BIBLIOGRAPHY

RHONDA M. OLOAN. October 2006. <u>Etiology of the Red Stele Disease of</u> <u>Strawberry (*Fragaria x ananassa* Duch.) in Benguet</u>. Benguet State University, La Trinidad, Benguet

Adviser: Luciana M. Villanueva, Ph.D.

ABSTRACT

This study was conducted to (a) describe the symptom variations in all strawberry cultivars observed to be infected in the field (b) assess the incidence of the disease in the strawberry growing areas of La Trinidad and Tuba, Benguet, (c) determine the best culture medium that support both mycelial growth and oospore/sporangia/zoospore production, (d) describe the morphological characteristics of the pathogen and (e) determine zoospore inoculum level that affects the growth and yield of strawberry.

Symptoms of stunting, wilting, abnormal change of leaf color, reddening of the core and eventual death of severely infected plants were observed on cultivars' Tioga and Sweet Charlie that were planted in the field. High incidence of the disease was observed in strawberry growing areas at Swamp Area, BSU, La Trinidad ranging from 20 - 43% infection while lower incidence was recorded in Longlong, La Trinidad and Sto. Tomas, Tuba with 19.80 and 8.91%, respectively.

Rapid growth of mycelia was observed in carrot broth agar (CBA) and oatmeal agar (OMA) with mean colony diameter of 27.45mm and 24.17mm in 7 days,

respectively. On the other hand, the mycelial growth in potato dextrose agar (PDA) was very slow with mean colony diameter of 7.53mm. The mycelia are white in color in PDA and OMA while brown to white in CBA and V8 juice agar (V8J-A). Mycelial growth was very dense in PDA, slightly dense in CBA and V8J-A while in OMA they were very scarce and could hardly be seen on culture.

Morphological studies showed that the hyphae are hyaline, coenocytic and slender with few swelling of hyphae in CBA, OMA and V8J-A culture media while profuse swelling was observed on PDA. The oospores are round and readily observed on CBA, V8J-A and OMA media with antheridia attached on the base. Sporangia are ovoid to pear shape, hyaline and non-papillate. The zoospores are very motile with two flagella attached but hardly seen. It has round shape when at rest.

Based on the cultural and morphological characteristics, the pathogen of the red stele disease of strawberry in Benguet was identified to be *Phytophthora fragariae*.

Inoculated plants exhibited symptoms of stunting, wilting, off-colored leaves, yellowing of immature leaves, and death of severely infected plants. Newly developed feeder roots have red to brick red color of the root core.

Plants inoculated with the highest concentration of zoospores (20×10^{3}) significantly gave the shortest mean height of 139.58cm and the lowest marketable yield of 117.91g. Yield reduction from 38-57% as a result of infection was noted on inoculated plants with disease severity rating of 3.67 to 5 equivalent to 26 - 100% root infection.

ii

TABLE OF CONTENTS

Bibliography	i
Abstract	i
Table of contents	iii
INTRODUCTION	
Importance of the Study	1
Objectives of the Study	4
Time and Place of the Study	4
REVIEW OF LITERATURE	
Strawberry	6
Red Stele Disease	7
The Pathogen and its Life Cycle	8
Disease Symptoms	10
MATERIALS AND METHODS	
Field Study	12
Survey	12
Symptomatology	14
Assessment of Disease Incidence	14
Laboratory Study	14
Collection of Diseased Plants	15
Isolation of the Organism	15

Identification of the Organism	15
Cultural Characteristic of the Organism	16
Morphological Characteristic of the Organism	16
Pathogenicity test	17
Preparation of Inoculum	17
Standardization of the Inoculum	18
Inoculation	18
Re-isolation of the Organism	19
Data Gathered	20
RESULTS AND DISCUSSION	
Field Study	23
Symptomatology	23
Disease Incidence	26
Laboratory Study	29
Characterization and Identification of the Organism	29
Mycelial Growth of the pathogen in different Culture Media	29
Cultural Characteristic of the organism	30
Morphological Characteristics of the organism	32
Pathogenicity Test	36
Symptoms on Inoculated Plants	36
Plant Height	40
Yield	41

Fresh Weight of Plants	43
Oven Dry Weight of Plants	45
Disease Severity at Harvest	45
SUMMARY, CONCLUSION AND RECOMMENDATION	
Summary	48
Conclusion	49
Recommendations	50
LITERATURE CITED	51
APPENDICES	54
BIOGRAPHICAL SKETCH	80



INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) production in the highlands of the northern Philippines started about almost a century. The cultivation of this crop was estimated to have started sometime in the early 19th century. It was introduced by the Americans most probably when an agricultural school in La Trinidad, Benguet (presently Benguet State University) was established in 1916. It has been a lucrative source of income for farmers and adds to the revenue of the province of Benguet. It is cultivated for its delicious berries, which are sold as fresh fruits or as preserves.

Strawberries have been in demand not only locally but also in other countries. According to Hermano (1995), The Baguio Growers and Marketing International Corp. (BGIMC) exported 6.4 tons of fresh strawberries to Hongkong in 1992. The volume increased to 9.1 tons in 1994. Strawberry is also in demand among processing factories such as the Magnolia Dairy Products, Inc., Lady's Choice consolidated Food Company and Baguio Strawberry House including local jam and wine makers.

Importance of the study

At present, strawberry is the number one and the most popular fruit produced in Baguio City and Benguet Province. It is estimated to rank number



six in sales among all farm crops grown in the area, surpassed only by potato, cabbage, carrots, Chinese cabbage, and beans (HARRDEC, 1996).

To meet the market demand several new cultivars which are high yielding have been introduced in Benguet and Baguio City which include Tioga, Hecker, Aliso, Mission bells, Fukuba, Haranoka, Toyonoka and many more (Hermano, 1995).

However, due to the presence of pests and diseases and other limiting factors, production is not yet enough to meet the increasing demand. Numerous diseases attack strawberries and the organisms causing the disease maybe on the plants when they are purchased or in the soil where the plants are set. One of the most serious threats in strawberry production are soil borne diseases like black root rot caused by several fungi, *Verticillium* sp., *Fusarium* sp., *Pythium* sp. and *Phytophthora* sp. Farmers interviewed at the Swamp area revealed that soil borne diseases had caused them severe yield loss. According to them, some of their plants show symptoms of stunting and wilting starting from December, and produced small berries. Severe infection was observed from February to April especially when the weather is very hot and dry.

Red stele or red core disease of strawberry is a major factor limiting strawberry fruit production in the northern two – thirds of the United States and in many areas of the world. The disease is most destructive in heavy clay soil that is saturated with water during cool weather (Ries, 1996).



The disease is caused by a soil-borne fungus and is not a natural inhabitant of most agricultural soils but introduced into a previously uninfected site through infected planting stocks or contaminated planting equipment. The red stele fungus is very persistent and can survive in the soil for up to 13 years or longer once it becomes established in the field or garden (Ellis, 1996). Once introduced to a site, the fungus spreads in a field via soil and water movement. Soil movement is usually accomplished through contaminated farm tools such as plows, hoes and trowels. Soil and water movement will often carry spores from higher areas to lower areas (Scopes and Stables, 1989; Ellis, 1996).

The red stele root rot disease attacks strawberry, loganberry, and potentilla (a genus of rosaceous herbs commonly called cinquefoil). Loganberry is a host. However, most infections are passed directly from strawberry plants to strawberry plants (Ellis, 1996).

In the Philippines, the red stele disease has not yet been reported but symptoms such as production of few runners, stunting, wilting and eventual death of severely infected plants were observed in the late 1980's. Furthermore, roots of plants just starting to wilt when dug showed reddening of the stele or core which is the most reliable symptom of red stele disease which was observed at the Swamp Area in La Trinidad, Benguet. According to Anderson (1956) and Ries (1996), the introduction of the disease to any strawberry-growing area is a threat because it renders the land useless for strawberry production over a period of 10 to 13 years or more. Losses are not great for the first 2-3 years after the



introduction of the disease in a field but occurrence of such disease if not properly managed may cause severe yield loss to farmers.

Hence, identification of the causal organism of red stele infecting strawberry in Benguet is very necessary for the implementation of control measures to prevent the spread of the disease.

Objectives of the Study

This study was conducted to:

- a. Describe symptom variations in all strawberry cultivars observed to be infected in the field;
- Assess the incidence of the disease in the strawberry growing areas of La Trinidad and Tuba, Benguet;
- c. Determine the best culture medium that would support mycelial growth and oospore/ sporangia/zoospore production;
- d. Describe the morphological characteristics of the pathogen, and
- e. Determine the zoospore inoculum levels that affect the growth and yield of strawberry.

Time and Place of the study

The survey was conducted in the strawberry growing areas at La Trinidad and Tuba, Benguet from November 2005 to May 2006. The pathogenicity test and laboratory studies were conducted at the Baguio National Crop Research and



Development Center (BNCRDC)-Bureau of Plant Industry (BPI), Guisad, Baguio City and the Department of Plant Pathology (DPP), College of Agriculture (CA) Benguet State University (BSU), La Trinidad, Benguet from November 2005 to August 2006.





REVIEW OF LITERATURE

Strawberry

The cultivated strawberry belongs to the genus *Fragaria* of the family *Rosacea*. Strawberry is locally known as *atakbang*, *duting* or *kubkubot* and *Fresa* in Spanish (Coronel, 1983). Strawberry production in Benguet is very successful because of its cool climate. The best temperature range for strawberry production is from 14 to 23°C (57-73°F). Strawberries normally flower as early as November; 2 to 3 months after planting.

Strawberry is a slow-growing herbaceous plant with a short crown or rootstock on which the leaves are produced together. Branch crowns and runners are produced in the axils of leaves which are normally trifoliate. The fruit trusses are produced terminally on the crown. The flower trusses may each have few or many flowers on branching stalk (Hermano, 1995; Encarta Encyclopedia, 2006)

Botanically, the berry is not a true fruit because it consists of swollen receptacles on the surface of which are embedded the numerous seeds (Hermano, 1999). The red part of a strawberry holds the actual fruit which is the tiny dark seeds on the outside of the strawberry. The red part is just the tip of the strawberry stem that's enlarged (Encarta Encyclopedia, 2006)

Several cultivars of strawberry have been introduced in Baguio City and La Trinidad, Benguet. According to Hermano (1995) cultivars Tioga, Aliso, Ceascape, Chandler, Haranoka and Toyonoka have been introduced. At present



cultivars, Tioga, Sweet Charlie and a newly imported variety Camarosa are the varieties commonly planted by farmers.

Red Stele Disease

Several authors used the names "red core", "red root rot", "red stele", "brown stele" and "black stele" to describe the disease. It was first named "Lanarkshire strawberry disease" in Scotland where the disease was first observed. The first definite reports of this disease were from Scotland in 1927 where Wardlaw (1921) as cited by Anderson (1956) attributed the trouble in Lanarkshire to poor cultural conditions followed by the entry of weak root parasites. The causal organism was named *Phytophthora fragaria* by Hickman in 1940 (Anderson, 1956; Milholland *et.al.* 1989).

Ries (1996) reported that red stele disease is a serious root disease of strawberries in the United States. It occurs during the late winter and spring. The fungus attacks strawberries, loganberry, and potentilla (a genus of rosaceous herbs commonly called cinquefoil) and is spread from one area to another primarily through diseased plants. Red stele usually does not appear in a new planting site until the spring of the first bearing year, from about full bloom to harvest. Minor symptoms of root infection may appear, however, in late fall of the first growing season. The disease is prevalent in poorly drained areas.



The Pathogen and Its Disease Cycle

Red stele is caused by soil-borne fungal-like organism *Phytophthora fragariae* (Westcott, 1978; Ferguson, 1982; Childers, 1983; Clark, 1984; Agrios, 2005; Holliday, 1989). This is very persistent and can survive in the field for 13 years or longer once it has become established, regardless of the crop rotation used or even if no strawberries are grown during that time (Ellis, 1989; Ries, 1996).

According to Ries (1996), the optimum temperature for growth and infection of the red stele fungus is 14 ^oC. It is inactive at 4.5 ^oC and above 30 ^oC. When the soil moisture is high and the temperature is cool, plants show typical symptoms with in 10 days after infection. He added that heavy clay soils, which retain moisture for long periods of time, provide a favorable environment for the development of the disease because the zoospore can spread greater distance and produce more infection sites. Horsfall (1978) added that infection of red stele is higher in coarse soil than in fine soil. However, Ellis (1996) reported that soil types do not affect the presence of the red stele fungus. The fungus is active at pH of 4.0 to 7.6 but will not grow in alkaline soil with pH of 8.0 or above. Likewise, Holliday (1989) observed that more disease is found in acid soil and there is a long survival of oospores. In culture media, it grows well with pH as low as 4.0 but no growth occurs at a pH of 8.0 or higher (Anderson, 1956). According to Anderson (1956) the causal organism of the red stele disease grows on very limited culture media.



George and Milholland (1986) reported that the optimum temperature range for both zoospore germination on host roots and oospore development in host tissue was 15-20 0 C. A temperature of 25 $^{\circ}$ C encouraged zoospore germination but greatly inhibit oospore formation.

Phytophthora fragariae persists in the soil as thick walled, resting spores called oospores. The oospore, not the chlamydospore is the primary survival structure in the field (Hickman, 1940 as cited by Law *et.al.* in 1991).

According to Ellis (1996), the oospores germinate and form sporangia with the infectious spores of the fungus called zoospores when the soil is wet and moist. Once the microscopic zoospores are released into the soil when the area is flooded it swims to the tips of strawberry roots, to which they are chemically attracted. Zoospores activity may occur at soil temperature ranging from 4 to 25°C, but is most significant from 7 to 15°C. He added that once the zoospore have infected the root tips, the pathogen begins to grow up into other parts of the roots, causing the characteristic dark rot and red stele symptom. Ries (1996) added that zoospores infecting the tips of the young and fleshy roots destroy the water-and-food conducting tissues. Infection and growth of the fungus in the roots reduces the flow of water and nutrients to the developing leaves and fruit causing drought-like symptoms in the plant. Ellis (1996) reported also that new sporangia are formed along the outside of infected root tissue and release additional zoospores whenever the soil is saturated, thereby continuing the spread of the disease. He added that oospores are also produced within infected roots



and when roots begin to rot and die oospores are released into the soil when the roots decay, thus completing the disease cycle.

Disease Symptoms

<u>Above ground</u>. Infected strawberry plants lack vigor or grow poorly, are stunted, and frequently wilt. The young leaves often have a metallic bluish-green while older leaves sometimes turn yellow or red prematurely. They produce small berries and a poor runner growth. During the hot, dry weather of early summer, infected plants wilt, collapse and die prematurely (Wescotte, 1978; Childers, 1983; Smith, 1984; Holliday, 1989; Alford and Locke, 1989; Los and Schroeder, 1992; Swift and Dickens, 1995; Ellis, 1996; Agrios, 2005).

Below ground. The most reliable symptom of red stele is found within the root. Plants with red stele have few lateral roots and the main fleshy roots have a "rat-tail" appearance. During intermediate stages of disease development, these fleshy roots will be white near the crown of the plant but will show dark rot progressing upward from the tips. In normal root, both the center (stele) and the part surrounding the stele is distinctively brownish-white while plant infected with red stele disease has distinctive brownish-red, pink or brick red. The red color may show only near the dead tip, or it may extend the length of the root (Wescotte, 1978; Holliday; Alford and Locke, 1989; Swift and Dickens, 1995; and Ellis, 1996). Ries (1995) reported that no other disease of strawberry shows this particular symptom. However, Khanizadeh and Buzard (1992) mentioned



that reddening of the root in the field-grown plants may be used as a preliminary diagnostic feature, concluding that visual symptoms alone should not be used as criterion to assess susceptibility. Resistant cultivars are not immune from infection and relatively high number of oopores may be observed in the symptomless root tissues of inoculated resistant types within two weeks.





MATERIALS AND METHODS

Field Study

<u>Survey</u>

The disease was assessed and recorded from the different strawberry growing areas namely Swamp Area, BSU Balili Experiment Area, and Longlong, all situated at La Trinidad and Sto. Tomas in Tuba, Benguet (Plates 2-5

A total area of 31,426 sq.m strawberry farms was surveyed to determine the incidence of the red stele disease at the Swamp Area (24,679 sq.m.), Balili Experiment Station (3,240 sq.m.), and Longlong (2,313 sq. m.) La Trinidad and Sto. Tomas, Tuba (1,194 sq. m.), Benguet.



Plate 1. Overview of strawberry farms at Swamp Area, BSU, La Trinidad, Benguet





Plate 2. Overview of strawberry farms at Balili Experiment Station, BSU, La Trinidad, Benguet



Plate 3. Overview of strawberry farms at Longlong, Puguis, La Trinidad, Benguet



Plate 4. Overview of strawberry farms at Sto. Tomas, Tuba, Benguet

Symptomatology

Reported symptoms of the red stele disease such as stunting, wilting and abnormal color of young leaves were observed and documented on cultivars Tioga and Sweet Charlie grown by farmers. Strawberry plants showing these above ground symptoms were uprooted and the roots were examined for symptom of reddening of the core to be declared as infected with red stele disease.

Assessment of Disease Incidence

The incidence of the disease was assessed in the different areas surveyed. In the Swamp area, the lot was divided into 4 blocks, each block consisting of 10 fields. To each field, ten sample plots were randomly selected. The total number of plants and the number of infected plants per plot were counted. Based on field symptoms, incidence of the disease was assessed based on Waller *et al.* (2002):

% Disease Incidence =

Number of infected plants x 100%

Laboratory Study

Total numbers of plants

Laboratory experiments were conducted at the BNCRDC-BPI, Baguio City and Department of Plant Pathology, College of Agriculture, BSU, La Trinidad.

14

Collection of Diseased Plants

Samples of strawberry plants showing characteristic symptoms of the disease were collected in La Trinidad particularly at the Swamp Area and BSU Balili Experiment Station. The samples were carefully dug up to avoid damage of roots and examined for symptoms of red stele and were placed individually in properly labeled plastic bags for further diagnosis, isolation and identification.

Isolation of the Organism

In the laboratory, infected strawberry plants collected from the field were washed thoroughly to remove adhering soil. Roots were peeled off to expose the root core for the presence of reddish-brown stele appearance. The roots were cut measuring 1 cm and surface-sterilized with 1% NaOCl, blotted dry and washed three times with sterile distilled water. Infected roots were isolated in PARPH, a selective medium for *Phytophthora* sp.. The plates were incubated for six days at 18-22 °C to allow the growth of the causal organism. The organism was re-isolated in carrot agar to obtain a pure culture.

Identification of the Organism

The causal organism of the red stele disease was identified through the cultural and morphological characteristics including coenocytic mycelia. sporangia, oospore and zoospore. The growth of the organism was compared from four different culture media.



<u>Cultural characteristics of the organism</u>. To determine the effect of culture media on the growth of the causal agent, four treatments were laid out following the completely randomized design (CRD) with 5 replications. The different culture media used as treatments were as follows.

TREATMENTSMEDIAT1Potato Dextose Agar (PDA)T2Carrot Broth Agar (CBA)T3V-8 Juice Agar (V8JA)T4Oatmeal Agar (OMA)

The growth of the causal organism on the different culture media was observed for a week.

<u>Morphological characteristics of the organism</u>. Sporangia and zoospores were produced following the procedure used by George and Milholland (1986). Agar discs were cut from a two-weeks old culture using a cork borer, transferred to a sterilized and petri plate, flooded with 10 ml sterilized 5% V8 juice instead of soil leachate and incubated for three days under continuous light at 18-22 ^oC. Likewise, zoospores were produced by chilling the agar disc containing sporangia to 5 ^oC temperature for 30 minutes and rewarmed at room temperature from 30 minutes to one hour.

The oospore and sporangia were measured following procedures used in microscopic measurement by Benzon (1998). Calibration was done by inserting



the ocular micrometer on the eyepiece of the microscope while the stage micrometer was placed on the stage of the microscope. Focusing was done on the stage micrometer using the low power objectives (LPO) and then on the high power objective (HPO) of the microscope. Zero (0) point of the stage micrometer was set to coincide with that of the ocular micrometer. The lines on the ocular micrometer that coincides exactly with the lines of the stage micrometer were counted. Calibration factor (CF) or calibration constant (CC) was calculated using this formula:

 $CF = \frac{n \text{ division of stage micrometer}}{20 \text{ divisions of ocular micrometer}} \times 0.01 \text{ mm}$

0.01 mm – divisions of the stage micrometer

Pathogenicity Test

To confirm the results on the identification of the pathogen, a pathogenicity test was conducted.

Preparation of inoculum

Pure culture of *P. fragariae* grown in carrot agar for two weeks at 24 °C was used as inoculum. Petri plate cultures were filled with 10 ml rain water sufficient to barely cover the agar. The cultures were incubated for three days under continuous light to induce the production of additional sporangia after



which these were chilled for 30 minutes at 5°C and re-warmed at room temperature from 30 minutes to one hour to induce the release of zoospores.

Standardization of the inoculum

Suspensions containing zoospores were prepared and spore concentrations were estimated using a hemacytometer. One ml of the zoospore suspension was sucked using a micropipette and deposited on the ridge of the hemacytometer. After the suspension had flow automatically to the counting chamber, spore counting was done in the five squares of the nine squares, labeled A which contains 16 small squares. Standardization was done three times. The average count of the five squares per trial was multiplied with 20,000 to obtain the total spore/ml. The treatments were prepared by dilution adjusting the desired concentrations by adding sterile distilled water and inoculated into the test plants.

Inoculation

Healthy strawberry runners of cv. Sweet Charlie produced from BSU were surfaced sterilized with 1% NaOCl for a minute and rinsed three times with sterile distilled water. Strawberry plants were inoculated with varying concentrations of zoospores to obtain a range of disease infection. The roots of each test plant were sprayed with the corresponding zoospore concentration to the assigned treatments. The control plants were sprayed with distilled water alone. Inoculated plants were wrapped with tissue paper and placed in clear plastic bags before storing in the dark for 48 hours to enhance zoospore penetration before planting. Plants in



all treatments were planted in plastic pots measuring 20 x 25 cm diameter filled with heat sterilized soil.

The treatments were laid out following the CRD design replicated four times with three sample plants per replicate.

TREATMENTSINOCULUM LEVEL (zoospores/ml) T_0 0 (Uninoculated) T_1 $2 \ge 10^3$ T_2 $5 \ge 10^3$ T_3 $10 \ge 10^3$ T_4 $15 \ge 10^3$ T_5 $20 \ge 10^3$

The treatments were the following:

All cultural management practices for commercial strawberry production like watering, fertilization, weeding and control of insect pests and foliar diseases were employed.

Re-isolation of the Organism from Inoculated Plants.

The roots were further observed in the laboratory to confirm if the rotting symptoms were due to *P. fragariae*. Roots measuring 5-7 mm were excised and observed under the microscope for the presence of oospores and reddening of the root core.



Re-isolation of the organism from the inoculated plants was done further using the same procedure used earlier in the isolation of the organism. Characteristics of the isolate obtained were compared to the original inoculum used.

Data Gathered:

- Field symptoms. These include symptoms of red stele disease reported in other countries.
- 2. Disease incidence in the field (%). This refers to healthy and infected strawberry plants counted and computed following the formula by Waller *et. a.l* (2002)

Disease Incidence (%) = No. of infected plant units x 100% Total no. of plant units

- 3. Cultural and morphological characteristics of the pathogen. The growth including the structure of the pathogen in different culture media were described.
- 4. Pathogenicity Test
 - a. Monthly plant height (cm). This was obtained by measuring the initial and monthly height after transplanting to harvesting stage for a period of four months.



- b. Fresh top weight at harvest (g). This was taken by weighing the tops of strawberry plants after uprooting.
- c. Fresh root weight at harvest (g). The roots were washed to remove adhering soils, blotted dry in tissue paper and weighed.
- d. Oven dry top weight (g). This was done by weighing the tops of the plant after it was oven-dried at 70° C for 2 days.
- e. Oven dry root weight. This was done by weighing the roots after it was oven-dried at 70° C for 2 days.
- f. Yield components (g). The yield was classified as marketable and nonmarketable. Marketable yield are berries with big sizes (17mm and above) and of good quality. Berries with very small sizes with poor quality and those attacked by insects and other airborne fungal diseases were considered as non-marketable.
- g. Disease severity All plants in pots were uprooted and rated based on the rotting of the roots using the rating scale below.

Rating	% root infection
1	No reddening of the root core
2	1-25% rotten roots and with reddening of root core
3	26-50% rotten roots and with reddening of root core
4	51-75% rotten roots and with reddening of root core
5	76-100% rotten roots and with reddening of root core





Plate 5. Disease rating scale used in assessing the severity of root infection due to *P. fragariae*



RESULTS AND DISCUSSION

Field study

Symptomatology

Table 1. Field symptoms of infected strawberry plants in La Trinidad and
Sto.Tomas, Tuba, Benguet.

	SYMPTOMS				
Location	Stunting	Change in Leaf color	Wilting	Small Berries	Reddening of root core
La Trinidad	Viz				
Swamp block I	+		+	+	+
Swamp block II	auc+	Safee	et.	+	+
Swamp block III	+	+	+ 01	+	+
Swamp block IV	+	+	+	+	+
Balili expt'l area	+	+ -	+	ā + r	+
Long-long	+	+		+	+
Tuba					
Sto. Tomas	t,		2001-	t	+

Table 1 shows the different symptoms associated with red stele disease in strawberry plants grown in La Trinidad and Tuba, Benguet. Wilting, stunting, abnormal leaf colors, production of small berries and the reddening of the root core, which are characteristics of red stele disease as reported in earlier studies (Wescotte, 1978; Childers, 1983; Smith, 1984; Holliday, 1989; Alford and Locke, 1989; Swift and Dickens, 1995; and Ellis, 1996) were commonly observed in the strawberry growing areas visited. However, wilting was not observed in Sto.



Tomas and Longlong farms. Further, root samples taken from Sto. Tomas exhibited reddening of stele mostly on decaying old roots but not on newly developed roots. Uprooted plants from Swamp Area showed severe rotting in old roots and reddening of the core in newly developed roots.

Cultivars Tioga and Sweet Charlie showed no variation on symptoms of the disease (Plates 6-8). However, according to the farmers who were interviewed the old Tioga cultivar was more resistant than Sweet Charlie. They changed from Tioga to Sweet Charlie which is high yielding to meet the market demand. However, farmers observed that variety Sweet Charlie is susceptible to diseases. According to Philley (1995) Tioga which is currently grown in the United States is susceptible to red stele disease and *Verticillium* wilt.

Symptoms of wilting and stunting are commonly observed starting from the month of December until the end of summer where the weather is warm and dry or after heavy rains when the area is flooded. According to some farmers, they also observed symptoms similar to burning effect on the leaves and plant eventually dies after several days. Roots of plants showing these symptoms when dug up carefully have rotted roots and very few new roots. Roots of wilted plant showed some "rat tail" appearance as described previously (Ellis,1996 and Ries , 1995.

The symptoms observed in the field have some resemblance to other fungal diseases like *Verticillium* sp., *Pythium* sp. and *Fusarium* sp. According to Los (1992), Ries (1996) and Anderson (1956), the most reliable symptom is

found within the root. During the survey, several infected roots were peeled off exposing the core of the roots. The fleshy roots appeared white near the crown of the plant but a dark rot progressing upward from the tips was observed. Some of the roots, however, did not show reddening of the core but oospores were present when observed under the microscope. According to Khanizadeh and Buzard (1992), visual symptoms alone should not be used as criterion to assess susceptibility. Resistant cultivars are not immune from infection and relatively high number of oospores may be observed in the symptomless root tissues of inoculated resistant types within two weeks.

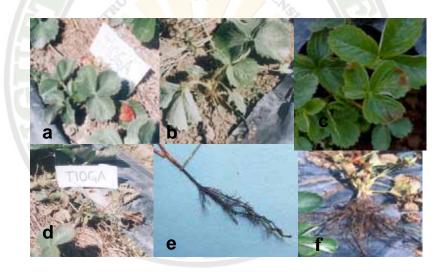


Plate 6. Symptoms of red stele disease on cv. Tioga (a) stunting, (b) wilting, (c) abnormal color of leaves, (d) death of severely infected plants (e) reddening of the core and (f) rotten roots





Plate 7. Symptoms of red stele on cv. Sweet Charlie (a) stunting, (b) wilting, (c) red colored stele, (d) abnormal color of leaves (e) death of severely infected plants and (f) rotten roots

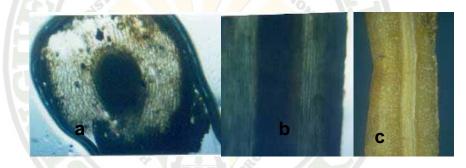


Plate 8. (a) Cross section of an infected root, x400 (b) longitudinal section of an infected strawberry root, x400 (c) healthy root with a yellow-white color of core, x400

Disease Incidence

Table 2 shows the incidence of the red stele disease in strawberry growing areas in La Trinidad and Tuba, Benguet. The lowest disease incidence was noted in Sto. Tomas, Tuba followed by Longlong, La Trinidad with 8.91% and 19.80%, respectively.



The root samples taken from Sto. Tomas area showed symptoms of the disease only in the old roots but not on newly developed ones. The infection in the old roots probably originated from the source of the planting materials. According to the farmers (personal interview, 2005), planting materials were obtained from the Swamp Area, BSU, La Trinidad, Benguet where the disease was first observed. However, the practice of overhead irrigation method and planting of strawberry in newly operated farms including the distance of one farm to the other area which is far enough to cause contamination might have contributed to the low incidence of the disease in this area.

On the other hand, strawberries grown in Swamp Area, BSU at La Trinidad, Benguet registered the highest infection particularly in Blocks II and III with 39.78% and 43.51% disease incidence, respectively. The high incidence of the disease in the Swamp Area could be attributed to the flat terrain and management practices of the farmers. During the rainy season, the farms are usually flooded for several days which are favorable for the organism to cause infection. It was also noted during the survey that farms with "dead rows" or no canals have higher infection compared to well maintain farms with raised beds of 7 up to 10 cm high. This confirms the findings of Ellis (1986) raising the beds to at least 8 to 10 cm prevents the roots from touching the water that favors the zoospore to swim and infect roots. Moreover, farmers at swamp and Balili areas at La Trinidad mostly irrigate their plants by flooding. According to Ries (1995), oospores from decayed roots are released into the soil when the soil is wet and



germinate by producing sporangia. Ellis (1996) added that the infective zoospores produced from the sporangia are disseminated by moving water and infect the tips of young fleshy roots of strawberry to which these are chemically attracted.

Another contributing factor in the disease development is the improper disposal of infected plant materials by farmers. Uprooted diseased plants are just left at the sides of the field which serve as source of inocula when condition is favorable.

Likewise, higher infection was observed in fields where planting materials were obtained from previously grown and harvested plants called daughter crowns which is a common practice to several farmers.

Location	AREA(sq.m.)	DISEASE INCIDENCE (%)
La Trinidad		
Swamp Block I	5,765	26.30
Swamp Block II	5,191	21.80
Swamp Block III	6438	43.51
Swamp Block IV	7,285	39.78
Balili	3,240	20.38
Long-long	2,313	19.80
Tuba		
Sto. Tomas	1194	8.91

 Table 2.
 Incidence of red stele disease in La Trinidad and Sto. Tomas, Tuba, Benguet



Laboratory Study

Characterization and Identification of the Pathogen

Mycelial growth of the pathogen on different culture media

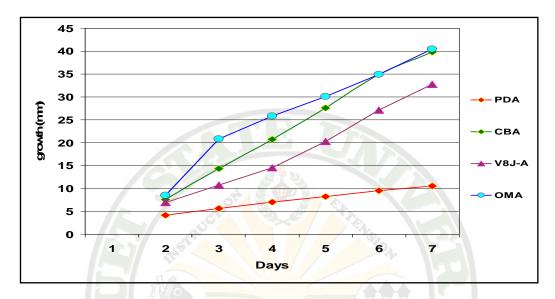


Figure 1. Average daily growth of the pathogen in different culture media

The average daily mycelial growth of the pathogen on different culture media is shown in Figure 1. The growth of the isolated organism is very slow compared to other fungal organisms which confirm the earlier report of Anderson (1956) that growth of *Phytophthora fragariae* is very slow when compared to other species of *Phytopthora* and grew only in very limited culture media.

Of the four culture media tested, OMA gave the widest colony diameter from 2nd to 7th day of incubation with a mean of 27.45mm after a week followed by CBA with a mean of 24.17 mm. V8J-A had a mean colony diameter of 18.71 mm and the smallest was observed on PDA with a mean 7.53 mm.



Cultural Characteristics of the Organism

	CULTURE MEDIA			
Criterion	PDA	CBA	OMA	V8J-A
Colony diameter after a week of incubation (mm)	10.55 ^c	39.85 ^a	40.45 ^a	32.75 ^b
Growth characteristic after a week of incubation	Very slow growth	Fast growth	Fast growth	Slightly fast growth
Characteristics of mycelia	Very dense, irregular and spreading pattern	Dense, fluffy	Very scarce, barely seen on media	Slightly dense, round with radiating margin
Color of mycelia	White	Brown to white	White	Brown to white

Table 3. Cultural characteristics of the pathogen in different culture media

The cultural characteristics of the pathogen in different culture media are shown in Table 3. Rapid mycelial growth was noted on OMA and CBA with colony diameters of 40.45mm and 39.85mm, respectively. On the other hand, the growth on PDA is very slow with a colony diameter of 10.55 mm. In PDA, the organism has very dense mycelia, with irregular and spreading pattern and white in color (Plate 9). On the other hand, mycelial growth on CBA is dense, fluffy, with round pattern and brown to white in color (Plate 10). Although rapid growth was observed on OMA, the mycelia are very scarce and could hardly be seen on media a week after isolation but after one month a very thin cotton-like appearance on the surface of the media with white color was observed. Growth



of the organism in V8J-A is slightly faster with brown to white mycelia and mycelial growth pattern of round with radiating margins (Plate 11).



Plate 9. Mycelial growth of the pathogen on PDA after a week of incubation

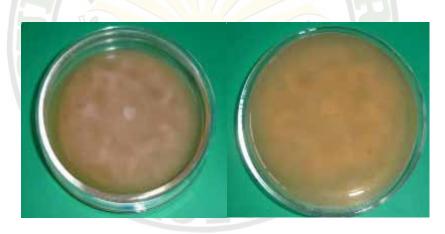


Plate 10. Mycelial growth of the pathogen on CBA after a week of incubation



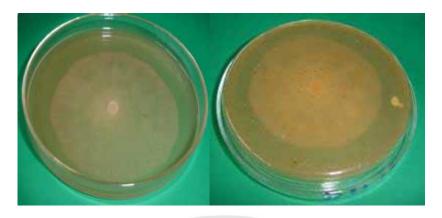


Plate 11. Mycelial growth of the pathogen on V8J-A after a week of incubation



Plate 12. Mycelial growth of the pathogen on OMA after a week of incubation

Morphological characteristics of the organism

<u>Hypha</u>. The hyphae are coenocytic, hyaline and slender with few swelling of hyphae on media CBA, OMA and V8JA, as compared to PDA where profuse swellings are observed (Plate 13).

<u>Oospore</u>. The oospores are round in shape, with diameter ranging from 33-41 *um*. They are found readily in culture grown in CBA, V8J-A and OMA but not on PDA. The oospores are surrounded by wrinkled, dark red oogonial



envelops. The antheridia were found attached to the base of the oospore. Oospores are also observed on excised roots from infected plants (Plate 14).

<u>Sporangia</u>. Sporangia were not readily observed on the four culture media used. These were produced after incubation of agar disc in 5% V8 juice or rainwater for 3 days. Sporangia were observed on CBA, V8J-A and OMA but not on PDA. The number of sporangia increased as the incubation period was prolonged. Likewise, sporangia from infected roots were produced by incubating excised roots in rain water. Sporangia were found to grow along the infected tissue. The sporangia are non-papillate, inversely pear shaped or oval, with measurement ranging from 53-72 *um* (Plate 15).

Zoospore. The zoospores germinated abundantly after chilling the agar disc containing sporangia at a 5° C temperature. These are very motile and swam immediately after coming out from the sporangia but encysted after an hour. The zoospore has 2 flagella and has round shape when at rest (Plate 16). According to Anderson (1956) zoospore measures 12 *um* and each sporangium contains 40 to 50 zoospores. Based from the reports of CABI (undated), the motile zoospore swims to the root tips of the host plant where they encysted, attach itself and form germ tubes which penetrate into the roots. The organsim traverses the cortex inter- and intracellularly to the stele, mainly colonizing the pericycle and the phloem. Growth is mostly concentrated on the stele but hyphae may grow out from the roots and form new sporangia.



The morphological and cultural characteristics of the organism are very similar to *Phytophthora fragariae* as described by Anderson (1956) and Stamps *et. al* (1990).

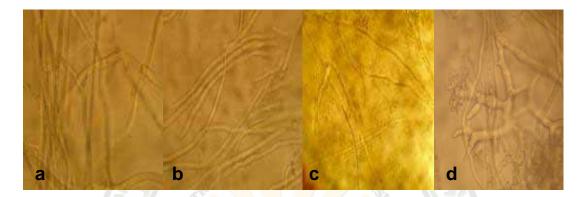


Plate 13. Hyphae, x400 (a) CBA, (b) V8J-A, (c) OMA, (D) PDA

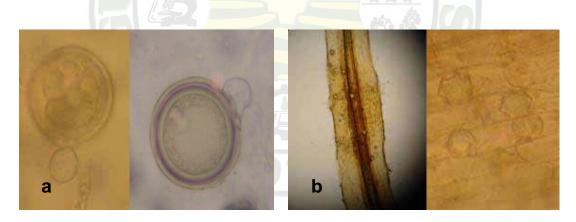


Plate 14. Ooospore (a) from culture media, x1000 (b) infected root, x400



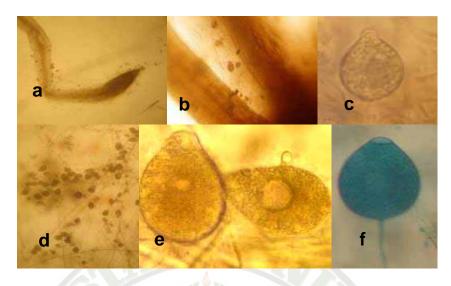


Plate 15. Sporangia from (a) infected root, x100 (b) infected root, x400 (c) infected root, x1000 (d) culture media, x400 (e) culture media, x1000 (f) stained sporangium showing the wide pore opening (non- papillate), x1000

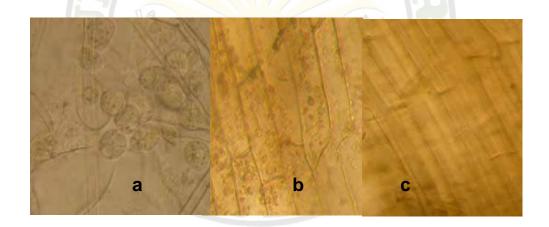


Plate 16. (a) Encysted zoospores released from a sporangium, x1000 (b) encysted zoospores inside cells of infected root, x400 (c) healthy plant cell



Pathogenicity Test

Symptoms on inoculated plants

The causal organism was inoculated to healthy plants and symptoms of stunting were observed a month after inoculation on some of the treated plants. The leaves were small, cupped and not spreading compared to the control. Some plants appeared healthy but when observed closely the leaves were off colored. They had blue green color compared to the uninoculated plants where leaves were shiny green. Other symptoms like wilting, yellowing of older leaves and productions of small berries were noted during the harvesting stage. Wilting occurred during warm weather. Abnormal change of color on leaves from green to red or purple was observed but on very few plants. Plants inoculated with 15 x 10^3 and 20 x 10^3 zoospores/ml were stunted and produced small The above observations were similar to the findings of Wescotte, (1978), berries. Childers (1983), Swift and Dickens (1995), Ries (1995) and Ellis (1996). According to Ries (1996), the stunting of the plant is due to the infection of the zoospore on the roots causing rotting which destroyed the water-and-food conducting tissues. The infection and growth of the fungus in the plant roots reduced the flow of water and nutrients to the developing leaves and fruit causing drought-like symptoms in the plant.





Plate 17. Strawberry plants 7 months after inoculation



Plate 18. (a) Symptom of stunting on inoculated plants (b) uninoculated plants



Plate 19. (a) Symptom of abnormal color on the shoot (b) uninoculated plant





Plate 20. (a) Symptom of yellowing of leaves on inoculated plants (b) uninoculated plant



Plate 21. (a) Symptom of wilting on inoculated plants (b) uninoculated plant



Plate 22. (a) Production of small berries and (b) death of severely infected plant



Inoculated plants when uprooted produced very few new roots and most of the main roots were rotten as compared to the uninoculated plants where roots are thick and bushy with several secondary feeder roots. The "rat tail" appearance was observed on infected roots (Plate 24a). Infected roots have brick red to brownish red stele progressing upward from the tip (Plate 24b) compared to healthy roots with yellowish white core (Plate 24c). The crown of the strawberry plant was not infected which confirms reports of Ellis (1996) and several authors that the fungus does not attack the crown. Microscopic examination of infected plants confirmed that the causal organism is *P. fragaria* as evidenced by the production of several oospores on infected roots. In addition, several zoospores were also found feeding on the root tissues. George and Milholland (1986) reported that the number of oospore per root segment in a susceptible variety inoculated with 1, 5, and 10×10^3 zoospores/ml was 17, 22 and 29, respectively. Results of the pathogenicity test further confirms the previous reports that P. fragariae is the causal organism of red stele disease.



Plate 23. (a) Symptoms of rotting of strawberry roots due to *P. fragariae* (b) root of uninoculated plants



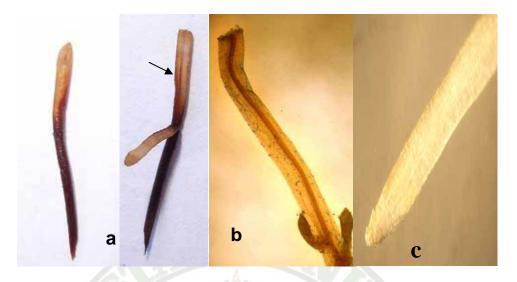


Plate 24. Excised roots showing the (a) "rat tail" appearance (b) reddening of the core, x400 (c) root of a healthy plant, x400

Plant Height (mm)

Result shows that the different zoospore concentrations influenced the growth of the crop (Figure 2). Plants inoculated with the highest concentration of 20 x 10^3 were the shortest with mean height of 139.58 mm after 4 months followed by those plants inoculated with 15 x 10^3 and 10 x 10^3 with 157.84 and 159.17mm, respectively. Early symptom of stunting was observed after a month on the treated plants. Plants inoculated with lower zoospore concentrations of 2 x 10^3 and 5 x 10^3 showed slight stunting and wilting only at harvest. The uninoculated plants were the tallest with mean height of 254.17 mm.

Inoculation of 20 x 10^3 zoospores/ml caused higher infection to the roots resulting to early stunting of the plant. The main roots were rotten and very few



new developed roots were produced compared to the uninoculated plants with thick and bushy roots with several secondary feeder roots.

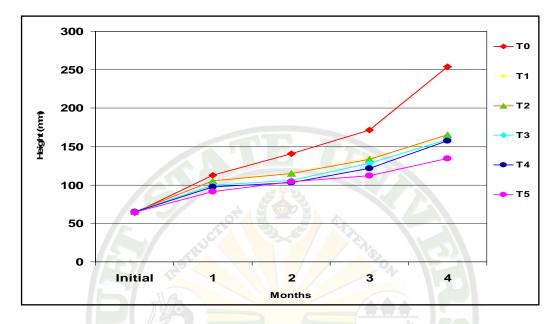


Figure 2. Average monthly height (mm) of strawberry plants as affected by inoculum level (Note: 0 = uninoculated, $1 = 2 \times 10^3$ zoospore/ml, $2 = 5 \times 10^3$ zoospore/ml, $3 = 10 \times 10^3$ zoospore/ml, $4 = 15 \times 10^3$ zoospore/ml, $5 = 20 \times 10^3$ zoospore/ml)

Yield

The infection caused by the disease on the inoculated plants greatly influenced the yield of strawberry (Figure 3). There was a reduction in yield from 38.03 to 57.74% on inoculated plants compared to the uninoculated ones. The different zoospore concentrations showed significant effect on the inoculated plants. Plants with the highest concentration of 20×10^3 produced the lowest mean yield of 117.91g while the highest was obtained from the uninoculated



plants with 279.05g. However, the yield of the plants inoculated with 2 x 10^3 , 5 x 10^3 and 10 x 10^3 zoospopres/ml were comparable.

On the other hand, the highest non-marketable yield was obtained from plants inoculated with 20 x 10^3 zoospores/ml with 75.93g but did not differ significantly with those plants inoculated with lower inoculum levels. The lowest non-marketable yield was obtained from the uninoculated and in plants that received the lowest inoculum level with 55.54 and 56.06g, respectively.

Results reveal that inoculum density of the organism directly affected the yield of the crop. The mean yield was observed to decrease significantly as the inoculum density was increased. High inoculum density resulted to the production of few and small berries. This is dew to the rotting of the roots and production of few secondary feeder roots resulting to early symptoms of stunting. According to Reid, (1949) as cited by CABI (undated), damage of the disease is most severe after winters with yield as low as 1 t/ha, mostly of small fruit of poor quality. In Nova Scotia (Canada), it was estimated that in one season 78% of the strawberry area was rendered unproductive causing losses to growers more than 1500 Can\$ per hectare (Gourly and Delbridge, 1972 as cited by CABI).



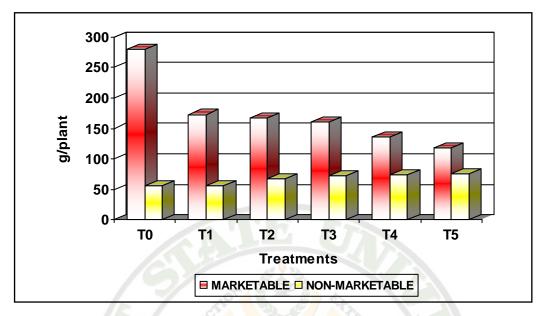


Figure 3. Effect of inoculum level of *P. fragariae* on the yield of strawberry (Note: 0 = uninoculated, 1= 2 x10³ zoospore/ml, 2 = 5 x10³ zoospore/ml, 3 = 10 x10³ zoospore/ml, 4 = 15 x10³ zoospore/ml, 5 = 20 x10³ zoospore/ml)

Fresh Weight of Plants

The fresh weight of the plant after harvest was taken to determine the effect of the different zoospore concentrations on the weight of strawberry plant (Figure 4). The lowest fresh top weight was obtained from plants inoculated with 15×10^3 and 20×10^3 zoospores/ml with means of 16.18 and 16.83g, respectively. This was due to severe stunting of inoculated plants. Inoculated plants were stunted and have small and cupped leaves as compared to the uninoculated ones with fully expanded leaves. Plants inoculated with 2×10^3 and 5×10^3 zoospores per ml showed slight symptom of stunting and wilting during the latter part of the harvesting period. However, statistical analysis shows insignificant differences



on plants inoculated with 2 x 10^3 , 5 x 10^3 and 10 x 10^3 zoospores/ml. The uninoculated plants significantly had the heaviest weight of 53.53g.

Similarly, fresh root weight was significantly affected by the fungal infection (Figure 4). Roots of inoculated plants showed rotting or decaying of the main roots. Very few secondary feeder roots were also produced. Plants inoculated with the highest level of inoculum were severely infected and gave the lowest fresh root weight of 6.05g followed by those inoculated with 15 x 10^3 and 10×10^3 zoospores/ml with 10.53 and 11.02g, respectively. On the other hand, the roots of uninoculated plants which were thick and bushy with several secondary feeder roots had the heaviest fresh root weight of 28.28g.

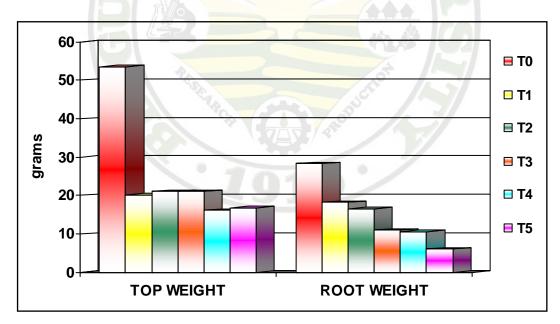


Figure 4. Effect of varying levels of *P. fragariae* inoculum on the fresh weight of strawberry (Note: 0= uninoculated, $1=2 \times 10^3$ zoospore/ml, $2 = 5 \times 10^3$ zoospore/ml, $3 = 10 \times 10^3$ zoospore/ml, $4 = 15 \times 10^3$ zoospore/ml, $5 = 20 \times 10^3$ zoospore/ml)

44

Oven dry weight of plants

The uninoculated plants gave significantly heavier top and root oven dry weights of 16.07 and 6.75, respectively compared to the inoculated plants (Figure 5). Inoculated plants exhibited rotting of the roots caused by the fungus resulting to stunting and wilting thus having lighter top and root weight. Plants that received the highest inoculum level of 20×10^3 zoospores/ml had the lowest top and root weights with 2.44 and 2.12g, respectively.

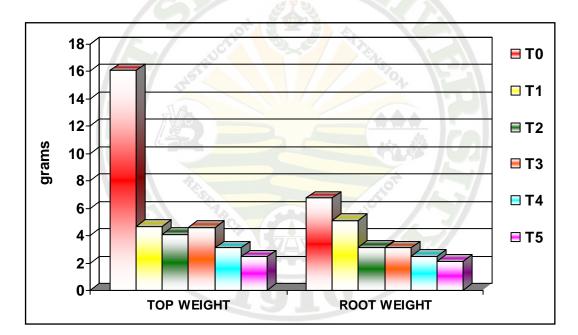


Figure 5. Effect pf varying levels of *P. fragariae* inoculum on the dry weight of strawberry (Note: 0= uninoculated, 1= 2 x10³ zoospore/ml, 2 = 5 x10³ zoospore/ml, 3 = 10 x10³ zoospore/ml, 4 = 15 x10³ zoospore/ml, 5 = 20 x10³ zoospore/ml)

Disease Severity at Harvest

An arbitrary rating scale was developed to assess the effect of the inoculum density on the severity of the disease on inoculated plants under pot test.



Result shows that inoculated plants have disease rating ranging from 3.67 to 5 equivalent to 26 up to 100% root infection (Figure 6).

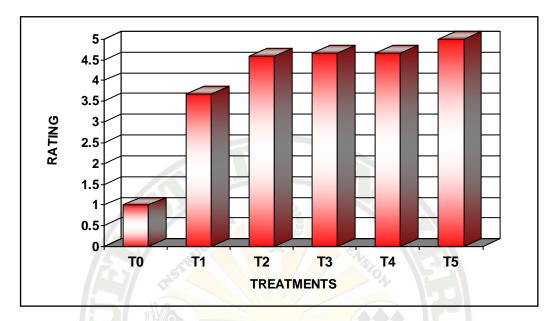


Figure 6. Disease severity as affected by inoculum density (Note: 0 = uninoculated, $1 = 2 \times 10^3$ zoospore/ml, $2 = 5 \times 10^3$ zoospore/ml, $3 = 10 \times 10^3$ zoospore/ml, $4 = 15 \times 10^3$ zoospore/ml, $5 = 20 \times 10^3$ zoospore/ml)

Infected plants within these range showed yellowing of leaves, wilting and stunting. Very few new roots were produced and most of the feeder roots have infected cores with reddening symptom on stele. Moreover, excised roots incubated in rain water for 3 days produced several oospores and sporangia.

According to George and Milholland (1986) oospore development by *P*. *fragariae* in roots of susceptible plants varied with inoculum density. Plants inoculated with 1, 5 and 10 x 10^3 zoospores/ml produced 17, 22 and 29 oospores per root segment. There was no significant difference in oospore production or percentage of root segments containing oospore when susceptible cultivar



Tennessee and resistant cultivar Stelemaster were inoculated with 1, 5, or 10×10^3 zoospores per milliliter.

Furthermore, significant difference was observed in oospore development between the two cultivars when inoculated with 10, 30 or $100 \ge 10^3$ zoopores/ml. The susceptible cultivar gave significantly greater number of oospores than resistant Stelemaster plants inoculated with the same concentrations of zoospores. Linear regression analysis had no significant effect on mean oospore production in the susceptible or resistant cultivars, but differences did occur in the percentage of susceptible root segments containing oospores among the three concentrations tested.





SUMMARY, CONCLUSION AND RECOMMENDATION

<u>Summary</u>

Symptoms of the red stele disease such as stunting, wilting, off-colored leaves, abnormal color of leaves from green to purple, yellowing of immature leaves, production of small berries and reddening of the core were mostly observed in the areas surveyed. High incidence of the disease was observed in Swamp area, BSU, La Trinidad, Benguet ranging from 23-43% infection while lower infection was observed in Longlong, La Trinidad and Sto. Tomas, Tuba with 19.80 and 8.91%, respectively.

The pathogen grows rapidly on OMA and CBA with mean mycelial colony diameter of 27.45 and 24.17mm, respectively. On the other hand, the growth on PDA is very poor with mean colony diameter of 7.53mm. The mycelia are white in color on PDA and OMA while brown to white on CBA and V8J-A media. Mycelial growth is very dense on PDA, slightly dense on CBA and V8J-A while mycelia on OMA are very scarce and can hardly be seen on culture.

The causal organism was identified as *Phytophthora fragariae*. The hyphae are coenocytic, hyaline and slender but profuse swelling of hyphae was observed on PDA. The oospores of the pathogen were readily observed on CBA and V8J-A and OMA but not on PDA. The oospores are round in shape and surrounded by a dark, red, wrinkled oogonial envelop. Several sporangia were observed on CBA and V8J-A but very few on OMA after incubating agar disc in



5% V8 juice for 4 days. No sporangial growth was observed on PDA. The sporangia are non-papillate with oval or inverted pear-shape. Zoospores are very motile with two flagella and are in round shape when encysted.

Strawberry plants inoculated with different concentrations of inoculum developed symptoms of stunting, wilting, off-colored leaves from shiny green to bluish - green, yellowing of immature leaves, small berries and death of severely infected plants. Abnormal discoloration of the leaves from green to purple was observed but on very few plants. Rotting of the main roots and reddening of the stele of new roots which is the most distinct symptoms of the disease was observed. Plants inoculated with the highest zoospore concentration of 20×10^3 significantly had the shortest mean height of 139.58mm after 4 months, the lowest fresh and oven dry weight of top and roots and the lowest marketable yield of 117.91g.

Inoculated plants have disease rating ranging from 3.67 to 5 which is equivalent to 26 - 100% root infection.

Conclusions

Results of the investigation revealed that no symptom variations were observed in strawberry cultivars infected in the field The high incidence of the disease observed at swamp area is attributed to its flat terrain with poor drainage and the cultural management of the farmers.



The causal organism was identified as *Phytophthora fragariae* based on morphological and cultural characteristics as earlier reported. The pathogen grows best on carrot broth agar.

Higher concentration of zoospores inoculated on strawberry plants resulted to severe infection. The disease can cause a yield reduction of 38 to 57% on strawberry plants and can be considered a threat to strawberry production in La Trinidad and Tuba, Benguet.

Recommendations

Based on the above findings, the following are recommended:

- 1. Infected plants showing symptoms of stunting and wilting with reddening of the root core/stele can be used as basis in the early detection of red stele disease in the field.
- 2. Proper cultural management and strict quarantine measures should be implemented to prevent the further spread of the disease and introduction of new diseases.
- 3. Further studies should focus on the development of cost effective and ecofriendly management strategies not only for *Phytophthora fragariae* but for all soilborne diseases of strawberry.
- 4. Verification studies should be done to determine the possible involvement of *P. fragariae* in a soil-borne disease complex currently observed in the field.



LITERATURE CITED

- AGRIOS, N. G. 2005. Plant Pathology, 5th ed. Elsevier Academic Press.London. Pp. 414-420.
- ALFORD, D.O and T. LOCKE. 1989. Pest and Diseases of fruits and hops. Pest and Disease Control Handbook 2nd ed.. Crop Protection Council. Pp 389-390.
- ANDERSON, H.W. 1956. Diseases of strawberries. McGraw-Hill Book Company, Inc. Pp. 411-420.
- BENSON, H.J. 1998. Microscopic Measurements. Microbiological Application 7th Ed. McGraw-Hill. Pp22-24.
- CABI (undated). *Phytophthora fragariae*. Data Sheets on Quarantine Pests.
- CLARK, D.E. 1984. How to grow fruits, nuts and berries. California Lans Publishing Company. P. 77.
- CHILDERS, N.F. 1983. Modern fruit science. University of Florida, Gainsville. P. 477.
- CORONEL, R.E. 1983. Promising fruits of the Philippines. University of the Philippines, Los Banos, College of Agriculture, Pp. 431-447.
- ELLIS, M.A. 1996. Red stele on strawberry. Ohio State University Extension Fact sheet. Plant Pathology, Columbus, Ohio. http://www.ag.ohio-state.edu~ohioline/hyg-fact/3000/3014.html.
- ENCARTA ENCYCLOPEDIA. 2006. Strawberry. Bruce Coleman Incorporation.
- FERGUSON, B. 1982. All about growing fruits, nuts and berries. San Francisco: Chevron Chemical Company. P. 103.
- GEORGE. S.W. and R.D., MILHOLLAND. 1986. Inoculation and Evaluation of Strawberry Plants with *Phytophthora fragariae*. Plant Disease 70.371-375.



- HARRDEC. 1996. Benguet Strawberry Techno guide. La Trinidad, Benguet HARRDEC-PARRFI. 46pp.
- HERMANO, G.F. 1995. Strawberry Production Technology and Management in the Highlands. A paper presented during the National Fruit Congress and Exhibition. Cagayan de Oro. 8Pp.
- HERMANO, G.F. 1999. Strawberry Production Management and Technoguide Ist ed., La Trinidad, Benguet. 51pp.
- HOLLIDAY, P. 1989. DICTIONARY OF Plant pathology. Kew. U.K. CAB International Mycological Institute, P. 236.
- HORSFALL, J.G. 1978. Plant Disease: An Advance Treatise. Vol. 2. p.215.
- KHANIZADEH. S. AND D. BUZARD. 1992. Red Stele Resistant Strawberries and their Role in Epidemiology of *Phytophthora fragariariae*. HortScience 27:870-871. www.octahort.org.
- LAW, T.F. and R.D., MILHOLLAND. 1991. Production of sporangia and oospores of *Phytophthora fragariae* in roots of strawberry plants. Plant Disease 75:475-478.
- LOS, L.M. and M.L., SCHROEDER. 1992. Red stele of strawberries. dentification Sheet No. 2, University of Connecticut, New york. http://www.hort.uconn.edu/ipm/homegrnd/htms/26strstl.htm
- STAMPS. D.J, G.M. WATERHOUSE, F.J. NEWHOOK AND G.S. HALL. 1990. Revised Tabular Key to the Species of *Phytophthora*. CAB International Mycological Institute. U.K. 28pp.
- REIS, S.M. 1996. Strawberry Red Stele Root Rot. Report on Plant Disease No. 701. <u>http://www.ipm.uiuc.edu/fruit/diseases/red-stele-root-rot/index.html</u>
- SCOPES, N. and L., STABLES. 1989. Pest and Disease Handbook 3rd Ed. British Crop Protection Council, England. Pp. 389-390.
- SWIFT, C.E and L.E., DICKENS. 1995. Strawberry diseases. Colorado State University, fort Collins, Colorado.
- SMITH, M.D. 1984. The Ortho Problem Solver. 2nd Ed. San Francisco, California. Ortho Information Service. P. 766.



- WALLER, J.M, J.M. LENNE and S.J. WALLER. 2002. Plant Pathologist Pocketbook. 3rd. ed., CABI Publishing, Wallington, UK. Pp. 27, 322.
- WESCOTTE, C. 1978. Plant Disease Handbook. 2nd Ed. Canada. D.V. Nostrand Comapany. P.336.





APPENDICES

APPENDIX TABLE 1. Components of culture media used

a. PARPH

Components	Amount
Pimaricin	5 mg
Ampicilin	200 mg
Rifampicin	10 mg
PCNB	100 mg
Hymexazol	10 mg
cornmeal agar	17 g
Distilled water	1 liter
b. CARROT BROTH AGAR	
Components	Amount
Carrot 7916	200 g
Bacto-agar	15 g
Distilled water	200 ml

c. V8 JUICE AGAR

<u>Components</u>	Amount
V8 juice	200 ml
CaCO3	2 g
Agar	17 g
Distilled water	800 ml

d. POTATO DEXTROSE AGAR

Components	Amount
Potato	250 g
Agar	20 g
Dextrose	20 g
Distilled water	1 liter



					Fie	eld					_	
Media	1	2	3	4	5	6	7	8	9	10	TOTAL	MEAN
LA TRINIDAD												
Swamp Area												
Block I	6.11	36.15	14.54	13.54	79.72	12.81	9.11	14.94	23.80	52.29	263.01	26.30
Block II	23.28	34.09	14.87	17.22	36.70	<mark>44.09</mark>	14.73	6.34	16.63	10.04	217.99	21.80
Block III	9.21	54.46	40.08	73.24	<mark>40.</mark> 36	21.28	65.66	68.88	30.64	33.30	437.11	43.51
Block IV	18.46	43.54	63	69.00	70.72	52.29	48.34	7.96	13.66	10.78	397.75	39.78
Balili, La												
Trinidad ^{1/}	9.68	4.63	21.50	20.33	11.77	23.66			-	-	91.57	20.38
Long Long ^{1/}	21.94	26.11	21.03	10.15		-	150.0	<u>a</u> [7	n -	-	79.23	19.81
TUBA												
Sto. Tomas ^{$1/$}	6.50	10.00	7.15	12.00	-	-	.07		- 1	-	35.65	8.91
GRAND TOTAL											1522.31	
GRAND MEAN				C.		205						18.05
^{1/} - Few farms were plan	nted with s	strawberry			NA)		1	5/				

APPENDIX TABLE 2. Incidence of red stele disease in the different strawberry growing areas of La Trinidad and Sto. Tomas, Tuba, Benguet



		1	Replicatio				
Media	1	2	3	4	5	Total	Mean
T ₁ - PDA	4.50	4.25	3.50	4.75	4.00	21.00	4.20 ^b
T ₂ - CBA	8.50	9.50	7.25	6.50	6.00	37.75	7.55 ^a
T ₃ - V8J-A	6.50	7.00	10.00	4.75	6.25	34.50	6.90 ^a
T ₄ - OMA	9.50	10.50	8.25	7.50	7.00	42.75	8.55 ^a
GRAND TOTAL	67	2	113.			136.00	
GRAND MEAN	THOTH	3055		THEN.			6.80
GRAND MEAN		ANALYS	SIS OF V	ARIANO	CE		6.80
Source of	Degree o	f Sum	of Me	eans of	, of	Tał	
	ALES &	f Sum	of Me		CE Computed F		6.80 Dular F 1%
Source of	Degree o	f Sum	of Me are so	eans of	Computed		oular F
Source of Variance	Degree o Freedom	f Sum 1 squa	of Me are so 98 1	eans of quare	Computed F	5%	oular F 1%

APPENDIX TABLE 3. Mycelial growth of the pathogen 2 days after isolation

**Highly significant

CV = 20.19%



Media	1	2	3	4	5	Total	Mean
$T_1 - PDA$	5.50	6.25	5.88	5.35	5.10	28.08	5.62 ^d
$T_2 - CBA$	17.60	15.75	13.25	12.75	12.50	71.85	14.37 ^b
T ₃ - V8J-A	10.75	10.50	15.00	8.75	8.50	53.50	10.70 ^c
T ₄ - OMA	20.25	22.50	23.75	20.00	17.75	104.25	20.85 ^a
GRAND TOTAL			10			257.68	
GRAND MEAN							12.88
	A	NALYS	IS OF V	ARIANC	E	RS	
Source of Variance	Degree of Freedom			leans of square	Comput	ted T	abular F
variance	Fieedom	squ		-	F	5%	
TREATMENT	3 •	616	.30	205.43	47.27*	** 3.24	4 5.29
ERROR	16	69.	54	4.35			
TOTAL	19	685	.84				

APPENDIX TABLE 4. Mycelial growth of the pathogen 3 days after isolation

** Highly significant

CV = 16.18%



Media	1	2	3	4	5	Total	Mean
$T_1 - PDA$	6.50	8.25	8.25	6.00	6.25	35.25	7.05 ^d
$T_2 - CBA$	22.75	22.00	19.25	19.25	20.25	103.50	20.70 ^b
T ₃ - V8J-A	14.75	14.25	20.00	12.75	11.00	72.75	14.55 ^c
T ₄ - OMA	25.25	27.50	28.75	25.00	22.75	129.25	25.85 ^a
GRAND TOTAL		1		ZV.		340.75	
GRAND MEAN							17.04

APPENDIX TABLE 5. Mycelial growth of the pathogen 4 days after isolation

ANALYSIS	OF VARIAN	NCE	

Source of Variance	Degree of Freedom			Computed	Tabı	ılar F
			square	F	5%	1%
TREATMENT	3	985.06	328.35	63.50**	3.24	5.29
ERROR	16	82.73	5.17			
TOTAL	19	1,067.79				

**Highly significant

CV = 13.35%



Replication									
Media	1	2	3	4	5	Total	Mean		
$T_1 - PDA$	8.00	8.50	9.35	8.75	6.75	41.35	8.27 ^c		
$T_2 - CBA$	28.00	29.25	26.25	26.25	28.25	138.00	27.60 ^a		
T ₃ - V8J-A	19.75	20.00	26.50	17.75	17.25	101.25	20.25 ^b		
T ₄ - OMA	30.25	31.50	32.75	28.00	28.00	150.50	30.10 ^a		
GRAND TOTAL	653		4 32.	×.		431.10			
GRAND MEAN							21.55		
		NALYSIS							
Source of Variance	Degree of Freedom	Sum of squar		eans of quare	Computed	Tab	ular F		
v arrance		Squar		quare	F	5%	1%		
TREATMENT	3	1,438.7	77 4	179.59	92.20**	3.24	5.29		
ERROR	16	83.2	23	5.20					

1,522.00

19

APPENDIX TABLE 6. Mycelial growth of the pathogen 5 days after isolation

** Highly significant

TOTAL

CV = 10.58%



Replication								
Media	1	2	3	4	5	Total	Mean	
T ₁ - PDA	9.50	8.75	10.50	11.50	7.25	47.50	9.50 ^c	
T ₂ - CBA	35.25	36.50	33.25	33.25	36.50	174.75	34.95 ^a	
T ₃ - V8J-A	25.50	25.75	33.00	27.75	23.50	135.50	27.10 ^b	
T ₄ - OMA	35.25	35.75	37.00	32.25	34.25	174.50	34.90 ^a	
GRAND TOTAL		1	4.			532.25		
GRAND MEAN							26.61	
	INSTITU			Stor				
	A	NALYSIS	S OF VAR	IANCE				
Source of Degree of Sum of Means of Variance Freedom square Computed Tabular F								

APPENDIX TABLE 7. Mycelial growth of the pathogen 6 days after isolation

e e	10			to A		
Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tabu	llar F
		745		F	5%	1%
TREATMENT	3	2,156.36	718.79	132.83**	3.24	5.29
ERROR	16	86.58	5.41			
TOTAL	19	2,242.94				

** Highly significant

CV = 8.74%



Media	1	2	3	4	5	Total	Mean		
T ₁ - PDA	11.00	9.25	11.75	12.50	8.25	52.75	10.55 ^c		
T ₂ - CBA	40.25	40.00	38.00	39.25	41.75	199.25	39.85 ^a		
T ₃ - V8J-A	31.00	31.25	40.00	32.50	29.00	163.75	32.75 ^b		
T ₄ - OMA	40.50	40.75	41.25	40.00	39.75	202.25	40.45 ^a		
GRAND TOTAL		1	4.			618.00			
GRAND MEAN	7	CTION		EATRES.			30.90		
ANALYSIS OF VARIANCE									

APPENDIX TABLE 8. Mycelial growth of the pathogen 7 days after isolation

ANALYSIS OF VARIANCE									
Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed _	Tabular F 5% 1%				
TREATMENT	3	2,944.25	981.42	168.09**	3.24	5.29			
ERROR	16	93.42	5.84						
TOTAL	19	3,037.67							

** Highly significant

CV = 7.82%



	Replication						
1	2	3	4	Total	Mean		
50.67	67.00	65.67	73.00	256.34	64.09 ^a		
58.33	63.00	65.00	70.33	256.66	64.17 ^a		
50.66	73.33	70.12	66.66	260.77	65.19 ^a		
57.33	70.67	65.00	66.67	259.67	64.92 ^a		
69.67	64.33	58.00	67.33	259.33	64.83 ^a		
50 <mark>.3</mark> 3	77.00	62.00	69.33	258.66	64.67 ^a		
	1/	Stor of	3	1551.43			
					64.64		
	50.67 58.33 50.66 57.33 69.67	50.6767.0058.3363.0050.6673.3357.3370.6769.6764.33	50.6767.0065.6758.3363.0065.0050.6673.3370.1257.3370.6765.0069.6764.3358.00	50.6767.0065.6773.0058.3363.0065.0070.3350.6673.3370.1266.6657.3370.6765.0066.6769.6764.3358.0067.33	50.67 67.00 65.67 73.00 256.34 58.33 63.00 65.00 70.33 256.66 50.66 73.33 70.12 66.66 260.77 57.33 70.67 65.00 66.67 259.67 69.67 64.33 58.00 67.33 259.33 50.33 77.00 62.00 69.33 258.66		

APPENDIX TABLE 9. Initial plant height of strawberry plants

ANALYSIS OF VARIANCE

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed F	Tabı 5%	ılar F 1%
TREATMENT	5	3.81	0.76	0.01 ^{ns}	2.77	4.25
ERROR	18	,205.23	66.96			
TOTAL	23	,209.04				

ns- not significant

CV = 12.66%



Inoculum Density		Repl	ication			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	53.33	90.67	88.33	105.00	337.33	112.44 ^a
$T_1 - 2 \ x \ 10^3$	73.33	75.33	80.00	90.00	318.66	106.22 ^a
$T_2 - 5 \ge 10^3$	76.67	90.67	73.33	75.00	315.67	105.22 ^a
$T_3 - 10 \ge 10^3$	59.33	85.67	68.33	85.67	299.00	99.67 ^a
$T_4 - 15 \times 10^3$	56.67	78.33	64.33	93.33	292.66	97.55 ^a
$T_5 - 20 \times 10^3$	<mark>61.67</mark>	65.67	75.00	71.67	274.01	91.34 ^a
GRAND TOTAL			-ON		1837.33	
GRAND MEAN		É				102.07

APPENDIX TABLE 10. Effect of inoculum levels of *P. fragariae* on plant height of strawberry 1 month after planting

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed F	<u>Tabı</u> 5%	ular F 1%
TREATMENT	5	621.34	124.27	0.70 ^{ns}	2.77	4.25
ERROR	18	3,207.92	78.22			
TOTAL	23	3,829.26				

ns-not significant

CV = 17.44%



Inoculum Density		Replic	cation			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (uninoculated)	91.67	118.33	98.33	113.33	421.66	105.42 ^a
$T_1 - 2 \ x \ 10^3$	75.33	78.00	92.33	103.33	348.99	87.25 ^{ab}
$T_2 - 5x \ 10^3$	80.00	103.33	75.33	86.00	344.66	86.17 ^{ab}
$T_3 - 10 \ge 10^3$	60.00	93.33	69.67	95.00	318.00	79.50 ^b
$T_4 - 15 \times 10^3$	63.33	80.33	68.33	97.67	309.66	77.42 ^b
$T_5 - 20 \times 10^3$	<mark>68.33</mark>	75.33	93.67	76.33	313.66	78.42 ^b
GRAND TOTAL		V/	O.V		2056.63	
GRAND MEAN						85.70

APPENDIX TABLE 11. Effect of inoculum levels of *P. fragariae* on plant height of strawberry 2 months after planting

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed F	<u>Tabi</u> 5%	ular F 1%
TREATMENT	5	4,654.26	930.85	3.03*	2.77	4.25
ERROR	18	5,536.07	07.56			
TOTAL	23	0,190.33				

*Significant

CV = 16.01



Inoculum Density		Repli	cation			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (uninoculated)	123.33	136.67	125.00	130.00	515.00	128.75 ^a
$T_1 - 2 \ x \ 10^3$	93.33	96.67	106.67	110.00	406.67	101.67 ^{ab}
$T_2 - 5 \ge 10^3$	91.67	121.67	76.67	110.00	400.01	100.00 ^{ab}
$T_3 - 10 \times 10^3$	67.33	131.67	79.67	106.67	385.34	96.34 ^b
$T_4 - 15 \times 10^3$	79.99	81.00	90.67	114.00	365.66	91.42 ^b
$T_5 - 20 \times 10^3$	70.00	80.00	108.33	78.67	337.00	84.25 ^b
GRAND TOTAL			TO T		2409.68	
GRAND MEAN						100.40

APPENDIX TABLE 12. Effect of inoculum levels of *P. fragariae* on plant height of strawberry 3 months after planting

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tabi	ılar F
		-	-	F	5%	1%
TREATMENT	5	9,254.79	1 850.96	2.36**	2.77	4.25
ERROR	18	14,091.34	782.85			
	23	3,346.13				

** Highly significant

CV = 18.06

Etiology of the Red Stele Disease of Strawberry (Fragaria x ananassa Duch.) in Benguet / Rhonda M. Oloan. 2006

Inoculum Density		Repli	cation			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	270.00	283.00	237.67	226.00	1016.67	254.17 ^a
$T_1 - 2 \ x \ 10^3$	164.00	130.00	180.00	173.00	647.00	161.75 ^b
$T_2 - 5 \times 10^3$	156.67	191.00	143.00	170.00	660.67	165.17 ^b
$T_3 - 10 \ge 10^3$	140.00	165.00	160.67	171.00	636.67	159.17 ^b
$T_4 - 15 \times 10^3$	157.00	16 <mark>5.</mark> 00	157.67	151.67	631.34	157.84 ^b
$T_5 - 20 \times 10^3$	89.67	<mark>159.33</mark>	173.33	136.00	558.33	139.58 ^b
GRAND TOTAL		2			4150.68	
GRAND MEAN						1037.68

APPENDIX TABLE 13. Effect of inoculum levels of *P. fragariae* on plant height of strawberry 4 months after planting

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed		ular F
				F	5%	1%
TREATMENT	5	3,256.40	6,651.28	12.55**	2.77	4.25
ERROR	18	9,541.28	530.07			
TOTAL	23	2,797.68				

** Highly significant



Inoculum Density		Repl	ication			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	26.92	51.48	48.53	47.63	174.56	43.64 ^a
$T_1 - 2 \ge 10^3$	37.53	27.52	26.67	37.56	129.28	32.32 ^b
$T_2 - 5 \times 10^3$	28.87	31.61	20.69	26.52	107.69	26.92 ^b
$T_3 - 10 \times 10^3$	24.91	30.17	24.30	31.09	110.47	27.62 ^b
$T_4 - 15 \times 10^3$	21.93	31.58	25.92	21.85	101.28	25.32 ^b
$T_5 - 20 \times 10^3$	29 <mark>.9</mark> 0	25.71	25.14	26.41	107.16	26.79 ^b
GRAND TOTAL			Stor	3	730.44	
GRAND MEAN						30.43
					1	
			107			

APPENDIX TABLE 14. Marketable yield of strawberry plants (February, 2006)

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tab	ular F
		J (U 1		F	5%	1%
TREATMENT	5	950.60	190.12	5.12**	2.77	4.25
ERROR	18	668.89	7.16			
TOTAL	23	1,619.49				

** Highly significant

CV = 20.03%



Inoculum Density		Repl	ication					
(zoospores/ml)	1	2	3	4	Total	Mean		
T ₀ – Control (Uninoculated)	70.70	84.55	97.58	63.45	316.28	79.07 ^a		
$T_1 - 2 \ x \ 10^3$	55.66	55.21	55.59	60.05	226.51	56.63 ^b		
$T_2 - 5 \times 10^3$	76.00	44.12	44.18	56.46	220.76	55.19 ^b		
$T_3 - 10 \ge 10^3$	64.82	48.01	48.79	57.75	219.37	54.84 ^b		
$T_4 - 15 \ x 10^3$	62.60	57.00	49.58	54.92	224.10	56.03 ^b		
$T_5 - 20 \times 10^3$	44 <mark>.6</mark> 9	53.3 <mark>6</mark>	46.97	55.22	200.24	50.06 ^b		
GRAND TOTAL			Stor	153	1407.36			
GRAND MEAN						58.64		
C			6	\$ U				
ANALYSIS OF VARIANCE								

APPENDIX TABLE 15. Marketable yield of strawberry plants (March, 2006)

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tabu	ılar F
				F	5%	1%
TREATMENT	5	2,112.85	422.57	4.38**	2.77	4.25
ERROR	18	1,735.02	96.39			
TOTAL	23	3,847.87				

** Highly significant

CV = 16.74%



Inoculum Density		Repl	ication			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	74.14	53.62	77.40	68.92	274.08	68.52 ^a
$T_1 - 2 \ x \ 10^3$	41.52	33.85	43.16	33.92	152.45	38.11 ^{bc}
$T_2 - 5 \ge 10^3$	44.46	41.67	45.16	31.09	162.38	40.60 ^b
$T_3 - 10 \ge 10^3$	29.97	37.10	32.96	41.25	141.28	35.32 ^{bc}
$T_4 - 15 \times 10^3$	27.79	22.05	20.73	20.24	90.81	22.70 ^d
$T_5 - 20 \times 10^3$	15. <mark>8</mark> 5	23.67	18.04	26.83	84.39	21.10 ^d
GRAND TOTAL		17	S. O.I.		905.39	
GRAND MEAN						37.72

APPENDIX TABLE 16. Marketable yield of strawberry plants (April,2006)

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed F	Tabi 5%	ular F 1%
TREATMENT	5	5,858.61	1,171.72	29.38**	2.77	4.25
ERROR	18	717.87	39.88			
TOTAL	23	6,576.48				

** Highly significant

CV = 16.74%



Inoculum Density		Repl	ication					
(zoospores/ml)	1	2	3	4	Total	Mean		
T ₀ – Control (Uninoculated)	46.40	58.55	57.31	44.40	206.66	51.67 ^a		
$T_1 - 2 \ x \ 10^3$	21.03	31.92	26.42	41.12	120.49	30.12 ^b		
$T_2 - 5 \ x \ 10^3$	25.51	23.61	45.51	44.37	139.00	34.75 ^b		
$T_3 - 10 \ge 10^3$	37.20	42.46	32.64	28.37	140.67	35.17 ^b		
$T_4 - 15 \ge 10^3$	23.96	26.37	37.85	15.26	103.44	25.86 ^b		
$T_5 - 20 \times 10^3$	14 <mark>.6</mark> 6	11.82	23.85	18.17	68.50	17.13 ^c		
GRAND TOTAL			Ston	154	778.76			
GRAND MEAN						32.45		
G				¢ U				
ANALYSIS OF VARAIANCE								

APPENDIX TABLE 17. Marketable yield of strawberry plants (May,2006)

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed _	Tabu	lar F
				F	5%	1%
TREATMENT	5	5	2,662.37	532.47	7.68**	4.25
ERROR	18	18	1,248.56	69.36		
TOTAL	23	23	,910.93			

** Highly significant

CV = 25.67%



Inoculum Density		Repl	ication			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	30.53	39.02	33.00	42.07	144.62	36.15 ^a
$T_1 - 2 \ x \ 10^3$	15.67	23.37	15.03	8.89	62.96	15.74 ^b
$T_2 - 5 \ge 10^3$	9.96	3.91	8.70	15.27	37.84	9.46 ^{bc}
$T_3 - 10 \ge 10^3$	3.96	9.99	7.64	10.67	32.26	8.07 ^c
$T_4 - 15 \times 10^3$	5.77	1.33	14.69	4.27	26.06	6.52 ^c
$T_5 - 20 \times 10^3$	4. <mark>2</mark> 5	3.40	2.47	1.24	11.36	2.84 ^c
GRAND TOTAL			Stor	3	315.10	
GRAND MEAN						13.13

APPENDIX TABLE 18. Marketable yield of strawberry plants (June, 2006)

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tabı	ular F
		531		F	5%	1%
TREATMENT	5	2,902.91	580.58	26.96**	2.77	4.25
ERROR	18	387.66	21.54			
TOTAL	23	3,290.57				

** Highly significant

CV = 35.35%



Inoculum Density		REPLIC	CATION			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	248.69	287.22	313.82	266.47	1046.20	279.05 ^a
$T_1 - 2 \ge 10^3$	171.41	171.87	166.87	181.54	691.69	172.92 ^b
$T_2 - 5 \ge 10^3$	184.80	144.92	164.24	173.71	667.67	166.92 ^b
$T_3 - 10 \ge 10^3$	160.86	167.73	146.33	169.13	644.05	161.01 ^b
$T_4 - 15 \ x \ 10^3$	142.05	138 <mark>.3</mark> 3	148.77	116.54	545.69	136.42 ^c
$T_5 - 20 \times 10^3$	109.35	<mark>117.96</mark>	116.47	122.87	471.65	117.91 ^d
GRAND TOTAL		12			4066.95	
GRAND MEAN						172.37

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tab	ular F
		1	1	F	5%	1%
TREATMENT	5	63,189.97	12,637.99	51.57**	2.77	4.25
ERROR	18	4,411.21	245.07			
TOTAL	23	67,601.18				

** Highly significant

Etiology of the Red Stele Disease of Strawberry (Fragaria x ananassa Duch.) in Benguet / Rhonda M. Oloan. 2006



Inoculum Density		REPLIC	CATION			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	53.55	61.72	54.60	52.30	222.17	55.54
$T_1 - 2 \ x \ 10^3$	60.060	42.790	61.000	60.390	224.24	56.06
$T_2 - 5 \ge 10^3$	74.63	42.96	76.23	76.23	270.05	67.51
$T_3 - 10 \ge 10^3$	92.08	79.48	66.05	50.75	288.36	72.09
$T_4 - 15 \times 10^3$	83.95	70.38	65.42	74.72	294.47	73.62
$T_5 - 20 \times 10^3$	60.27	123.03	60.13	60.27	303.70	75.93
GRAND TOTAL			YON		1602.99	
GRAND MEAN		É				66.79

APPENDIX TABLE 20.	Effect of inoculum levels of P. fragariae on the non-
	marketable yield of strawberry

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tab	ular F
				F	5%	1%
TREATMENT	5	1,601.26	320.25	1.11 ^{ns}	2.77	4.25
ERROR	18	5,184.52	288.03			
TOTAL	23	6,785.78				

ns- not significant

CV = 25.41%



Etiology of the Red Stele Disease of Strawberry (Fragaria x ananassa Duch.) in Benguet / Rhonda M. Oloan. 2006

Inoculum Density		REPLI				
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	45.55	61.24	42.80	64.54	214.13	53.53 ^a
$T_1 - 2 \ x \ 10^3$	24.59	27.12	12.41	16.66	80.78	20.20 ^d
$T_2 - 5 \ge 10^3$	22.39	13.77	31.79	16.30	84.25	21.06 ^d
$T_3 - 10 \times 10^3$	20.25	20.50	23.33	20.00	84.08	21.02 ^d
$T_4 - 15 \ x \ 10^3$	13 <mark>.9</mark> 0	18. <mark>34</mark>	16.17	16.30	64.71	16.18 ^b
$T_5 - 20 \times 10^3$	17.08	<mark>16.42</mark>	16.58	17.25	67.33	16.83 ^{bc}
GRAND TOTAL		12			595.28	
GRAND MEAN						24.80

APPENDIX TABLE 21. Effect of varying levels of *P. fragariae* inoculum on the top fresh weight of strawberry plants

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tabı	ular F
		1	1	F	5%	1%
TREATMENT	5	4,051.39	810.28	20.54	2.77	4.25
ERROR	18	710.14	39.45			
TOTAL	23	4,761.53				

** Highly significant



Inoculum Density		Repl	ication			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	24.1	31.18	23.62	34.21	113.11	28.28 ^a
$T_1 - 2 \ge 10^3$	10.31	18.15	12.74	31.34	72.54	18.14 ^{ab}
$T_2 - 5 \ x \ 10^3$	11.85	12.12	32.59	9.58	66.14	16.54 ^b
$T_3 - 10 \ge 10^3$	9.81	11.21	14.16	8.88	44.06	11.02 ^b
$T_4 - 15 \times 10^3$	9.86	11.06	9.63	11.56	42.11	10.53 ^b
$T_5 - 20 \times 10^3$	5.21	6.3	4.93	7.74	24.18	6.05 ^c
GRAND TOTAL			- Cot		362.14	
MEAN TOTAL						15.09

APPENDIX TABLE 22. Effect of varying levels of *P. fragariae* inoculum on the root fresh weight of strawberry plants

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tab	ular F
		-		F	5%	1%
TREATMENT	5	1,218.02	243.60	6.10**	2.77	4.25
ERROR	18	718.47	39.92			
TOTAL	23	1,936.49				

** Highly significant

CV = 41.87%



Inoculum Density		Repli				
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	15.17	18.78	15.20	15.13	64.28	16.07
$T_1 - 2 \ x \ 10^3$	5.53	4.79	3.42	4.98	18.71	4.68 ^{ab}
$T_2 - 5 \ x \ 10^3$	4.09	3.41	4.75	4.19	16.44	4.11 ^{bc}
$T_3 - 10 \ge 10^3$	3.57	3.19	6.85	4.78	18.39	4.60 ^{bc}
$T_4 - 15 \times 10^3$	2.48	3.89	3.17	2.92	12.46	3.11 ^c
$T_5 - 20 \times 10^3$	2.97	2.90	1.10	2.79	9.76	2.44 ^c
GRAND TOTAL		1/	10th	50	140.04	
MEAN TOTAL						5.83

APPENDIX TABLE 23. Effect of varying levels of *P. fragariae* inoculum on the top dry weight of strawberry plant

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed F	Tabul 5%	<u>ar F</u> 1%
TREATMENT	5	5	518.08	103.62	75.48**	4.25
ERROR	18	18	24.71	1.37		
TOTAL	23	23	542.80			

Etiology of the Red Stele Disease of Strawberry

** Highly significant

CV = 20.08%



Inoculum Density		Repli	ication			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (Uninoculated)	7.88	5.61	6.23	7.27	26.99	6.75 ^a
$T_1 - 2 \ge 10^3$	6.31	4.94	2.26	6.90	20.41	5.10 ^{ab}
$T_2 - 5 \ge 10^3$	1.39	3.22	5.96	2.00	12.57	3.14 ^{bc}
$T_3 - 10 \times 10^3$	2.00	2.27	5.57	2.56	12.41	3.10 ^{bc}
$T_4 - 15 \times 10^3$	1.69	3.30	2.73	2.19	9.90	2.48 ^c
$T_5 - 20 \times 10^3$	2.46	1.78	1.20	3.05	8.49	2.12 ^a
GRAND TOTAL					90.77	
GRAND MEAN						22.69

APPENDIX TABLE 24. Effect of varying levels of *P. fragariae* inoculum on the dry weight of strawberry roots

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tabı	ılar F
		-		F	5%	1%
TREATMENT	5	63.47	12.69	5.73**	2.77	4.25
ERROR	18	39.91	2.22			
TOTAL	23	103.38				

Etiology of the Red Stele Disease of Strawberry

** Highly significant

CV = 39.37%



Inoculum Density		REPLIC	CATION			
(zoospores/ml)	1	2	3	4	Total	Mean
T ₀ – Control (uninoculated)	1.00	1.00	1.00	1.00	4.00	1.00 ^d
$T_1 - 2 \ x \ 10^3$	3.33	3.33	4.00	4.00	14.66	3.67 ^c
$T_2 - 5 \ge 10^3$	4.67	5.00	4.33	4.33	18.33	4.58 ^{ab}
$T_3 - 10 \ge 10^3$	5.00	4.67	4.33	4.67	18.67	4.67 ^{ab}
$T_4 - 15 \times 10^3$	4.33	5.00	5.00	4.33	18.66	4.66 ^{ab}
$T_5 - 20 \times 10^3$	5.0 <mark>0</mark>	5.00	5.00	5.00	20.00	5.00 ^a
GRAND TOTAL			Stor.	33	94.32	
GRAND MEAN						3.93
65						

APPENDIX TABLE 25. Disease severity as affected by inoculum density

Source of Variance	Degree of Freedom	Sum of square	Means of square	Computed	Tabular F	
				F	5%	1%
TREATMENT	5	45.24	9.0	113.75**	2.77	4.25
ERROR	18	1.43	0.08			
TOTAL	23	46.67				

** Highly significant

CV = 7.18%

