BIBLIOGRAPHY

TAGWAY, KAREN S. MARCH 2006. Antibiotic Resistance of *Xanthomonas axonopodis pv*. *Diffenbachiae* (Anthurium Bacterial Blight) in La Trinidad, Benguet and Baguio City. Benguet State University, La Trinidad, Benguet.

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ABSTRACT

Fifteen *Xad* isolates were obtained from 10 anthurium cultivars grown at 7 locations. The *Xad* isolates differed in ability to degrade starch.

In general, most of the *Xad* isolates were resistant only to 50-200 ppm concentration of penicillin and streptomycin. The isolates were susceptible to concentrations from 500 to 2000 ppm. All the isolates were resistant to the recommended rates of Nordox (Cu_2O) and Funguran (CuOH).

The management measures most frequently employed by farmers were: twice weekly uprooting/removing and disposing of infected plants/leaves and weekly application of chemicals.

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INTRODUCTION

Anthurium (*Anthurium andraeanun Linden ex Andre*) is one of the most widely grown cutflower crop in Benguet. It has gained popularity in the Philippines as well because of its durability and attractiveness. According to DAMAS (2002), the top producers of anthurium in the Philippines are CAR (23%), Southern Mindanao (22%) and Southern Tagalog (17%).

One of the major constraints to anthurium production in Benguet is the bacterial blight disease caused by *Xanthomonas axonopodis* pv *dieffenbachiae* (McCulloch and Pirone, 1939) Vauterin *et.al*. 1995. Formerly known as *Xanthomonas campestris* pv *dieffenbachiae* (EPPO, 2005), the pathogen causes the same blight of other ornamental and food crops in the family *Araceae* including *Spathiphyllum, Aglaonema,* and *Xanthosoma* (Alvarez, 2000). This family also includes *Diffenbachia, Philodendron, Caladium,* and *Zantedeschia* sp. (Calla Lily) - all of which are grown in Benguet.

The disease has taken a heavy toll on the anthurium population worldwide. This disease infects the leaves, stems, shoots, and roots leading to the death of the whole plant (Rosario, 1988). The European Plant Protection Organization (EPPO, 2005) identifies the Philippines as the only Asian country where the disease can be found.

According to an FRLD study (1993), Benguet anthurium growers are predominantly monoculturists. This practice increases the probability of severe blight infection within their plantings. Infected planting materials can transmit the pathogen and the most common planting materials used in Benguet are suckers. Furthermore, the disease can spread to uncontaminated areas through splashing rain, irrigation water, infected tools, wet clothing and infected soil on footwear (WebMan, 2000).

Recommended measures to control the disease include the use of antibiotics (streptomycin and oxytetracycline) and the selection for resistant varieties. Currently, there are no identified resistant varieties for the Philippines.

Application of antibiotics in the agroecosystem could result in the build up and persistence of resistance genes in the environment (McManus, 2004). In fact, Henny et al. (2001) reported earlier that some *Xad* isolates have developed resistance to the recommended antibiotics.

It is therefore necessary to determine whether such resistant isolates can be found within the anthurium plantations in the locality. This information will have an impact on the development of integrated management measures against bacterial blight of anthurium to prevent further evolution and dissemination of resistant strains of the bacterium and reduce the spread of the disease.

This study aimed to isolate and characterize *Xad* from symptomatic anthurium, determine resistance of *Xad* isolates to various levels of streptomycin and penicillin, and determine the management measures employed by anthurium growers against bacterial blight.

Samples of symptomatic plants were collected from major planting sites in La Trinidad and Baguio City. Samples were then processed at the laboratory of the Department of Plant Pathology, College of Agriculture, Benguet State University. The study was conducted from August 2005 to March 2006.

REVIEW OF LITERATURE

The Disease, Its Cause and Symptoms

Bacterial blight of anthurium is a vascular disease. In the Philippines, the disease was first reported by Divinagracia and Enrique (1984). It is caused by *Xanthomonas axonopodis* pv *diffenbachiae* (McCulloch & Pirone, 1939) Vauterin *et al.* 1995 formerly known as *Xanthomonas campestris* pv d*ieffenbachiae* (McCulloch & Pirone, 1939) Dye. The bacteria live in the xylem of the host plant and from there induce symptoms of wilting and necrosis. After xylem and marginal necrosis begins, the pathogens are able to breakdown cell walls and cytoplasm and obtain nutrition from sources other than the xylem sap.

Symptoms include small scattered angular water-soaked spots near the margin most visible from the undersides of the leaf and a faint chlorosis when viewed from the upper side (McCallow and Leonhardt, 1983).

In the advanced stage, as more tissues are killed, the spots become circular to irregular brown areas, surrounded by a light yellow border. The necrotic center becomes rough, dry and sometimes curled. The leaves turn generally yellow before defoliation. If the infected leaves are not removed, the bacterium will go down the petiole into the stem manifesting itself in the systematic stage. The systemic or vascular infections first appear as general yellowing of the entire leaf blades of older leaves. The petioles of these leaves have infected vascular bundles showing that the bacterium has been moving up from the stem through the petiole and the leaf. Eventually, other parts of the plant are infected and the entire plant is killed (McCallow and Leonhardt, 1983). .

The Site of Infection

The principal infection sites of *Xad* are the hydathodes of the anthurium leaf. These hydathodes are located on the (lower surface) outer margin of the leaf and occupy only a small portion of the total leaf surface. Two reasons can be given for bacterial infection through hydathodes. First, the bacterial cells have one flagellum and are motile. Hydathodes have a continuous water pathway from the outside of the leaf to the xylem. Stomata on the other hand, may have water near their openings but free water is not usually present in the intercellular spaces. Thus, bacteria can swim into stomata, but then are not able to swim further (Imamura and Higaki, 1981).

Second, is that guttation liquid contains nutrients such as amino compounds. Virulent strains of *Xanthomonas campestries pv. oryzae* on rice have been shown to multiply readily on the nutrients in the guttation liquid of the hydathodes. This process is called chemotaxis and was first demonstrated by Macnab (1978).

Epidemiology of the Disease

Generally, the bacteria originate from previously infected plants and they are carried by tiny water droplets (aerosols) through dew that drips from one plant to another or by rain splash to healthy plants (Alvarez,1990). Likewise, Edmund (1978) further stated that the disease can be transferred by cutting tools or contaminated clothing or hands of workers.

In the case of rice (Pavgi et al) as cited by Singh, (1978) *Xanthomonas campestris pv oryzae* can be carried over by infected seeds from previous crops. It is possible that such seeds are initiated into the nursery where seedlings catch the infection and carry it to the field. According Nishijima (1990), this pathogen survives principally on infected

plant debris and only as a free living organism in the soil. Also, it can survive for a long period on plant tissues without causing symptoms; this is referred to as the latent period.

In anthurium there is evidence that the latent period maybe as long as ten months. Thus, Sakai (1990) stated that the plant may look healthy in between but when subjected and placed under temperature, nutritional or moisture stress, they may suddenly manifest severe symptoms of the disease. Moreover, Harfacre (1979) citing the work of Fang, et al (1956) added that temperature has something to do with the occurrence and how it is manifested to a certain degree. Farms at higher elevation have been observed with lower incidence of blight. Although plants produce flowers more slowly at cooler temperature and may compromise with yield this may go a long way in controlling the disease (Harfacre, 1979).

In relation to this, topography, climate and cultural practices affect the development of the disease. In Japan, Singh (1978) observed that poorly drained field along rivers or lakes and mountainous basin with excess of rainfall and humidity, floods and typhoons are conducive for the disease development.

The soil pH on the other hand, also largely influences the growth of *Xad* as studied by Sakai (1990). He found out that low pH severely lowers the growth of the bacteria; at pH 4-6 the disease is stagnant and at pH 4-5 and below growth occurred. In contrast, mild acidity to mild neutral (6 - 7) enhanced and favored the growth of the pathogen (McCallow and Leonhardt, 1983).

Control

Antibiotics were used in Hawaii and found to be effective (Alvarez, 1990). The method involves removing and destroying all systematically infected plants and blighted leaves and spraying with the antibiotic streptomycin sulfate at 200 ppm. Furthermore, the antibiotic was effective in controlling anthurium blight when sprayed after sanitizing.

However, these antibiotics could not be used in a normal maintenance/preventive operation or used extendedly because of the resistance problem and their possible detrimental effects on beneficial bacteria. Sakai (1990) cited though that proper cultural practice and control techniques applied can reduced the level of disease to a minimum and easily manage their impact on production.

Long-term disease management measures can also be done for the control of the said disease such as sanitation, biological control, cultural practices and developing anthurium cultivars/hybrids that may be resistant or tolerant to the disease. Sanitation, combined with the use of resistant or tolerant varieties is the most effective method of combating the disease (WebMan, 2000).

Resistance to Antibiotics

Certain bacteria are naturally resistant to a specific antibiotic, and this characteristic property limits a drug's spectrum of activity: this is referred to as intrinsic resistance. As antibiotics became more frequently used, a new problem emerged. Specific bacteria previously considered sensitive to a drug had become resistant and were responsible for treatment failures: this is referred to us acquired resistance. Such was the case for *Staphylococcus aureus*, which was originally sensitive to penicillin G; most strains are now resistant. The mechanism of resistance in this case was due to the

production of an enzyme by the bacteria, called a penicillinase that was able to destroy the antibiotic (Nishijima, WT., 1990).

Another particularly worrisome form of acquired resistance was first described in Japan by Teruhiko Akiba, a Japanese microbiologist, in 1959. It resulted in bacteria becoming simultaneously resistant to several different antibiotics. Of special concern to scientists was the observation that this type of resistance could be transferred from one bacterium to another. Furthermore, this transmissible resistance to multiple antibiotics could be passed between completely unrelated types of bacteria. The mechanism by which this is achieved is now understood to involve the transfer between bacteria of small pieces of DNA called plasmids that carry all the genetic information necessary to make the bacteria resistant (Macnab, R.M, 1978).

Generally this type of resistance has resulted from an indiscriminate use of antibiotics. When this phenomenon was first discovered, there was serious concern that it would lead to a loss of utility for antibiotics (Macnab, R. M., 1978).

Although antibiotic use on plants is minor relative to total use, MacManus (2004) said that application of antibiotics in the agroecosystem presents unique circumstances that could impact the build up and persistence of resistance genes in the environment. She further points out that no apparent human health issues have arisen after four decades of antibiotic use on plants in the farm setting. However, despite the controlled conditions in clinical settings, medical experts have witnessed the failure of one antibiotic after another in clinical settings (Immamura, J. & T. Higaki, 1981).

MATERIALS AND METHODS

Collection, Isolation and Maintenance Of *Xad* isolates

Leaf samples of anthurium plants infected with bacterial blight was collected randomly from different nurseries and greenhouses around La Trinidad, Benguet and Baguio City. The samples were surface-sterilized with 10% sodium hypochlorite for 3-5 minutes and rinsed with sterile distilled water. Sections (2-3 mm) from the margin of actively growing lesions were cut and placed in 1-2 drops of sterile distilled water on a flame-sterilized glass slide. The bacterial cells were allowed to ooze from the cut tissues, and loopfuls from this suspension were streaked onto agar slants.

The tetrazolium chloride agar (TCA) medium was modified by eliminating casein hydrolysate and reducing the final concentration of triphenyl tetrazolium chloride (TTC) to 0.001% (Alvarez et al, 1988). The plates were incubated for 2-3 days at room temperature. Single colonies that appeared on the medium were picked and streaked onto yeast dextrose calcium carbonate (YDC) agar slants.

Stock culture of the isolates were prepared by using a loopful each of 48-hour old pure cultures of each isolates and streaked onto YDC agar slants. The seeded slants were incubated at room temperature, and then stored in the refrigerator after good growth was obtained. The cultures were revived every 2 weeks on the same medium.

Cultural Characteristics of *Xad* Isolates

The cultural characteristics of the *Xad* isolates were noted on the YDCA and on the modified TCA, earlier mentioned. In addition, cultural characters on nutrient agar (NA), King's Medium B Agar (KMBA) and sucrose peptone agar (SPA) were also noted.

A loopful of suspension from the working cultures was streaked onto the different media. Plates were then incubated at 28^0C for 48 to 72 hours. Colony morphology was then described.

Sensitivity of Different Isolates of *Xad* to Streptomycin and Penicillin

The procedure modified by Dolores (2004) was adopted. The different isolates of *Xad* was tested for resistance to streptomycin and penicillin starting at 50 ppm. The isolates that were found to be resistant at this concentration were further tested to increasing concentrations of streptomycin sulfate and penicillin (at 200, 500, 1,000 and 2000 ppm). The isolates were also tested with two copper based fungicides: copper hydroxide (CuOH) and copper oxychloride (CuOCl).

Modified TCA media without 0.001% TTC but with different concentrations of streptomycin sulfate and penicillin was poured into the plates. One mL of a 48-hour old pure culture of the isolate was spread on the medium in triplicate plates. Growth of *Xad* isolates on different concentrations of streptomycin, penicillin and in copper-based fungicides was recorded after 3 days of incubation at 28-30^0C.

Test for Starch Hydrolysis

Loopfuls of 48-hour old cultures of the different isolates of *Xad* were streaked over the surface of starch agar plates (2% starch) and incubated at room temperature for 2 days. The surface of the agar plates was then flooded with dilute solution of Gram's iodine as test starch hydrolysis.

Determination of Growers' Management
Measures Against Bacterial Blight

The growers' practices in managing bacterial blight were determined through interviews. The instrument used in the interview is attached. Information obtained here was used to validate the findings on the antibiotic sensitivity of the *Xad* isolates.

Data Gathered

The data gathered are the following:

1. Cultural characteristics of *Xad* isolates on modified TCA, YDCA, NA, KMBA and SPA. The cultural characteristics of the *Xad* isolates on the various media were observed. Specifically, the following colony characters were noted: size, shape, color, elevation, margin, etc.

2. Sensitivity of isolates to antibiotics and copper-based fungicides. The sensitivity of the *Xad* isolates to the different levels of streptomycin sulfate, penicillin and copper-based fungicides were observed and reaction was noted as susceptible or resistant.

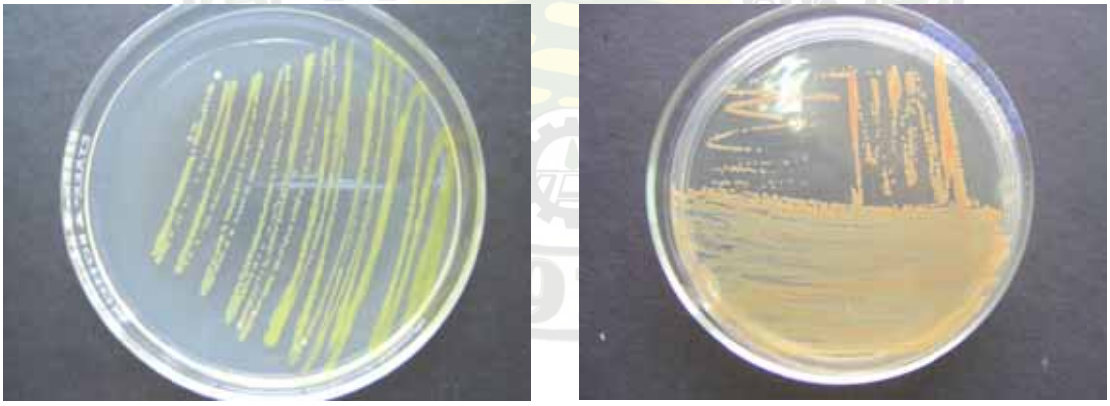
3. Ability of isolates to hydrolyze starch. The appearance of zones of clearing was noted around the *Xad* colonies.

4. Growers' management measures against bacterial blight. The management practice employed by the anthurium growers against bacterial blight was noted.

RESULTS AND DISCUSSION

Collection, Isolation and Maintenance of *Xad* Isolates

Leaf samples of anthurium plants infected with bacterial blight were collected randomly from different greenhouse around La Trinidad, Benguet and Baguio City. It was observed that the isolated bacteria from infected leaves grew well on modified TCA medium with 0.001% TTC. In 1998, Alvarez et.al. reported that the modified TCA is a good general differential medium which permits a fairly accurate presumptive identification of *Xanthomonas*. With 0.001% TTC, the colonies were red to orange at the center surrounded b a yellowish margin, smooth, almost circular, raised and glistening, 2-3 days after from small to very small colonies.



NA Modified TCA

Plate 1. Bacterial colonies on NA and modified TCA

There were 15 isolates collected from different location (Table 1). Most of the source cultivars were grown in greenhouse and in partially open sheds. Ten cultivars exhibited local symptoms, while 5 showed systemic symptoms.

Table 1. Sources and host cultivars of isolates collected for the study and the type of symptoms observed.

Isolate #	Source	Type of cultivation (open or greenhouse)	Host cultivars	Type of symptom (Local or systemic)
1	BSU greenhouse	Greenhouse	Sweetheart orange	Local
2	BPI Baguio	Greenhouse	Baguio white	Local
3	Baguio Orchidarim	Open	Kaumana	Local
4	Baguio Orchidarium	Open	Butterfly white	Local
5	BSU Orchidarium	Open	Tulip	Local
6	BSU greenhouse	Greenhouse	Kaumana	Local
7	Puguis, La Trinidad	Greenhouse and half open	Ivory white	Systemic
8	Puguis, La Trinidad	Greenhouse and half open	Ozaki	Systemic
9	Puguis, La Trinidad	Greenhouse and half open	Kaumana	Systemic
10	Puguis, La Trinidad	Greenhouse and half open	Obake	Systemic
11	Puguis, La Trinidad	Greenhouse and half open	Sweetheart orange	Systemic
12	BSU greenhouse	Greenhouse	Nitta	Local
13	Puguis, La Trinidad	Greenhouse and half open	Hawaiian red	Local
14	Puguis, La Trinidad	Greenhouse	Tulip	Local
15	Puguis, La Trinidad	Greenhouse	Nitta	Local

Cultural Characteristics of Xad Isolates

The cultural characteristics of the *Xad* isolates were shown in Table 2. A loopful of bacterial suspension from the working cultures was streaked on nutrient agar (NA), King's medium B Agar (KMBA), Sucrose Peptone Agar (SPA), Yeast Dextrose Calcium

Table 2. Summary table on the cultural characteristics of isolated bacteria on various media

Media	Basis	Isolate number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NA	Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
	Elevation	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
	Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Surface	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
	Color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
	Size	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7	70.7
KMBA	Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
	Elevation	Raised	Raised	Convex	Convex	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
	Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Surface	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
	Color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
	Size	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8	64.8
SPA	Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
	Elevation	Raised	Raised	Raised	Convex	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
	Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Surface	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
	Color	Yellow	Yellow	Yellow	Yellow	Yellow	Light yellow	Light yellow	Yellow	Light yellow	Yellow	yellow	Yellow	Light yellow	Light yellow	yellow
	Size	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
YDCA	Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
	Elevation	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
	Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Surface	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
	Color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
	Size	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1	74.1
TCA	Colony shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
	Elevation	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
	Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Surface	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
	Color	Light red	Light red	Red	Orange	Light orange	Light red	Light orange	Orange	Light pink	Yellow pink	Light red	Light orange	orange	Red	Light red
	Size	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9	81.9

Agar (YDCA), and the modified Tetrazolium Chloride Agar (TCA). Colony and cultural characteristics of Xad isolates were similar to those of *Xanthomonas dieffembachiae* as described by McCulloch & Pirone (1939), Hayward (1978), and Skerman et.al. (1980).

Sensitivity of Different Isolates of Xad to
Streptomycin, Penicillin and Copper-based Fungicide

The different isolates of Xad were tested for resistance to streptomycin and penicillin starting at 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm and 2000 ppm (Plate 2).

Most of the isolated were resistant to lower concentration of streptomycin and penicillin as observed at 50 ppm and 100 ppm (Table 3.1). Except for the tulip variety from BSU Orchidarium which was found resistant to streptomycin and penicillin.

The isolates were found to be sensitive to higher concentration of streptomycin and penicillin. It was found also that the isolates were very resistant to Nordox and Fungaran which were copper-based fungicide.

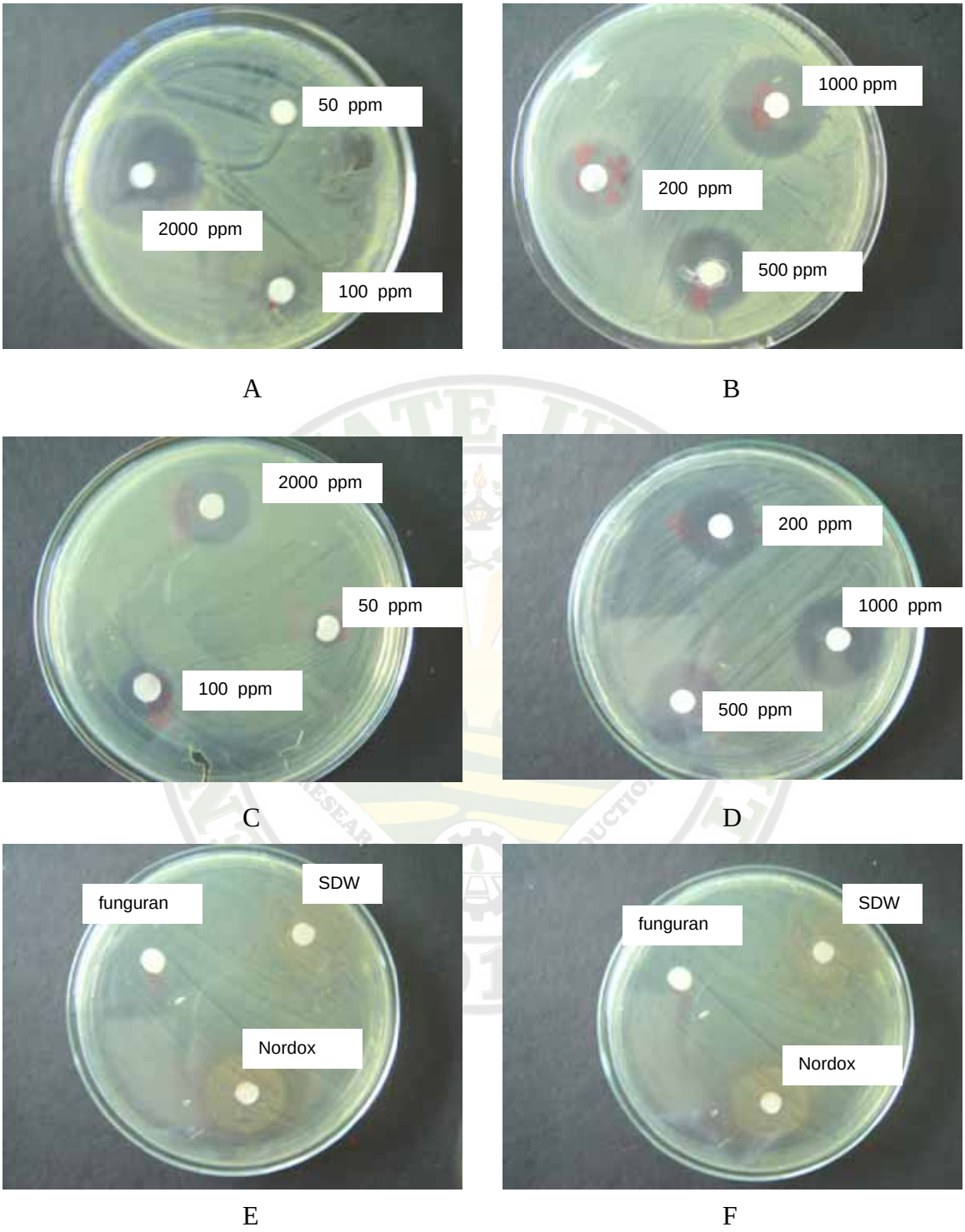


Plate 2. Inhibition zones affected by the various chemicals tested (A-B – Penicillin; C-D – Streptomycin; E-F – Funguran, Nordox & SDW)

Table 3.1a. Antibiotic sensitivity of *Xanthomonas axonopodis pv. diffenbachiae* isolates to Streptomycin

Isolate	Concentration of Streptomycin (ppm)					
	50	100	200	500	1000	2000
1	I	I	S	S	S	S
2	R	R	I	S	S	S
3	I	I	S	S	S	S
4	R	R	I	S	S	S
5	R	R	R	R	R	R
6	R	I	S	S	S	S
7	I	S	S	S	S	S
8	I	I	S	S	S	S
9	R	I	S	S	S	S
10	R	I	S	S	S	S
11	R	I	S	S	S	S
12	R	R	S	S	S	S
13	I	S	S	S	S	S
14	R	I	S	S	S	S
15	I	S	S	S	S	S

R - Resistant
I - Intermediate
S - Susceptible

* Standard Scale: >11 is R and <15 is S

Most of the isolates were resistant to 50 ppm and 100 ppm, except for isolates 2, 3, & 5 which reaches to 200 ppm, and they were all susceptible to higher concentration of penicillin (Table 3.1b)

Table 3.1b. Antibiotic sensitivity of *Xanthomonas axonopodis pv diffenbachiae* isolates to Penicillin

Isolate	Concentration of Penicillin (ppm)					
	50	100	200	500	1000	2000
1	R	R	R	S	S	S
2	R	R	R	R	R	S
3	R	R	R	S	S	S
4	R	R	S	S	S	S
5	R	R	R	R	R	R
6	R	R	S	S	S	S
7	R	R	S	S	S	S
8	R	R	S	S	S	S
9	R	R	S	S	S	S
10	R	R	S	S	S	S
11	R	R	S	S	S	S
12	R	R	S	S	S	S
13	R	R	S	S	S	S
14	R	R	S	S	S	S
15	R	R	S	S	S	S

R - Resistant
S - Susceptible

* Standard Scale: >14 is R and <15 is S

From Table 3.2a, the source of cultivars have different reactions on the concentration of streptomycin applied which indicates that there were no resistant cultivars so far.

Table 3.2a. Source of Cultivar

Cultivars	Concentration of Streptomycin (ppm)					
	50	100	200	500	1000	2000
Sweetheart Orange	R	R	S	S	S	S
Baguio White	R	R	I	S	S	S
Kaumana	R	R	S	S	S	S
Butterfly White	R	R	I	S	S	S
Tulip	R	R	R	S	S	S
Ivory White	R	S	S	S	S	S
Ozaki	I	I	S	S	S	S
Obake	R	I	S	S	S	S
Nitta	R	I	S	S	S	S
Hawaiian Red	I	S	S	S	S	S

The Baguio White, reach its resistance to 1000 ppm of penicillin, also with tulip to 200 ppm, most of the isolate were resistant to lower concentrations.

Table 3.2b. Source of Cultivars

Cultivars	Concentration of Penicillin (ppm)					
	50	100	200	500	1000	2000
Sweetheart orange	R	R	S	S	S	S
Baguio white	R	R	R	R	R	S
Kaumana	R	R	S	S	S	S
Butterfly white	R	R	S	S	S	S
Tulip	R	R	R	S	S	S
Ivory white	R	R	S	S	S	S
Ozaki	R	R	S	S	S	S
Obake	R	R	S	S	S	S
Nitta	R	R	S	S	S	S
Hawaiian red	R	R	S	S	S	S

Most of the source location of the isolates were resistant from 50 to 200 ppm streptomycin and they were susceptible to 500 to 2000 ppm which indicate that there were no resistance formed on the cultivars on the source location.

Table 3.3a. Source Location

Location	Streptomycin (ppm)					
	50	100	200	500	1000	2000
BSU greenhouse Floriculture	R	R	R	S	S	S
BSU Horticulture	R	R	R	S	S	S
BPI Baguio	R	R	R	S	S	S
Baguio Orchidarium	R	R	R	S	S	S
BSU Orchidarium	R	R	R	S	S	S
Puguis 1	R	R	R	S	S	S
PUguis 2	R	R	R	S	S	S

Same is true with penicillin, no resistance formed on the concerned locations, they were resistant only on lower concentration.

Table 3.3b. Source Location

Location	Penicillin (ppm)					
	50	100	200	500	1000	2000
BSU greenhouse Floriculture	R	R	S	S	S	S
BSU Horticulture	R	R	R	S	S	S
BPI Baguio	R	R	R	S	S	S
Baguio Orchidarium	R	R	R	S	S	S
BSU Orchidarium	R	R	R	S	S	S
Puguis 1	R	R	R	S	S	S
PUguis 2	R	R	R	S	S	S

Test for Starch Hydrolysis

The isolates were able to hydrolyze starch. Clear zones were found around or bordering the streaks of the isolates upon application of diluted Gram's iodine. Six isolates were found to react easily upon application of the Gram iodine. They produced wider clear zones.

Iodine solution (Gram's) is an indicator of starch. When iodine comes in contact with a medium containing starch, it turns blue. Starch is hydrolyzed and is no longer present however, if the medium will have a clear zone next to the growth. Bacteria that hydrolyzed starch produce amylases that yield molecules of maltose, glucose, and dextrins (Benson, 1998).

Valencia et al. (2004) observed that starch hydrolyzers grew more rapidly on starch agar medium than non-starch hydrolyzers. Nevertheless, they were equally virulent and they were found to be pathogenic on anthurium plants.

Table 4. Starch Hydrolysis by *Xanthomonas axonopodis pv diffenbachiae* isolates

Isolates	Clearing Zones	Isolates	Clearing Zones
1	+ + +	9	+
2	+ + +	10	+ +
3	+ + +	11	+
4	+ +	12	+
5	+ + +	13	+
6	+ +	14	+
7	+ + +	15	+ + +
8	+		

Legends:

+ + +	=	wide clearing
+ +	=	moderate
+	=	slight

Slight

Moderate

Wide

Plate 3. Variations in ability to hydrolyze starch among the isolates

Determination of Grower's Management Measures Against Bacterial Blight

Most of the anthurium growers practiced the removal and disposal of infected leaves also the addition of organic matter on the plants, most of it were compost of dried leaves, coconut husk and the use of animal manures especially cow manure (Table 5).

Some of the growers uproot the infected plant but disposed them near the uninfected plants. They make use also of the suckers of the infected plant. The greenhouse also is very moist because of the use of rainburst irrigation which favors the spread of the disease. The disease can not be controlled because of those existing practices done by farmers at Puguis.

Though they sprayed the plants twice a week with the use of fungicide, the disease symptoms observed was systemic (Puguis) already though some were localized.

The growers made use also of antibiotics but they just spray when there is available antibiotic.

Most of the growers were engaged with the use of hose as their irrigation system.

Table 5. Management practices employed by anthurium growers to control bacterial blight.

Management Practice	Frequency of Application
Removal and disposal of infected leaves	Twice a week (44/45)
Uprooting and disposal of infected plants	Twice a week (42/45)
Application of chemicals	Once a week (42/45)
Resistant variety	(1/45)
Use of clean planting materials	--
Addition of organic matter	Every month (44/45)
Careful irrigation	

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary

The study was conducted to test the sensitivity of different Xad isolates to antibiotics and copper-based fungicide, that were collected from different greenhouses around La Trinidad, Benguet and Baguio City.

Most of the Xad isolates were resistant to lower concentration and susceptible to higher concentration of streptomycin and penicillin. Except for the tulip variety from BSU Orchidarium which was observed to be resistant to the antibiotics.

The 15 Xad isolates differed in ability to degrade starch. The most frequently employed management measures were: uprooting/removing and disposing of infected plants/leaves and application of chemicals.

The isolates were found also to be very resistant to copper-based fungicides, the Nordox and Funguran.

Conclusion

The Xad isolates differ from each other. Most Xad isolates were generally not resistant to antibiotic concentration from 500 ppm and above. However, presence of isolates with intermediate reaction to 50-500 ppm concentrations poses a potential for development of resistance if antibiotic use is continued.

Growers do not associate watering with spread of the disease and still believe that the disease is caused by a fungus.

Recommendations

Based on the findings of this study, the following were recommended:

1. Thorough survey to cover bigger commercial farms and to identify cultivars used that may be sources of future infection.

2. Development and implementation of integrated disease management measures to address the variability in the *Xad* isolates.

3. Genetic analysis to determine whether the observed differences were due to genetic differences as well.

4. Familiarization of farmers with symptoms of the *Xad* infection to allow differentiation from *Ralstonia* infection.

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APPENDICES

Appendix 1: Communication Letter

College of Agriculture
Department of Plant Pathology
Benguet State University
La Trinidad, Benguet

October 2005

Dear respondents,

The undersigned is conducting a study entitled ‘’ANTIBIOTIC RESISTANCE OF XANTHOMONAS AXONOPODIS PV DIFFENBACHIAE (ANTHURIUM BACTERIAL BLIGHT) IN LA TRINIDAD, BENGUET AND BAGUIO CITY’’ in partial fulfillment of the degree Bachelor of Science in Agriculture (Plant Pathology).

In this regard, I am requesting your cooperation and support by sharing me a part of your time to give the necessary data by answering the questions properly.

Your honest answers will contribute so much to this study.

Thank you very much and may the Almighty God bless you always.

Respectfully yours,

KAREN S. TAGWAY
Researcher

APPENDIX 2. Questionnaire

1. Location of the farm _____
2. Area devoted to anthurium _____
3. Type of culture _____
    a. Open or protected _____
    b. If protected _____
        full or partial _____
        plastic or glass _____
4. Occurrence of bacterial blight _____
    Present or absent _____
5. If present, type of symptoms observed _____
    Local, systemic _____
6. Incidence (20-30 sample plants)_____
7. Severity of infection _____
    Rating:
    1. No infection
    2. Nearly plant affected with blight plant still retaining normal form
    3. When every plant affected and about ¼ of the area is destroyed by blight
    4. When every plant affected and about ½ of the area is destroyed by blight
    5. When about ¾ of the area are affected with blight
    6. Only few leaves left green
    7. When all leaves are dead including the stem
8. Anthurium cultivar used _____

9. Source of cultivar _____

10. Type of irrigation _____

11. Management of bacterial blight: Which of the following do you use against bacterial blight? Check as many as you use.

_____ a. Removal and disposal of infected leaves

_____ b. Uprooting and disposal of infected plants

_____ c. Application of chemicals, please specify _____

_____ d. Resistant variety, please specify _____

_____ e. Use of clean planting materials

_____ f. Addition of organic matter, specify _____

_____ g. Careful irrigation

_____ h. Other practices, please specify

_____

_____

_____

_____

APPENDIX 3. The recommended management measures for bacterial blight of anthurium.

a. Avoid using infected planting materials.

b. Remove all leaves with foliar infection by breaking or cutting the petrole near the base. Remove all systemically-infected plants.

c. Spray beds thoroughly with streptomycin sulfate or oxytetracyline weekly for 6-8 weeks at the rate of one and a half vial per five gallons of water.

d. Disinfect tools to prevent the spread of the disease during harvesting or leaf pruning.

e. Avoid using copper-based fungicides such as copper hydroxide and tribasic copper sulfate. Laboratory and field tests have shown that the bacterim is resistant to the copper-based compounds and that phytoxicity to anthurium my occur.

f. Avoid close contact with the plant during wet condition. Touching creates wounds the augmented by water, encourages disease spread.

g. Monitor plants continuously of diseased plants are found, remove them immediately.

h. Provide good aeration and good drainage system

i. Maintain strict sanitation.