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BROCCOLI ROTATION AND RESIDUE AMENDMENT: A SUSTAINABLE MANAGEMENT OPTION FOR SOIL-BORNE DISEASES OF STRAWBERRY

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ABSTRACT

Continuous strawberry monocropping increases the prevalence of soil-borne diseases caused by plant parasitic nematodes and fungi. This study was undertaken to verify the effects of broccoli rotation and residue amendment on the population of plant parasitic and free-living nematodes; to evaluate their effectiveness against major soil-borne fungal diseases of strawberry and to assess the profitability of the potential technology. Four rotation treatments were evaluated namely, Strawberry-strawberry (untreated); Broccoli-strawberry; Broccoli-broccoli-strawberry; and Strawberry-strawberry (The soil was applied with thiophanate methyl). The treatments were laid out using the Randomized Complete Block Design with four replications. Although not significant, broccoli-broccoli-strawberry and broccoli-strawberry rotations reduced the population of lesion nematodes (*Pratylenchus penetrans*) and foliar nematodes (*Aphelenchoides fragariae*) in the soil, roots and leaves, respectively. The same treatments increased the number of free-living nematodes in the soil which are necessary in the decomposition of organic materials. Significant reduction in root necrosis and disease severity of fungal diseases was noted in the above treatments resulting in significant increase in strawberry yield. The highest return on cash expense (ROCE) was obtained from broccoli-broccoli-strawberry, followed by broccoli-strawberry with 238.61% and 143.75 %, respectively and lower on two croppings of strawberry drenched with fungicide (43.79%) and the lowest on untreated strawberry monocropping having only 21.84%.

Keywords: *strawberry, broccoli rotation, Pratylenchus penetrans, Aphelenchoides fragariae*

INTRODUCTION

Strawberry production has been a lucrative source of income for Benguet farmers. The demand for locally produced berries far exceeds available supplies. Thus, small scale producers get higher returns from strawberries rather than from most other crops. The unique agro-climatic conditions of Benguet make the growth of the crop favorable, giving the province a comparative advantage in this industry. Benguet strawberries have become so popular too, that the province has gained the distinction, “Strawberry Capital of the Philippines”, courtesy of the capital town of La Trinidad. According to Padua and Ligat (2015), the biggest area planted to strawberry was in La Trinidad followed by Tuba and Tublay with 116.0, 5.0 and 1.5 hectares, respectively. However, other provinces like Bukidnon, Nueva Viscaya and Ifugao are starting to grow strawberries too (Padua and Ligat, 2015).

Due to the high demand for fresh and processed strawberry products, farmers continuously plant strawberries in the same area. This has resulted to the rapid increase in soil-borne diseases caused by fungi, bacteria and plant parasitic nematodes which are important limiting factors in strawberry production in the locality. Previous surveys showed the association of the following nematodes in strawberries in Benguet: *Meloidogyne javanica*, *M. hapla*, *Pratylenchus penetrans*, *Helicotylenchus*, *Trichodorus* and *Rotylenchus* (Villanueva, 1994; Ngaseo, 1987 and Kiat-ong, 2005). On the other hand, Nagpala *et al.* (2003) reported the presence of other diseases like leaf scorch (*Marsonina earlieanium*), leaf spot (*Mycosphaerella fragariae*), leaf blight/ anthracnose (*Colletotrichum* spp.), gray mold (*Botrytis cinerea*), Phytophthora rot (*Phytophthora* sp.), Fusarium wilt (*Fusarium oxysporum*) and crinkle mosaic virus. Villanueva *et al.* (2006) reported crimp disease (*Aphelenchoides fragarie*) and Verticillium wilt (*Verticillium* sp.) as emerging problems in strawberry production in Benguet. The occurrence of the red stele disease (*Phytophthora fragariae*) in strawberry growing areas in Benguet was reported by Oloan and Villanueva (2007). With the favorable climatic conditions in the area, the complex diseases caused by the combinations of the above pathogens are causing substantial yield losses in the crop.

Crop rotation is one of the oldest and most useful cultural management practices which involves the planned order of specific crops planted on the same field. It also means that the succeeding crop belongs to a family different from the previous one. The planned rotation may vary from 2 or 3 years or longer. Many diseases build up in the soil when the same crop is grown in the same field year after year. Rotation can break the cycle and stop the build up of disease organisms in the field. The advantages of crop rotation include the prevention of soil depletion, maintenance of soil fertility, reduction of soil erosion, management of pests and diseases, reduction of reliance on synthetic chemicals and control of weeds (Ashley, 1994).

In some production systems, crop rotation can be considered as an economically viable means of reducing soil-borne propagules of *Verticillium* and yield losses from wilt disease. The effective management of *Verticillium* wilt in cauliflower has been reported with broccoli rotation and residue amendment (Subbarao *et al.*, 1999; Xiao *et al.*, 1998). In California, USA, strawberry plants grown using broccoli rotation showed the lowest disease severity rate among the rotation treatments, next to methyl bromide + chloropicrin in effectiveness. Likewise, Njoroge *et al.* (2009) reported that yield of strawberry was greater in plots rotated with broccoli. Generally, most of the beneficial effects of amendments with *Brassica* spp. have been attributed to glucosinolates, which are sulfur-containing compounds present in all plant parts of species belonging to the family *Brassicaceae* (Brown and Morra, 1998). Glucosinolates are not toxic by themselves, but are converted to toxic isothiocyanates, thiocyanates, nitriles, epithionitriles, and oxazolidine-2-thiones as the plant tissues break down in soil (Fenwick *et al.*, 1983).

Allyl isothiocyanate, a break-down product of allyl glucosinolate present in *B. juncea*, completely suppresses in vitro radial growth of V. *dahliae* (Mayton *et al.*, 1996). Results from studies on rotation crop residue amendment suggest a biological mode of action; sustained suppression of soil-borne pathogens results from the activation of biological components that are already present in the soil (Davis *et al.*, 1996). In the rhizosphere, any stages of the pathogen life cycle could be susceptible to the biological suppressive components in a number of ways. It is possible that the microbial population changes resulting from broccoli residue decomposition also lead to greatly increased competition among root colonizers (Staple *et al.*, 1998). Increased microbial activity following broccoli amendment and the resulting competition for colonization of root cortical surface may also limit infection foci for V. *dahlia* (Subbarao *et al.*, 1999). The biological suppressive action may result from the effects of compounds released from the residue or of the decomposition products on population density and activity of antagonistic microorganisms (Lockwood, 1988). Mature broccoli crop residues are rich in lignin and the enzymes involved in lignin biodegradation can also degrade fungal melanin. Melanin is known to protect the fungus from various abiotic and biotic stresses (Butler *et al.*, 1998).

In the Philippines, the potential of broccoli rotation on the management of soil-borne diseases of strawberry at the Swamp Area, Benguet State University, La Trinidad, Benguet has been studied whereby significant reduction in diseases caused by fungi, bacteria and plant parasitic nematodes were noted. Broccoli-broccoli-strawberry rotation gave the highest strawberry yield which was at par with broccoli-strawberry and lettuce-lettuce-strawberry rotation (Villanueva *et al.*, 2008).

In the absence of available non-chemical methods of disease management in strawberry, farmers have resorted to the use of chemical pesticides. However, this practice is not only expensive but also dangerous to human health and the environment. Aside from the absence of effective chemicals to control these diseases, soil-borne pathogens are very difficult to manage once introduced in an area. Thus, there is an urgent need to develop an alternative disease management strategy for these diseases.

Objectives

The study was conducted to verify the effect of broccoli rotation and residue amendment on the population of plant parasitic and free-living nematodes; to evaluate their effectiveness against major soil-borne fungal diseases of strawberry and to assess the profitability of the potential technology.

MATERIALS AND METHODS

Treatments and experimental design

The experiment was conducted at the Balili Experimental Area, BSU, La Trinidad, Benguet. The area was previously planted with strawberry known to be infested with soil-borne diseases caused by *Fusarium* sp., *Verticillium* sp., *Phytophthora* sp., *Colletotrichum* sp. and plant parasitic nematodes. An area of about $80m_2$ was thoroughly prepared and divided into four blocks. Each block consisted of four plots with a dimension of 1m x 5m each representing the rotation treatments namely, 1) Strawberry-strawberry (untreated), 2)Broccoli-strawberry, 3)Broccoli-broccoli-strawberry and 4)Strawberry-strawberry (the soil was treated with fungicide, thiophanate methyl). The first and last treatments served as control. The treatments were laid out using Randomized Complete Block Design (RCBD) with four replications. The first crops were planted on December 08, 2008. The initial population of the pathogens was assessed before planting. After the harvest of broccoli, the crop residues were shredded and incorporated into the soil. About 4kg residues were applied per plot. At four weeks after incorporation, the beds were again prepared for the next crop cycle. The broccoli seedlings transplanted on May 19, 2009 for Treatment 3 were damaged by a typhoon and were allowed to decompose in the field. The strawberry plants for Treatments 1 and 4 were uprooted in June 2009. Before planting the second crops on November 18, 2009, soil samples were again collected and checked for any changes in nematode population after the typhoon. For the third cropping, strawberry runners were transplanted on November 5, 2010. Processed chicken manure was applied before planting at the rate of 20g per hill. Vermicompost tea enhanced with *Verticillium* sp., baking soda (168g/16 L water) and pepper (20g/16 L water) were sprayed to provide nutrients to plants and for the control of insects. A total of 16 sprayings was made throughout the growing period. In all the cropping periods, strawberry cv Sweet Charlie was used.

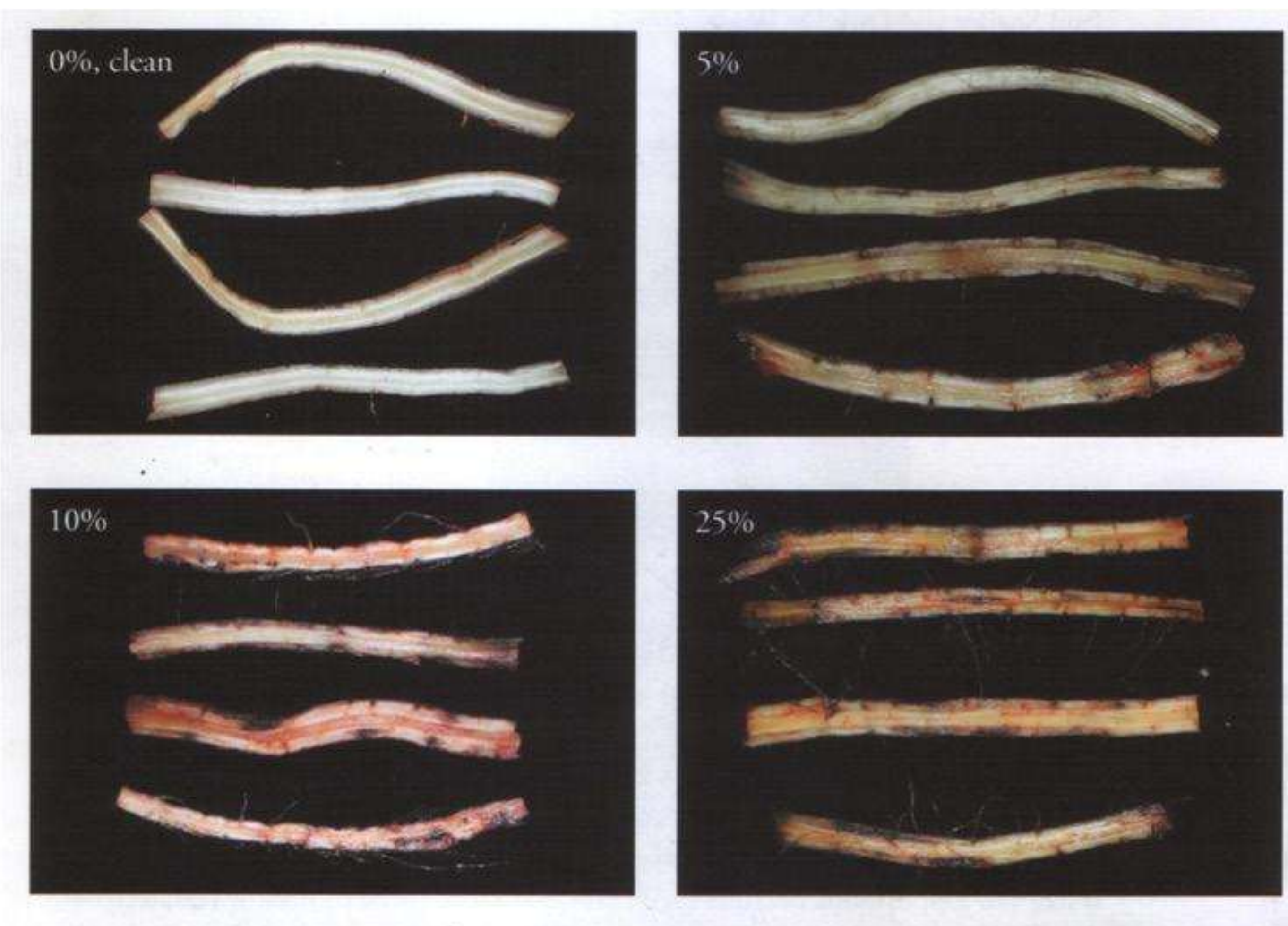
Sampling and data analysis

Four hundred cc soil samples (200 cc for plant parasites and 200 cc for the free-living nematodes) were collected before the start of the cropping periods and at harvest while the root (2g) and leaf samples (50g) were gathered only at the termination of the cropping periods. Nematode extraction and identification were carried out at the Biocontrol Laboratory, Benguet State University, La Trinidad, Benguet. Nematode taxa present in the soil, roots and leaves were identified using their morphological characteristics and taxonomic keys (Baldwin and Mundo-Ocampo, 2003; Shurtleff and Averre, 2000 and Siddiqi, 2000). The effect of the treatments on root necrosis was evaluated following the necrotic lesion scoring in banana roots caused by *P. penetrans* (Speijer and de Waele, 1997) (Fig. 1). On the other hand, weekly assessment of foliar diseases was done using the Florida Peanut leaf spot scoring system (Chiteka *et al.*, 1988) as follows:

SCALE	DESCRIPTION
1	No disease
2	Very few leaf spots in lower canopy (1 – 5 % loss)
3	Few leaf spots in lower and upper canopy (6 – 9 % loss)
4	Some leaf spotting in lower and upper canopy with light defoliation (≤10 % loss)
5	Leaf spotting noticeable in canopy with some defoliation (≤25 % loss)
6	Leaf spots numerous with significant defoliation (≤50 % loss)
7	Leaf spots numerous with heavy defoliation (≤75 %loss)
8	Numerous leaf spots on few remaining leaves with severe defoliation (≤90 % loss)
9	All but few heavily spotted leaves have been shed (≤95 % loss)
10	Plants defoliated or dead

In each plot, strawberry fruits were harvested two times a week from the 10 sample plants in each treatment replication. These were weighed then sorted as marketable or non-marketable.

The effects of treatments on the population of plant parasitic and free-living nematodes, disease severity and yield were determined by analysis of variance (ANOVA). Means were compared using Duncans Mutiple Range Test (DMRT).



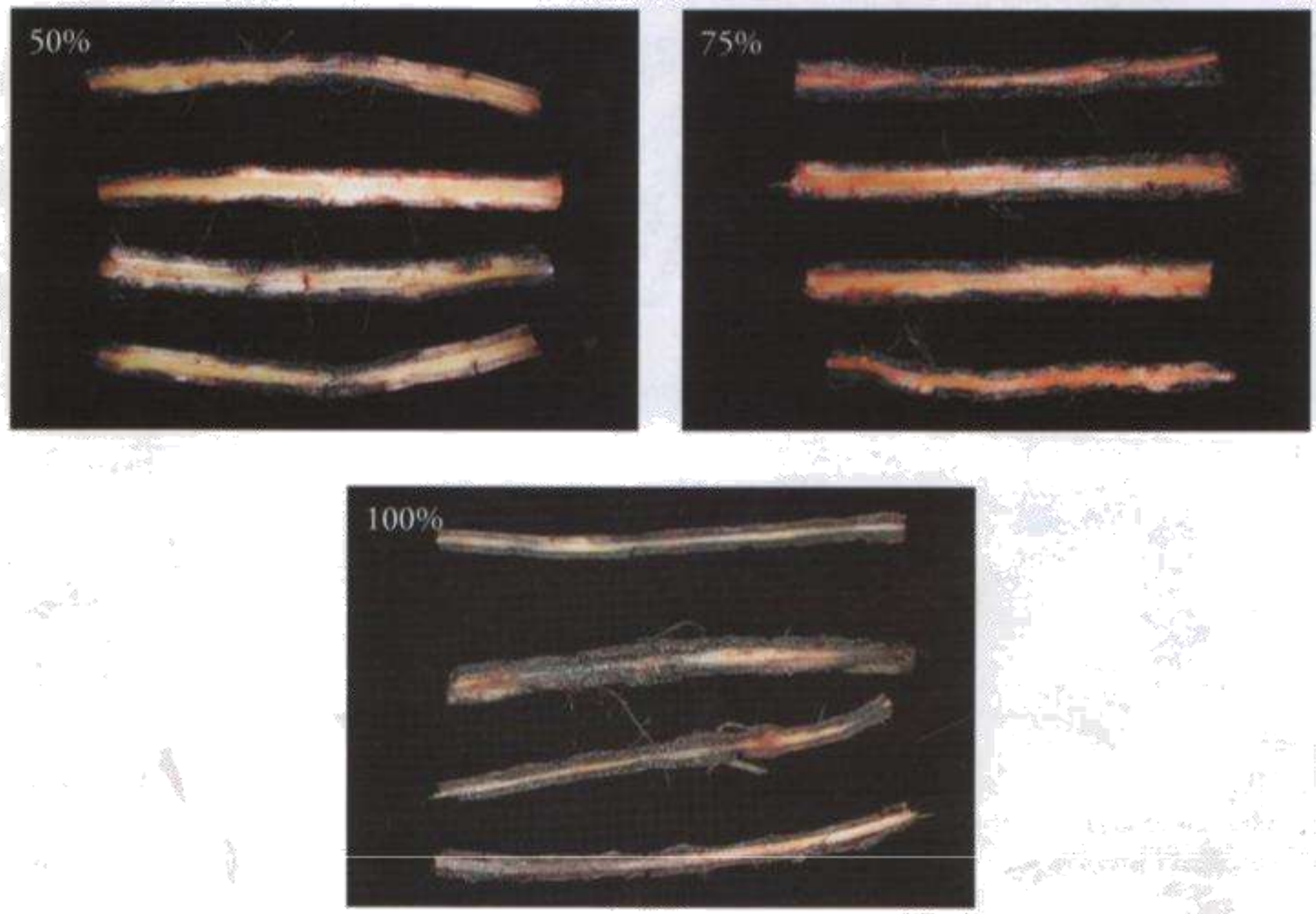


Figure 1. Necrotic lesion scoring in banana roots caused by P. *penetrans* (Speijer and De Waele, 1997)

RESULTS AND DISCUSSION

Effect on nematode population

Twelve genera of plant parasitic nematodes were found associated with strawberry in the Experimental Area. They were *P. penetrans*, *Helicotylenchus* sp., *Tylenchus* sp., A. *fragariae*, *Globodera rostochiensis*, *Meloidogyne* sp. *Psilenchus* sp., *Rotylenchulus* sp., *Tylenchorynchus* sp., *Paratylenchus* sp., *Paratrichodorus* sp., and *Criconomella* sp. The broccoli rotation treatments had little influence on the soil population of major plant parasitic nematodes namely, P. *penetrans*, *Helicotylenchus* sp. and G. *rostochiensis* (data not shown). Numerically, however, the number of P. *penetrans* in the roots was lower in broccoli rotations as compared to strawberry mono cropping with or without fungicide application (Fig. 2). On the other hand, the inoculum levels of A. *fragariae* were initially low at the start of the second cropping but increased substantially in the third cropping (Fig. 3). Broccoli rotations however and residue amendment were able to reduce A. *fragariae* populations than the strawberry mono cropping with or without thiophanate methyl application. Njoroge *et al.* (2009) reported that rotational plantings and incorporation of green manures have been used to suppress populations of P. *penetrans*, and to reduce populations of *Meloidogyne chitwood*. According to Johnson *et al.* (1992), lack of nematode suppression or inconsistencies among studies are often attributed to differences in glucosinolate concentrations of incorporated brassicaceous materials. Ploeg and Stapleton (2001) demonstrated that 2% weight per weight (w/w) broccoli incubated at 25_oC for seven hours did not compromise the ability of M. *javanica* to parasitize a plant. The practical interference of this conclusion is that when *Brassica* spp. with high glucosinolate content is used as amendment, sufficient biomass must be applied to allow uniform distribution through the soil profile for subsequent volatilization. This requirement may be even more critical in cooler climates.

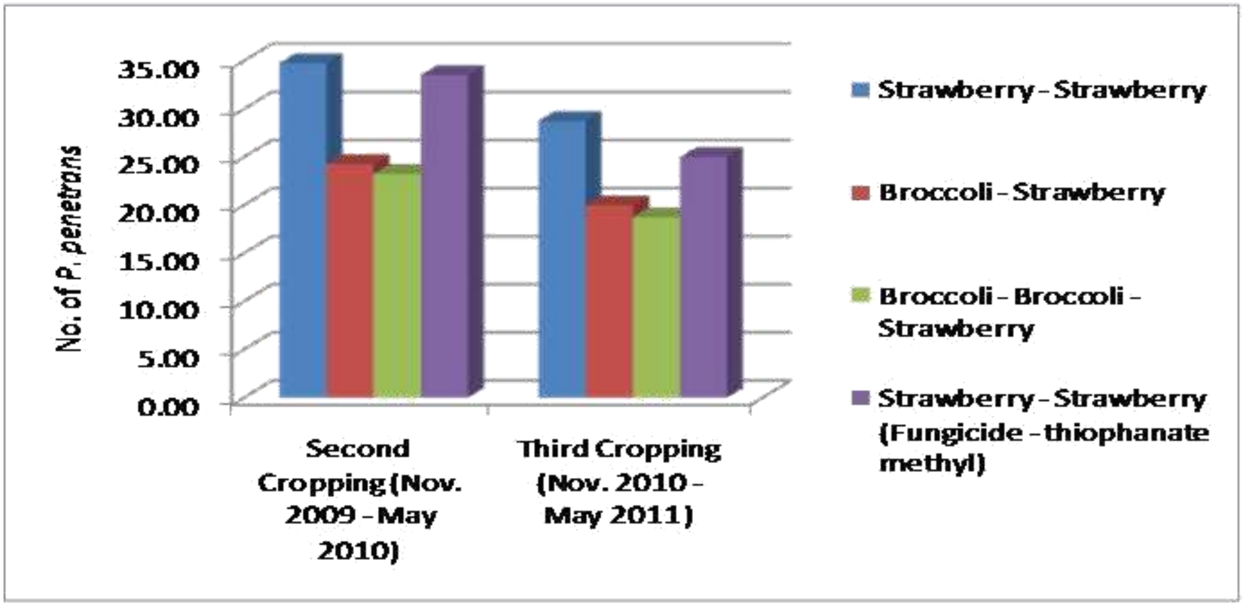


Figure 2. Number of lesion nematodes, P. *penetrans* in 2g roots as affected by the different treatments. Means are not significantly different at P≤ 0.05using DMRT.

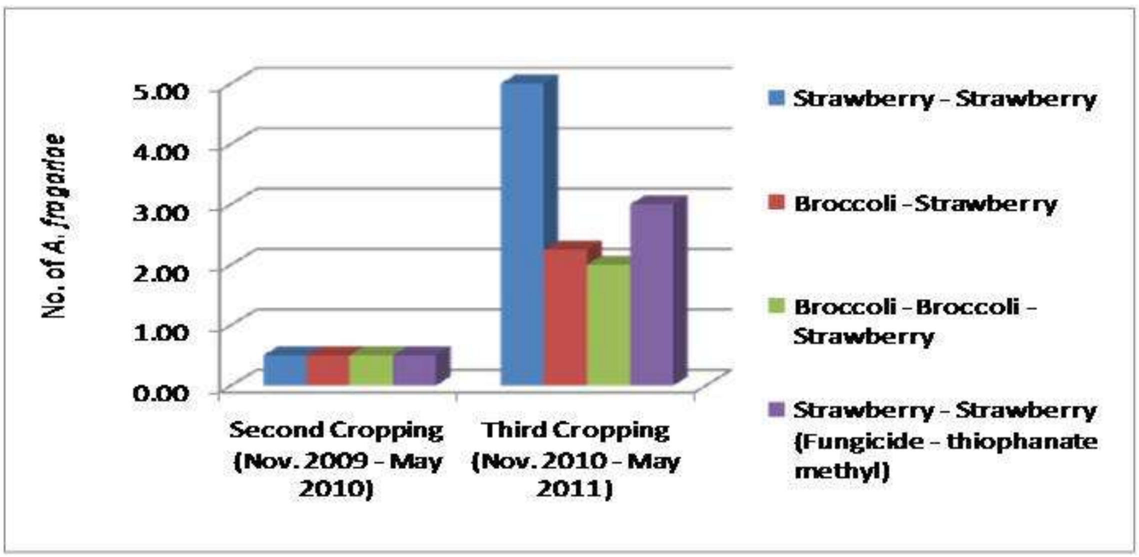


Figure 3. Number of foliar nematodes, A. *fragariae* in 50 g leaves as affected by the different treatments. Means are not significantly different at P≤ 0.055% level using DMRT.

Effect on root necrosis

Root necrosis is usually caused by root infecting fungi (*Verticillium* and *Fusarium*) and the root lesion nematode, P. *penetrans*. According to Chen and Rich (1962), nematodes increase the number of infection courts and the density of growth in infected tissues, presumably due to wounding and increased nutrition available to the fungal pathogen. Similarly, the local influence of P. *penetrans* on black root rot suggests increased numbers of infection sites due to wounding or to predisposition to limited areas of the cortex. Nematode feeding and movement directly result in cell damage and death. The indirect effects of lesion nematode infection are discoloration of the endodermis and early polyderm formation, followed by localized areas of secondary growth and cortical cell weakening or death. Weakened or dying cells resulting from direct or indirect effects of P. *penetrans* are more susceptible to root infecting fungi, thereby increasing infection and cortical root rot.

Development of root necrosis was noted in all the treatments. However, broccoli rotations resulted in significantly lower root necrosis compared to two croppings of strawberry whether untreated or treated with fungicide during the two cropping seasons (Fig. 4).

Brassicas are used as biofumigants most commonly through the use of green manures. They are known to produce secondary compounds from glucosinolate hydrolysis, sometimes referred to as allellochemicals, which can control or suppress soil-borne pests and diseases (Shetty *et al.*, 2000). Brassicas produce 30-40 different glucosinolates which when combined with the enzyme myrosinase, form breakdown products with nematode suppressive effects. These compounds work through interference with nematode reproductive cycles, growth inhibition or feeding deterence and direct toxicity.

Effect on the population of free -living nematodes

The different treatments had varying effects on the population of free–living nematodes. Broccoli rotations favored the build up of free-living nematode populations as compared to the other treatments during the first and third cropping seasons (Fig. 5). It has been reported that the addition of organic amendments may stimulate the entire soil food web including beneficial biological control organisms (Swift *et al.*, 1979). However, reduction in free living nematode population was observed in the second cropping in strawberry–broccoli rotation but increased again in the third cropping on broccoli-broccoli-strawberry rotation.

Halbrendt and Jing (1996) reported that non-plant parasitic nematodes were less sensitive to brassica-induced toxins than plant parasites. Therefore, although a number of free-living nematodes in the experiments may have declined shortly after the broccoli-amendment, the generally short generation time of saprophytic nematodes and ample food supply resulted in their quick recovery and in large populations, in the broccoli-amended soil in the third cropping. On the other hand, a low population of free-living nematodes was recorded in strawberry-strawberry rotation applied with thiophanate methyl. This might be due to the possible effect of the fungicide on the free-living nematodes. The sensitivity of free-living nematodes has been reported for other non-fumigant nematicides and fungicides with antihelminthic properties (Simpkin and Coles, 1981). Free living nematodes are very important in the decomposition of organic materials and recycling of nutrients in soil. Nematode bacterivores and fungivores do not feed directly on soil organic matter but on the bacteria and fungi which decompose organic matter. The presence and feeding of these nematodes accelerate the decomposition process and recycle minerals and other nutrients from bacteria, fungi and other substrates returning them to the soil where they are accessible to plant roots (Freckman, 1982).

Effect on disease severity

During the second cropping, broccoli-broccoli-strawberry rotation showed the lowest severity rating of fungal diseases caused by *Colletotrichum* sp., *Fusarium oxysporum*, *Verticillium* sp. and *Rhizoctonia* sp. (data not shown). Also, broccoli–strawberry rotation and two croppings of strawberry treated with fungicide lowered the disease severity as compared to untreated strawberry monocropping which increased tremendously. Significant reduction in the severity of fungal diseases was noted in broccoli rotations on the third cropping (Fig. 6). Residues of *Brassica* spp. have proven to be effective in reducing several soil-borne pathogens (Bending *et al.*, 2000). Keinath (1996) reported significant reductions of gummy stem blight of watermelon in soil amended with cabbage residue. Chan and Close (1987) demonstrated the control of Aphanomyces root rot from *Brassica* residue amendments. *Brassica* spp. are well known for their characteristic sulfur-containing compounds, known as glucosinolates, and for the disease-suppressive effects of the toxic by-products derived from the breakdown of these compounds (Sarwar *et al.*, 1998).

Effect on yield

The highest strawberry yield was obtained from broccoli-broccoli-strawberry rotation. This was significantly different from broccoli-strawberry and monocropping of strawberry applied with fungicide (Fig. 7a). However, the yield of plants in broccoli-strawberry rotation was significantly higher than the untreated strawberry monocropping. No significant differences were

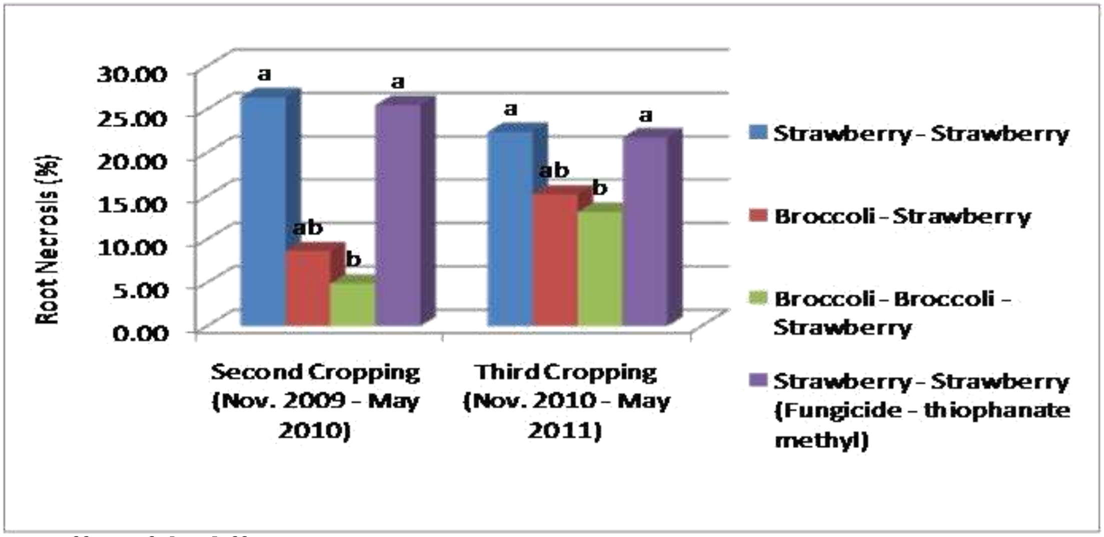


Figure 4. Effect of the different treatments on percentage root necrosis .
Means with the same letter are not significantly different at P≤ 0.05using DMRT.

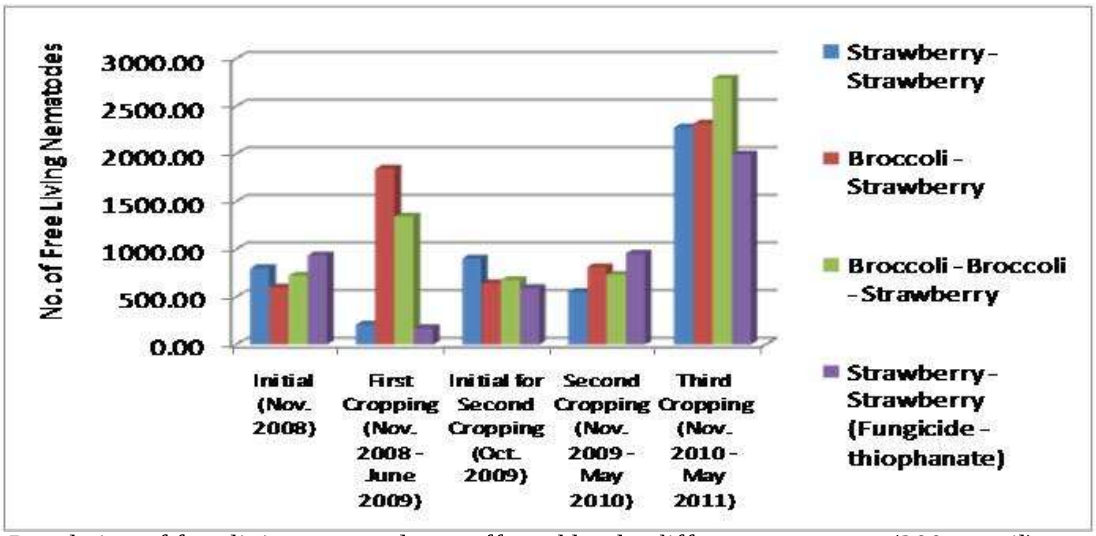


Figure 5. Population of free-living nematodes as affected by the different treatments (200 cc soil).
Means are not significantly different at P≤ 0.05using DMRT.

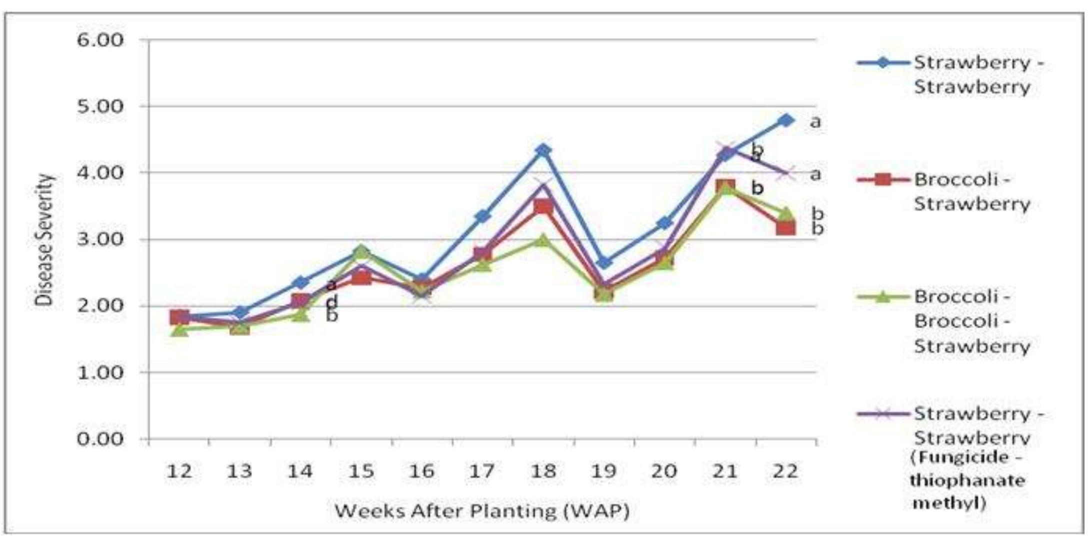


Figure 6. Severity of fungal diseases of strawberry during the third cropping season (November 2010 to May 2011).
Lines with the same letters are not significantly different at P≤ 0.05using DMRT.

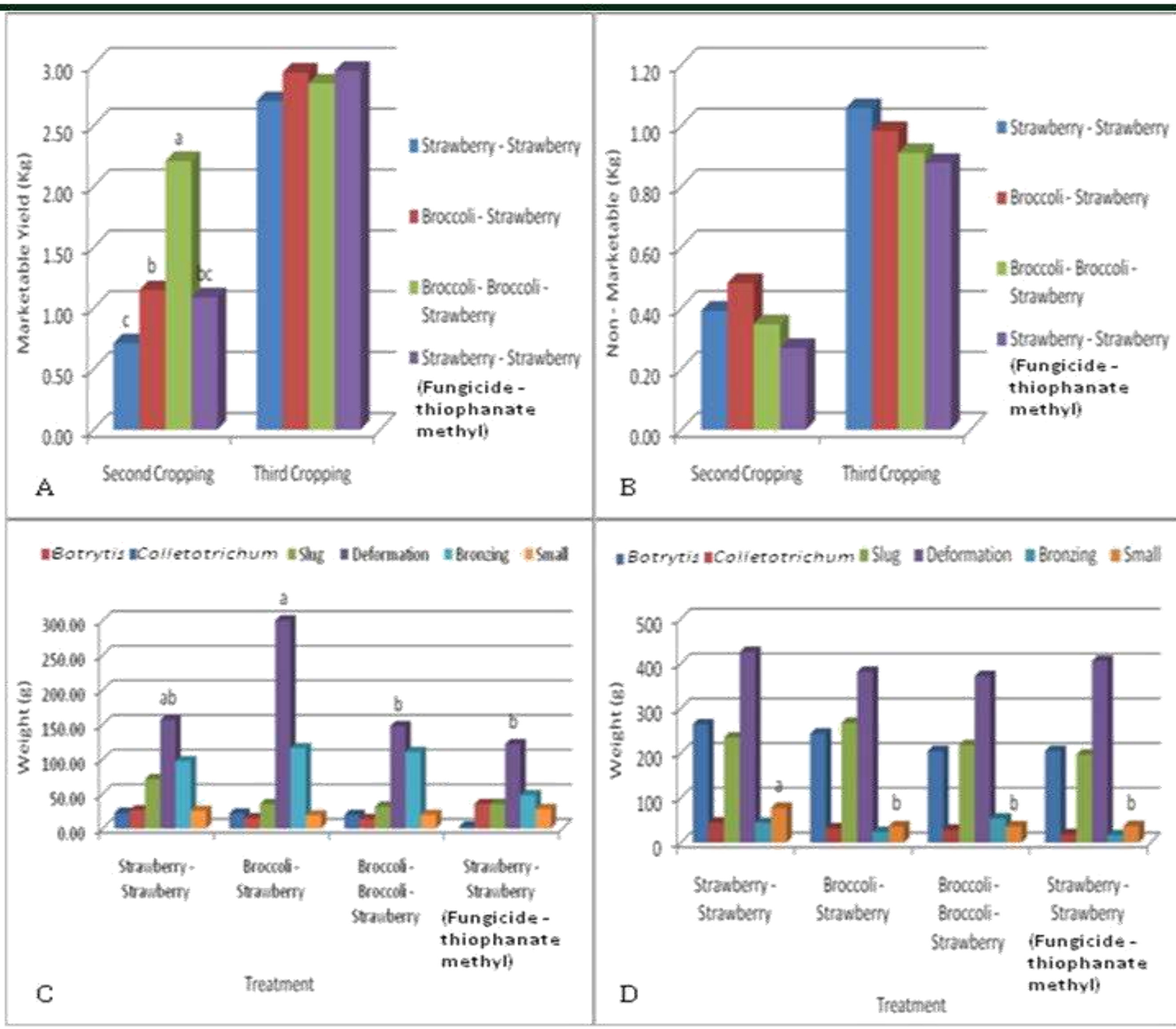


Figure 7. Strawberry yield: A) marketable, B) non-marketable, C) causes of non -marketability of yield during the second cropping and D) causes of non -marketability of yield during the third cropping. Different letters on the bars indicate significant differences among treatments according to DMRT at P≤ 0.05.

noted in terms of non-marketable yield (Fig. 7b). The highest non-marketable yield was caused by the deformation of fruits followed by bronzing of berries possibly as affected by feeding of mites and thrips (Fig. 7c). Ten or more mites per fruit are required to produce bronzing and when the number of thrips exceeds two per fruit at the early fruit maturity stage, the amount of unmarketable fruit can exceed 20%. Mites and thrips can be found together on the same fruit (Anonymous, 2010). The deformation of berries was also the major cause of non-marketability of yield during the third cropping followed by damage due to feeding by slugs and cutworms and the effect of gray mold caused by B. *cinerea* (Fig. 7d).

Cost-benefit analysis

Strawberry–strawberry rotation applied with fungicide incurred the highest production cost (P37, 372.75) but provided lower ROCE (43.79%). In contrast, the cost of untreated strawberry monocropping decreased to P35, 686.75 but gave the lowest ROCE of 21.84%. Production of strawberry following one broccoli rotation resulted in higher ROCE (143.75%) because of income generated from broccoli and higher strawberry yield. The highest ROCE (238.61%) was obtained from broccoli-broccoli-strawberry rotation (Table 1).

Table 1. Comparisons of production cost and returns of strawberry in broccoli-strawberry rotation with or without fungicide application (500m_2)

STRAWBERRY - STRAWBERRY		BROCCOLI - STRAWBERRY		BROCCOLI - BROCCOLI – STRAWBERRY		STRAWBERRY - STRAWBERRY (thiophanate methyl)	
Cost (PhP)							
Strawberry runners	33,800.00	Broccoli seedlings	5,200.00	Broccoli seedlings	10,400.00	Strawberry runners	33,800.00
Processed Chicken Manure (PCM)	1,086.75	Strawberry runners	20,800.00	Strawberry runners	20,800.00	PCM	1,086.75
Pepper	500.00	PCM	1,860.75	PCM	2,173.50	Pepper	500.00
Baking soda	300.00	Pepper	500.00	Pepper	500.00	Baking soda	300.00
		Baking soda	300.00	Baking soda	300.00	Fungitox 70 WP (thiophanate methyl)	1,686.00
Total Cost (PhP)	35,686.75		27,886.75		34,173.50		37,372.75
Strawberry Yields (kg)	543.50	Broccoli Yields (kg)	756.5	Broccoli Yields (kg)	1,714.50	Strawberry Yields (kg)	671.75
		Strawberry Yields (kg)	566.00	Strawberry Yields (kg)	803.50		
Strawberry Value/ kg	PhP 80.00	Broccoli Value/ kg	PhP 30.00	Broccoli Value/ kg	PhP 30.00	Strawberry Value/ kg	PhP 80.00
		Strawberry Value/ kg	PhP 80.00	Strawberry Value/ kg	PhP 80.00		
Total Value (PhP)	43,480.00		67,975.00		115,715.00		53,740.00
ROCE (%)	21.84		143.75		238.61		43.79

CONCLUSIONS AND RECOMMENDATION

Broccoli rotation and residue amendment are effective in reducing the soil-borne diseases of strawberry caused by fungi and plant parasitic nematodes. This could be attributed to the allelochemicals produced by the crop during decomposition. The practice also increases the population of free-living nematodes which are very important in the decomposition of organic materials and recycling of nutrients in the soil. Broccoli-broccoli-strawberry and broccoli-strawberry rotations are more profitable than strawberry monocropping with or without fungicide application.

Since the potential technology is environment-friendly, this could be recommended in conventional and organic strawberry production systems and could be integrated in the package of technology (POT) for disease management in strawberry. The use of *Aphis mellifera* to disseminate *Trichoderma* for the control of fruit rot caused by B. *cinerea* (Saclangan *et al*., 2015) may also be included in the POT. In addition, to minimize non-marketability of strawberry fruits due to mites, the release of endemic predatory mite, *Amblyseius longispinosus* which feeds on two spotted spider mite (*Tetranychus urticae* Koch) could also be used (Tanyag *et al*., 2015) .

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