote fruits and shoots are being sold in the price as low as 50 centavos when there is oversupply and may go as high as 15 to 20 pesos per kilogram (Velasco, 1994). Chayote has other important uses aside from food purposes. It could be used as wind breaker in small gullies and slopes destroyed by runoff water. It also protects the soil from direct rain and helps prevent massive leaching and soil erosion.

In any plant production venture, the occurrence of diseases becomes a limiting factor. The prevalence of diseases becomes one of the producers' main problems since this leads to losses in yield quality and quantity, and eventually loss in income. In order to boost the production of chayote to supply local market demands, its appropriate management measures can only be formulated after it has been properly understood. However, suitable management techniques could only be devised from an understanding of its etiology.

Further tests are then necessary to ascertain the identity of the causal organism causing stem rot of chayote so that appropriate disease management measures can be instituted.

This study was conceived and conducted to:

1. Describe the symptoms of the disease;

2. Identify the pathogen causing the stem rot of chayote; and

3. Conduct pathogenicity test using PDA culture as inoculums to a healthy chayote plant grown from healthy fruit.

The study was conducted from August 2010 to March 2011. Samples were collected from Mocgo and Puspusok of Sagubo, Kapangan, Benguet and were processed at the Department of Plant Pathology, BSU, La Trinidad, Benguet.

REVIEW OF LITERATURE

Plant Diseases and Their Causes

Plant diseases are deviations from the normal growth and development of plants incited by microorganisms, parasitic flowering plants, nematodes, viruses, or adverse environmental conditions (Stevenson, 2002). These are of paramount importance to humans because these damage plants and plant products on which humans depend for food, clothing, furniture, the environment, and housing (Agrios, 1997).

The four major consequences of plant diseases are: These may limit the kinds of plants and industries in an area, these reduce the quality and quantity of plant produce, these make plants poisonous to humans and animals, and these may cause financial losses.

Plant diseases are considered one of the many natural hazards in crop production. These undoubtedly arose and developed on earth. These are named according to the organisms which cause the diseases. On the other hand, these may also be named according to the appearance of the plant after supporting a particular disease organism.

The Host

Chayote (*Sechium edule*) is a perennial tropical vine. It grows in tropical climate and bears fruit over a period of several months.

Chayote grows best in rich, well drained, sandy loam soils Purseglove (1968) as cited by Payangdo (2008). It is also adapted to an altitude of 800 - 2,200 meters above sea level with temperature ranging from 16 - 25^{o}C. It is a warm season crop and a good bloom is enhanced by short day. Likewise, chayote could be grown in temperate climates by artificially controlling daylength (Aung and Kushad, 1990). After 6 to 8 weeks of growth, the vine can be shaded with dark cloth on a frame to keep sunlight to 8 hours each day for the next 4-6 weeks. The frame could be moved to shade the vines at about 4:00 P.M. and removed after sunrise at about 8:00 A.M. the following day. After flowers develop, the vines can grow under normal daylength. Moreover, observations revealed that chayote production from sunny side areas yield more fruits per vine than those grown in leeside areas. This lower yield noted from the leeside areas was considered as the effect of the lower temperature and less hours of sunlight intensity in the area. Pursegrove (1986) as cited by Payangdo (2008) claimed that chayote is a warm season crop that if grown in less favorable condition, it will have luxuriant vine growth but reduced fruit production.

Chayote plants are sensitive to wind damage during strong typhoons. Therefore, production should be located in areas that are not frequented by strong winds especially during typhoon months. The best place for chayote production is in between 800 and 1,800 meters above sea level with a temperature range of 16 - 25^{o}C.

Varieties of chayote that existed in some growing areas in the Philippines since the nineteenth century are: elongated fruit with deep to slight grooves, non-varieties; oblong with deep to slight grooves, non-spiny to spiny varieties; Monticello white-oval shaped and smooth peeled from Puerto Rico and Australian green-oblong with light groove from Australia.

<u>Fungal Diseases</u>

The chayote's principal problems in the sub-tropical climate are air-borne and soil-borne fungi, pathogens like powdery mildew, downy mildew, and anthracnose. Signs of fungal diseases vary but normally the leaves will develop yellow spotches and eventually the whole leaf will yellow. The infection is most likely in the spring after planting. The good news is that fungi do not like direct sunlight, extreme heat, and clean air so infected chayote tend to lose some stems during the summer (preceded by yellowing of leaves) but win the battle with plant disease in the heat of August and recuperate for the fall harvest (Rajnauth, 1996).

Fungi and Fungal Diseases

Fungi are organisms that have no true roots, leaves, stems or chlorophyll. Instead, these have hyphae (microscopic threads) of various types which can grow in the soil or in a host plant. These threads absorb food from the plant or organic material in the soil. Fungi cannot make certain necessary food materials so these must live on food products manufactured by other organisms. Fungi reproduce by various methods. These produce different types of spores. Some spores are spread by air currents and others by contact or rain splash. Fungi that cause leaf spots, downy mildew and gummy stem blight produce hundreds and even thousands of these spores in one spot on a leaf or stem.

Many of the fungi causing seedling blights, root rots, wilts and certain fruit rots do not produce “heavy duty” spores that are able to survive in the soil for many years. *Fusarium* spp. produce chalmydiospores and *Pythium* spp. produce oospores. This situation plus the lack of convenient and economical fungicides for use in the soil make it difficult to control this group of soil-borne fungi. Other fungi produce visible resting structures called sclerotia. Southern stem rot, caused by *Sclerotium rolsfsii*, produces mustard seed-sized sclerotia.

Fusarium which is suspected to cause stem rot of chayote have been characterized as causing the following symptoms: vascular wilt, yellows, corm rot, root rot, and damping-off. The most important of this is vascular wilt. Of the vascular wilt-causing fusaria, *Fusarium oxysporum* is the most important species (Agrios, 1988; Smith et al., 1988). In general, fusarium wilts first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasrty (downward drooping) of the older leaves. At the seedling stage, plants infected by *F.oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1988). Browning of the vascular tissue is strong evidence of fusarium wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Jones *et al*., 1982; Smith *et al*., 1988).

F.oxysporum has no known sexual reproductive stage. Asexual reproductive structures include both microconidia and macroconidia. Microconidia are abundant, oval to kidney-shaped, generally one-celled and are formed on short conidiophores. Microconidia are capable of infecting roots but probably play little role in initial infection in the field because of their ephemeral nature. Macroconidia are fusiform (“canoe-shaped”), typically having three to five cells and are produced in large numbers, often in clumps known as sporodochia. Macroconidia also may infect roots but their primary role maybe survival as these have the ability to form chlamydospores (sexual resting structures). Chlamydospores are the primary means of survival and typically form under conditions of sub-optimal growth for the fungus or death of the plant. *Fusarium oxysporum* forms two types of chlamydospores: one within the macroconidium and one form within the mycelium. Those formed from mycelia tend to occur singly or in pairs and may be either intercalary (within the mycelium) or terminal (occurring at the ends). Disease results from infection of the root or hypocotyls by hyphae resulting from either the macroconidia or chlamydospores once infection of the root or hypocotyls by hyphae results from either the macroconidia or chlamydospores. Once infection has occurred the fungus penetrates and grows within the xylem. Microconidia are produced within the vessel, dislodge, and are carried up by the xylem with the transpiration stream, sporadically germinating and producing more mycelia and microconidia. Wilting occurs as a result of the formation of tyloses within the xylem by the host. Tyloses are 'balloon-like' invaginations of the xylem parenchyma cells that block the vessel. Tyloses serve to slow the spread of the pathogen through the plant but also restrict water flow, resulting in wilting. In addition, the pathogen causes a general breakdown of the xylem parenchyma cells resulting in various types of gums that add to the blockage. The extent to which fungal toxins are involved in pathogenesis is not known. Once infection has occurred, FON is limited to the xylem and generally emerges only after the death of the plant (Agrios, 1988).

MATERIALS AND METHODS

Collection of Diseased Materials

Suspected diseased chayote stems were collected from Mocgo and Puspusok of Sagubo,Kapangan, Benguet. Symptoms of the disease were noted. The samples were placed individually in transparent plastic bags, labeled and brought for further diagnosis at the Department of Plant Pathology Laboratory.

Microscopic Examination of Samples

Thin tissue sections were cut from the advancing portion of the lesions and studied under the microscope. All observations were recorded.

Isolation and Identification of Fungal Isolate

In the laboratory, thin tissue sections were cut from the advancing portion of the collected samples showing stem rot symptom. These were isolated in potato dextrose agar (PDA) plates following standard procedures.

To obtain pure culture, the organism was re-isolated in PDA plates. Specimen was prepared from the pure culture for microscopic examination. It was compared to the specimen prepared from the natural stem showing the typical symptoms to assure that both are one and the same.

Pathogenicity Test

Inoculation technique. The suspected *Fusarium* sp. was isolated and tested for pathogenicity by inoculation into healthy chayote plants. Two inoculation techniques were done to compare their effectiveness with an inocolum concentration of 10^7 spores. Eight chayote plants were used in the experiment: 4 plants were assigned for each inoculation technique. The inoculated plants were laid in open field and symptoms developed were observed daily thereafter.

1. Pricking and spraying. Stems were pricked with a sterilized needle before spraying with fungal suspension prepared from the diseased stems. This method closely resembles inoculation under natural conditions.

2. Drenching. The soil covering the roots of germinated healthy chayote was sliced by a sterile knife to assure injury of the roots as avenue for entry of the inocula. The prepared fungal suspension was drenched to the roots.

Re-isolation of the pathogen. Re-isolation of the pathogen from inoculated test plants was done using standard procedures. Characteristics of the isolates obtained were then compared to those of the inoculums used.

Data Gathered

1. Symptoms of the disease. This refers to visual observation of abnormalities manifested by the infected chayote plant and plant parts. The symptoms seen on the stem were described, examined and compared to symptom description in available literature.

2. PDA culture identification of isolates. This refers to isolate identification based on characteristics observed after a week of incubation: colony diameter (mm), growth, and type and color of mycelia.

3. Microscopic characteristics of isolates. The isolates were characterized as to microscopic appearance of the hyphae and conidia.

4. Pathogenicity test results. These include the effectiveness of the inoculation method to produce disease and the compliance to the requirement of the disease produced by artificial inoculation to yield the same isolate as the original inoculum.

RESULTS AND DISCUSSION

Description of Collection Sites

Chayote stem rots were collected from Puspusok (Figure 1) and Mogco (Figure 2) of Sagubo, Kapangan, Benguet. An ocular survey of the sites showed widespread distribution of plants manifesting the symptoms of the disease. Disease incidence is estimated at about 40%.

Figure 1. Overview of chayote farm at Puspusok, Kapangan, Benguet where symptoms of *Fusarium* wilt was assessed at about 40%

Figure 2. Overview of chayote farm at Mocgo, Kapangan, Benguet where symptoms of *Fusarium* wilt was likewise assessed at about 40%

Disease Symptoms

Infection starts with light discoloration at the stem accompanied by wilting of the plant, which at first may recover during the evening, but plant eventually wilts permanently. Dull, gray green appearance of upper leaves precedes a loss of turgor pressure, and wilting and yellowing of the upper leaves. With age, the lesion turns brown then black (Figure 3). The younger stems are more susceptible than the mature stems.

Fusarium wilts first appear as slight vein - clearing on the outer portion of younger leaves, followed by (downward drooping) of older leaves. At the seedling stage,

Figure 3. Symptoms of naturally-infected chayote stem collected from sampling site

plants infected by *F. oxysporum* may wilt and die soon after symptoms appear. In older plants, vein - clearing and leaf epinasty are often followed by stunting, yellowing of lower leaves, and finally death of the entire plant (Agrios, 1988). Browning of the vascular tissue is a strong evidence of *Fusarium* wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Jones *et al*., 1982 and Smith *et al*., 1988).

Pathogen Identification

Growth in PDA. The fungus isolated in PDA plate produced fast-growing mycelia with a diameter of 9mm after a week of incubation at 23-24^{0}C, seen as cottony growth (Figure 4). Generally, the underside was white to orange. These characteristics coincide with the description of the fusaria including *Fusarium oxyporum* (Smith *et al.*, 1988). If sporodochia are abundant, the culture may appear cream or orange in color (Smith, 1988).

Figure 4. PDA culture of *F.oxysporum* taken in the seventh day of incubation at 23-24^{0}C

Microscopic Characterization

The fungus isolate conforms to *F.oxysporum* having conidiosphores in a sporodochium that are branching (Figure 5); conidiogenous cells; phialides with collarettes; and phialospores either macrospores or macroconidia which are usually slightly curved or boat-shaped which produce three to five cells (Agrios, 1988).

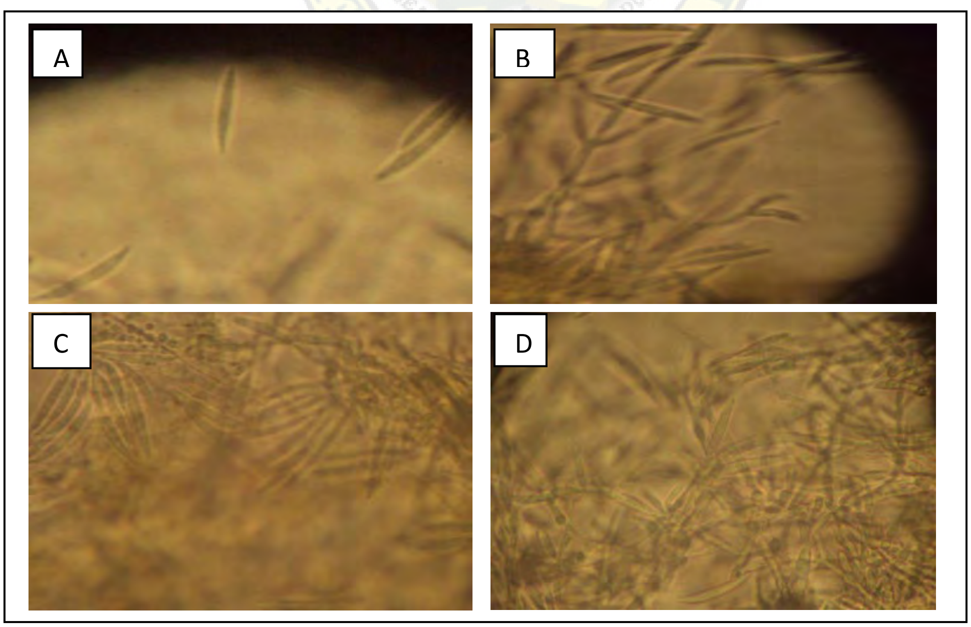

Figure 5. Morphological characteristics of the fungus A) Macroconidia; B and D) Branching conidiophores in a sporodochium; and C) Macroconidia in clump formation

Pathogenicity Test Results

Results of the inoculation methods experiment showed that pricking and spraying the cell suspension (10^7) into the cellular spaces with a syringe led to the appearance of disease symptoms in four days after inoculation (Table 1). However, the symptoms were manifested only in young stems (Figure 6). While the roots were injured before drenching the propagule, the non-production of the disease indicates that the pathogen does not enter the plant through root injury and that drenching the propagules does not ensure ability to cause infection. The direct pricking of the stem and spraying the propagules provides immediate contact of the propagules with the plant tissues being attacked by the pathogen. Pricking and spraying of the fungal suspension proved to be an effective and efficient way of introducing the pathogen to the host. Test plants that were pricked showed similar symptoms to the original symptoms observed in the source plant. The method may have been effective because it permits introduction of a definite number of fungi uniformly throughout the stem tissue.

Table 1. Pathogenicity of the fungal isolate comparing two inoculation methods with inoculum level of 10^7

		DAYS	AFTER	INOCULATION	
METHOD	4	7	10	13	16
Pricking and spraying [a]	+	++	+++	+++	+++
Drenching [b]	-	-	-	-	-

Disease rating on the chayote stem: - = no infection, + = stem start to rot, ++ = rotting on the stem with few visible white mycelia, +++ = stem rot and vascular wilt

[a] The stems were pricked first before the fungal suspension was sprayed.

[b] Fungal suspension was drenched to the roots given injury by slicing the soil cover.

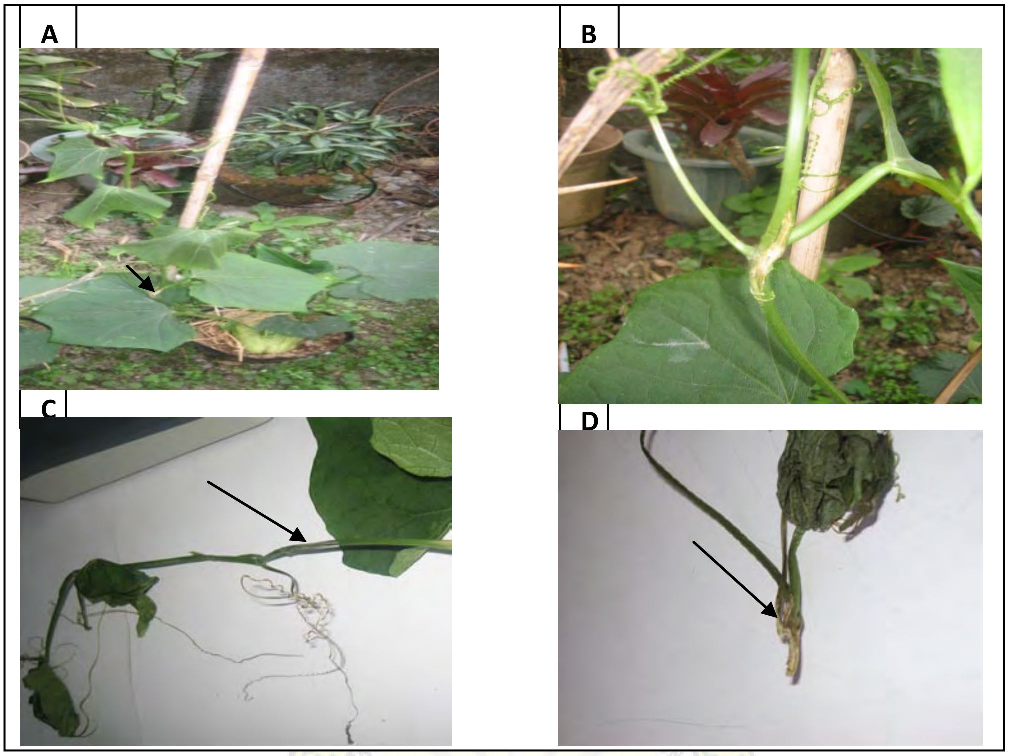

Figure 6. Symptoms of the disease on artificially inoculated chayote plants (A, B, C and D: Pricking and spraying inoculation)

Re-isolation of the Fungal Isolate from Artificially Inoculated Stems

To complete the proof of pathogenicity as set by Koch's postulates, fungi from the artificially inoculated symptomatic plants were re-isolated on PDA. The characteristics of the fungal isolate that was re-isolated were similar to the characteristics of the inoculum used (Figure 7). This therefore completes the Koch's postulates.

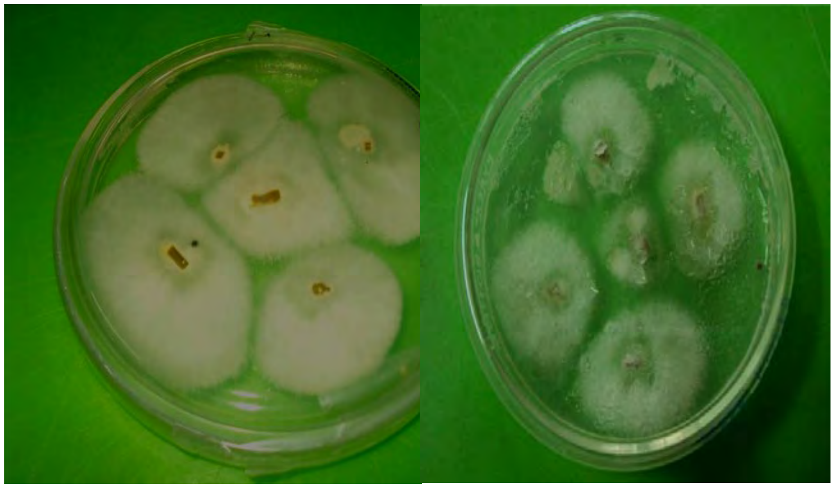

Figure 7. *Fusarium oxysporum* isolates re- isolated from the artificially inoculated chayote plant

Identity of the Re-isolated Fungus

On the basis of the cultural and microscopic characteristics of the fungus re-isolated from the infected chayote stems, the fungus is identified as *F. oxysporum*. This is corroborated by the findings of Smith *et al*. (1988) and Agrios (1988).

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

A pure culture of the fungus produced fast - growing cottony mycelia with white to orange color on the underside of the plate. The fungus has conidiophores in a sporodochium with an aggregate and branching formation. Moreover, macroconidia of the fungus are with phialides, slightly curved or boat-shaped which produce three to five cells and are produced in large number, often in clumps known as sporodochia. In addition, this fungus can also cause stem rot on artificially inoculated chayote stem in about four days through the pricking followed by spraying of spores as method of inoculation.

Conclusion

The pathogen is *F.oxysporum* as based on symptoms, microscopic observations, and growth pattern in PDA and as supported by pathogenicity test results.

Recommendation

Although we have gained a better perspective of the stem rot of chayote, we should, therefore, take advantage of the results obtained and make use of the information in developing a better management plan of attack.

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