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Preface

We would like to present, with great pleasure, the inaugural volume-5, Issue-4, April 2019, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development,* and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
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Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
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Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
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Maple syrup	Forestry Growth
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Presently working as Assistant Professor in Dept. of Plant Pathology, College of Agriculture polytechnic, NAU, Waghai.

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Production of barbatimão (Stryphnodendron adstringens) seedlings of increasing doses of domestic sewage effluent

Luiz Bruno Silva Constantino[1], Jayme Ferrari Neto[2], Beatriz Santos Machado[3], Fernando Jorge Correa Magalhães Filho[4], Denilson de Oliveira Guilherme[5]

[1]Graduate in agronomy, Dom Bosco Catholic University, Campo Grande/Mato Grosso do Sul
[2]Professor of agronomy graduate, Dom Bosco Catholic University, Campo Grande/Mato Grosso do Sul
[3,4,5]Postgraduate program in environmental sciences and agro-business sustainability, Dom Bosco Catholic University, Campo Grande/Mato Grosso do Sul.

Abstract— *The barbatimão is a traditional plant species of Brazilian cerrado. Barbatimão bark is widely used in the production of medicines and cosmetics. Because it is an extractive exploitation plant, the activity can be considered aggressive for plants that after having their shells removed may die. Another point to consider is the lack of a fertilization protocol. Aiming to improve the nutrition of barbatimão seedlings through the use of effluent of domestic exhaustion. This work had as objective to study the production of barbatimão seedlings in function of crescents doses of domestic exhaustion. The experiment was conducted in a randomized block design with 5 treatments and 10 replications. The seedlings were grown in plastic bags of 7.5 liters filled with substrates based on pinus bark. The biometric evaluations were initiated 30 days after transplanting when the plants were already established in the new container and consisted of: height of the plants (measured by means of millimeter ruler) being considered with initial point of the region of the collection until the apex of the seedling; diameter of the stem (measured by means of digital caliper) and number of leaves counted from the base to the apex of the plant, being considered as leaf the petiole of the composite leaf inserted in the branches of the plant. In general, it was concluded that barbatimão seedlings were negatively influenced by the addition of domestic effluent from household wastewater. However, studies with lower dosages than those tested in this study are suggested.*

***Keywords*—biomass, Agricultural production, nutritional management.**

I. INTRODUCTION

The Stryphnodendron adstringens, popularly known as Barbatimão, bark of virginity, tiller beard, among other synonyms, is a species of the Brazilian Cerrado and transition zones with other biomes, occurring between the states of Bahia, Goiás, Mato Grosso, Mato Grosso do South, Minas Gerais, São Paulo, Tocantins and Distrito Federal (ALMEIDA et al., 1998; LORENZI, 2002).

The barbatimão has high pharmaceutical demand due to the high production of tannin that concentrates in a higher content mainly in the bark of the plant with about 25 to 30% of tannin in aqueous extract (PANIZZA et al., 1988).

The high demand for secondary metabolites such as tannin present in this species makes it subject to disorderly extractivism (BORGES FILHO et al., 2003). This in many Brazilian states does not have a management plan that regulates the part of the plant to be collected, time of year to collect the plant and quantities of plants to be collected per year area.

In this way, alternatives for the cultivation of Barbatimão are necessary and of great importance.

Besides the great importance of Barbatimão in the pharmaceutical area, it is also indicated for the recovery of degraded areas and can be used for the extraction of wood, which is heavy, hard and resistant to the action of climatic inclement weather (LORENZI, 1992).

Another factor that hampers the production of barbatimão for its intensive commercial exploitation is the lack of seedlings for planting. Since the species propagates in a seminiferous way in nature, subject to edafoclimatic intempéries. The plant's nutrition is still unknown, a fact that would facilitate the use of fertilizers for its production, which also increases the process of seedling production.

The search for more sustainable agricultural practices has brought the tendency of reuse of effluents and biosolids from domestic exhaustion. This fact from the agronomic point of view is very beneficial for the agricultural crops, to promote greater organic matter input, slow and continuous release of nutrients, improvement of soil microbiological conditions, etc. The reuse of water in irrigation in Brazil is no longer an alternative and is becoming a necessity, due to the low rainfall levels observed in recent years and also that this type of effluent has been affecting the environment through the pollution of rivers (Queiroz et al. .

However, ANVISA regulates maximum levels for concentration of heavy metals in food, which can become contaminated with this form of fertilization. As barbatimão is not ingested by most humans in products that use its by-products, this concern is diminished.

Considering the above, it is hypothesized that the use of domestic effluent can significantly improve the growth of barbatimão seedlings, accelerating its growth in comparison to plants not fertilized with this effluent. Therefore, the objective of this work was to evaluate the growth of barbatimão seedlings as a function of increasing doses of domestic sewage effluent.

II. MATERIAL AND METHOD

The experiment was carried out in the city of Campo Grande-Mato Grosso do Sul, Brazil, and defined by the geographical coordinates "20º 23 '12" south latitude, "54º 36' 32" west longitude and 632 meters high.

The seeds were purchased via internet from a collector in the state of Santa Catarina - Brazil. The seeds were mechanically scarified by means of grooves, caused by the abrasion of a thick sandpaper in the seed coat. This practice was carried out in order to favor the entry of water into the seed and thus accelerate its germination process.

After the chiseling, two seeds per cell were planted with a volume of 280 cm3 filled with substrate with a coat of pinus with a volume of 280 cm3, without the use of mineral fertilization. Germination occurred approximately 35 days after sowing with germination rate of 65%. The initial irrigation of the trays was done by drinking water until the seedlings reached an average of 15 cm in height. At this moment the seedlings were transplants to plastic bags with volume of 7.5 liters, where they were cultivated until the end of the experiment.

The experimental design was a randomized block design, with five treatments and six replicates. The treatments consisted of increasing doses of domestic sewage effluent at doses (0%, 25%, 50%, 75% and 100%) consisting of dilutions of the diluted raw sewage in increasing proportions until complete irrigation by water without effluents. Each time the plants were irrigated 1.2 liters of the concentrations were applied. The pH of the soil on which the plants were cultivated was monitored periodically in order to avoid large oscillations. This fact would reduce the absorption of nutrients by the seedlings.

The biometric evaluations were initiated 30 days after transplanting when the plants were already established in the new container and consisted of: height of the plants (measured by means of millimeter ruler) being considered with initial point the region of the collection until the apex of the seedling; diameter of the stem (measured by means of digital caliper) and number of leaves counted from the base to the apex of the plant, being considered as leaf the petiole of the composite leaf inserted in the branches of the plant.

After 180 days of cultivation, the seedlings were sectioned and the following evaluations were carried out: wet weight of shoot, dry weight of shoot, root volume, root length, root weight, root dry weight. The plant materials were dried in a forced circulation oven for two days at a temperature of 70 ºC and afterwards weighed. The Dikson molt quality index (DICKSON et al., 1960) was also calculated by equation 1- Dickson Quality Index (DQI):

$$DQI = \frac{TDM\ (g)}{\frac{H\ (cm)}{D\ (mm)} + \frac{DSM\ (g)}{RDM\ (g)}} \tag{1}$$

This Dickson Quality Index (DQI) was determined as a function of total dry mass (TDM), height (H), diameter (D), dry shoot mass (DSM) and root dry mass (RDM).

At the end of the data collection, the analysis of variance and the averages of the treatments were performed using the Tukey test with significance level $p > 0.05$ and for the quantitative data performed the regression analysis.

III. RESULT AND DISCUSSION

Plant height was higher when water without effluent dilution was used, the diameter of the stem of the plants was higher when only drinking water was used (Table 1). The height evaluation of the shoot can be used to express the quality of the seedlings and is recognized as a good measure of the performance potential of the production. In the experiment, the height was highlighted in the treatments with 0% and 100% of sewage, as shown in Fig 1.

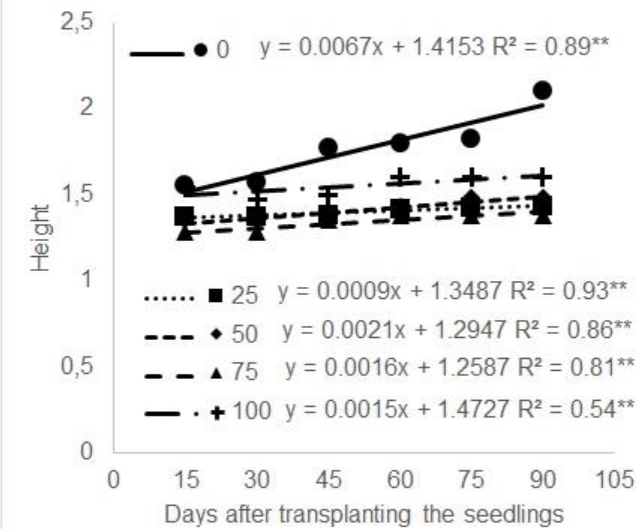

FIGURE 1 - Height of barbatimão seedlings as a function of increasing doses of domestic sewage effluent.

TABLE 1
DIAMETER, NUMBER OF LEAVES AND HEIGHT OF BARBATIMÃO PLANTS GROWN IN DIFFERENT CONCENTRATIONS OF DOMESTIC SEWAGE EFFLUENT.

Efluent (%)	Diâmeter (mm)	Number of leaves	Height (cm)
0	1.77 a	5.03 a	6.43 a
25	1.40 b	3.86 b	5.63 b
50	1.41 b	3.64 b	5.51 b
75	1.35 c	3.31 b	5.55 b
100	1.55 ab	4.14 ab	6.00 ab

TABLE 2
DRY AND WET WEIGHT, VOLUME AND LENGTH OF BARBATIMÃO VEGETATIVE MATERIAL, GROWN AT DIFFERENT CONCENTRATIONS OF DOMESTIC SEWAGE EFFLUENT.

Efluent (%)	HSW (g)	SDW (g)	RV (ml)	RL (cm)	MRW (g)	RDW (g)
	--------------------g------------------		Ml	cm	-------------g----------------	
0	0.80 a	0.45 a	0.75 a	23.67 a	0.44 a	0.24 a
25	0.25 b	0.14 b	0.20 b	16.58 b	0.16 b	0.10 b
50	0.55 b	0.28 b	0.37 b	17.84 b	0.34 b	0.16 b
75	0.34 b	0.17 b	0.20 b	14.54 b	0.19 b	0.10 b
100	0.56 b	0.27 b	0.47 b	14.67 b	0.36 b	0.18 b

HSW - humid shoot weight; SDW - shoot dry weight; RV - root volume; RL – root length - MRW-moist root weight; RDW - root dry weight

The photosynthetic process occurs mainly in leaves. They convert solar (luminous) energy into a usable chemical form, producing carbohydrates from CO_2 and H_2O. In addition, they perform perspiration, accumulate and redistribute nutrients. Thus, the individuals with the highest number of leaves have greater availability of assimilated photos and, consequently, they present greater growth.

In general, the increase in the number of leaves was higher when only water 0% and sequentially 100% of sewage were used, as shown in Fig 2, following the same results represented by the treatments in height.

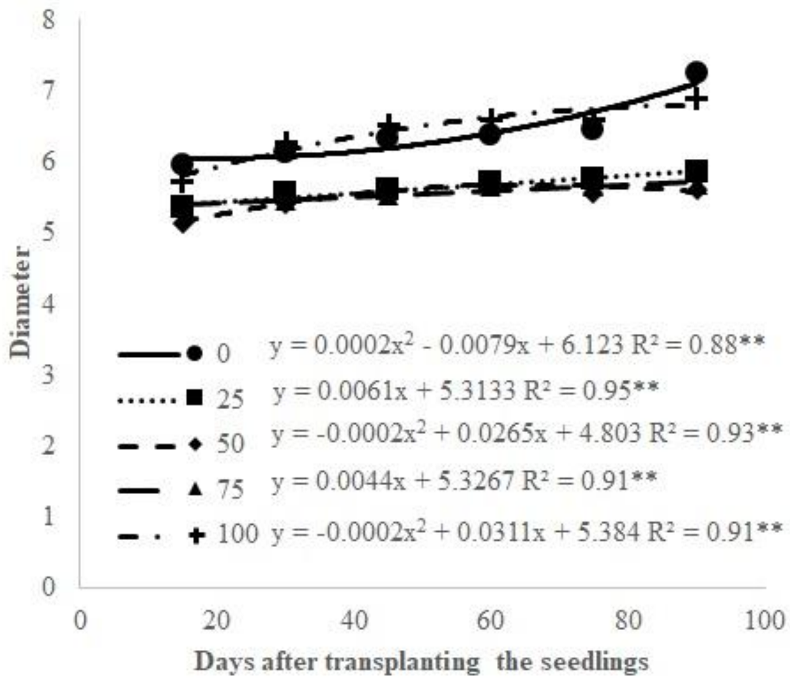

FIGURE 2 - Number of leaves of barbatimão seedlings as a function of the increasing doses of domestic sewage effluent.

The diameter of the lap is used to evaluate the survival capacity of seedlings and may also be indicated to help determine the fertilizer doses to be applied in the production of seedlings. In the present experiment, the results showed that the increase of effluent levels in the treatments did not allow a significant increase in stem diameter when compared to the use of only water, as shown by Fig 3.

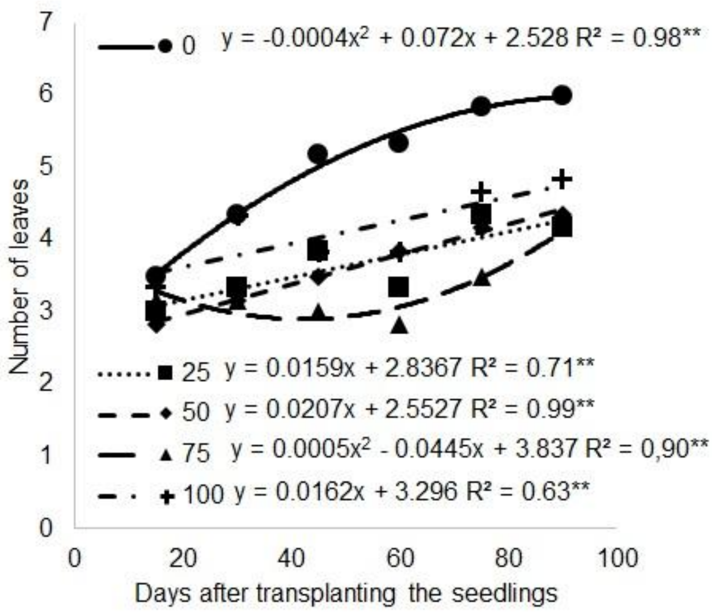

FIGURE 3 - Stem diameter of barbatimão seedlings as a function of increasing doses of domestic sewage effluent

In relation to the variables evaluated above, we observed that all the evaluated items did not diverge among themselves, with the exception of the root volume, showing a greater development when irrigated with only water (0% treatment).

With these results the Dickson quality index (IQD) was applied, which is recognized as a good quality indicator of seedlings, because the robustness (H / DC ratio) and the biomass distribution balance (MSPA ratio) / MSR) (TRAZZI, 2011), weighting the results of several important morphological characteristics used for quality assessment. For Caldeira et al. (2012), the higher the IQD, the better the quality of the seedling. According to Birchler et al. (1998) and Hunt (1990), the IQD value should be greater than 0.2, which was not observed among the means of this study. In this experiment, the highest mean was obtained in the treatment with 0% sewage, that is, IQD = 0.13 as Fig 4.

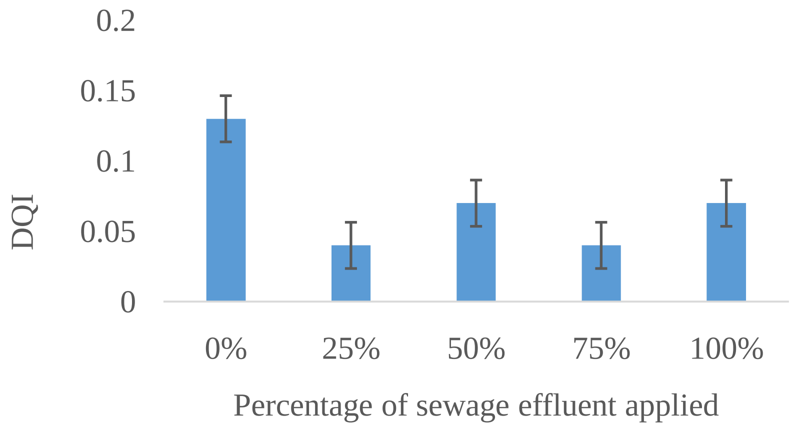

FIGURE 4 - Mean values and standard deviation for the Dicksom Quality Index (IQD) of barbatimão seedlings, as a function of the increasing doses of domestic sewage effluent.

However, several studies have demonstrated that IQD is a variable attribute, occurring differences depending on the species, the management, the type of substrate, the volume of the container and the age at which the seedlings were evaluated (Caldeira et al., 2005; TRAZZI, 2011).

The results indicate that the elevation of sewage levels in the substrate negatively influenced barbatimão seedlings. This fact was described by other authors who used this residue as a component of the substrate. Figueiró et al. (2005) observed a decrease in the emergence rate of melon seedlings grown on substrates containing sewage in proportions greater than 2.5%.

The use of sewage indicated that concentrations of this compound may be cytotoxic to the species. Thus, substrates with dosages of sewage sludge are not recommended for the production of barbatimão seedlings. These results coincide with those of Trigueiro and Guerriine (2003) and Morais et al. (1997), which found that composite substrates with sewage rates higher than 70% and 30% interfered negatively in the development of eucalyptus and cedar seedlings, respectively.

In general, the plants from the treatments with addition of sewage showed signs of intoxication such as necking and necrosis. This may have occurred because the substrate presents a higher concentration of organic matter and nutrients (GOMES et al., 2013). Another symptom observed in the barbatimão seedlings, cultivated in substrates with doses of sewage, is chlorosis, followed by necrosis at the tips of the leaves. Another observed symptom can be attributed to excess Zn, which in plants are characterized by reduced growth and leaf chlorosis (FONTES; COX, 1998). However, it was not possible to associate the symptoms of toxicity with a specific element because the nutritional requirements and the behavior of micronutrients in the development of this species still need to be further studied.

IV. CONCLUSION

In general, it was concluded that barbatimão seedlings were negatively influenced by the addition of domestic effluent from household wastewater. However, studies with lower dosages than those tested in this study are suggested.

V. AUTHORS RECOMMENDATION

O barbatimão é uma especie vegetal tradicional do cerrado brasileiro. A casca do barbatimão é muito utilizada na produção de medicamentos e cosméticos. Por ser uma planta de exploração extrativista, a atividade pode ser considerada agresssiva para as plantas que apos terem suas cascas tiradas podem morrer.Outro ponto a ser considerado é a falta de um protocol de adubação. Visando melhorar a nutrição de mudas de barbatimão em por meio do uso de effluent de esgotamento domestico.Este trabalho teve como objetivo estudar a produção de mudas de barbatimão em função de doses crescents de esgotamento domestic. O experiment foi conduzido em delinemanto de blocos ao acaso com 5 tratmentos e 10 repetiçoes . As mudas foram cultivadas em sacos plasticos de 7,5 litros preenchidos com substrates a base de casca de pinus. The biometric evaluations were initiated 30 days after transplanting when the plants were already established in the new container and consisted of: height of the plants (measured by means of millimeter ruler) being considered with initial point the region of the collection until the apex of the seedling; diameter of the stem (measured by means of digital caliper) and number of leaves counted from the base to the apex of the plant, being considered as leaf the petiole of the composite leaf inserted in the branches of the plant. In general, it was concluded that barbatimão seedlings were negatively influenced by the addition of domestic effluent from household wastewater. However, studies with lower dosages than those tested in this study are suggested.

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The Role of Salicylic Acid, Jasmonic Acid and Ethylene in Plant Defense

Firoozeh Chamandoosti

Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran PhD of Cellular and Developmental Biology, Assistant Professor of Iranian Research Institute of Plant Protection Department of Plant Diseases

Abstract— *A complex network of cross – talk between the salicylic acid (SA) and jasmonic acid (JA) pathways further fine tunes plant defense responses. SA can be formed from cinnamate via o – coumarate or benzoate depending on whether the hydroxylation of the aromatic ring takes place before or after the chain – shortening reactions. SA not only functions against biotrophs, but also activates plant resistance against the below – ground disease such as root – knot nematodes. The synthesis of jasmonates and many other oxylipins is initiated by lipoxygenases (LOXs), which catalyze the regio – and stereoselective dioxygenation of polyunsaturated fatty acids. JA activates plant immune responses to necrotrophic pathogens, some phloem – feeding insects and chewing herbivores. Also JA is also involved in other aspects of plant – pathogen interactions, including systemic acquired resistance (SAR). The role of ethylene (ET) in plant diseases resistance is dramatically different duo to type of pathogene and plant species. There are many evidence that show ethylene response is linked to gene for gene resistance. It is proven that there are a strong connection between different pathways related to SA, JA and ET for plant diseases resistance. So that SA – dependent and JA/ethylene – dependent pathwa ys induce expression of different sets of PR genes and result in plant resistance to different pathogens.*

Keywords*— *Jasmonic acid, Plant Resistance, Salicylic acid.

I. INTRODUCTION

Plant resistance to pathogenic agents usually operates through a complex defense mechanism network. Compounds such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) regulate plant defense pathways to trigger appropriate responses to different pathogens [4]. Whereas the SA signaling pathway is mainly activated against biotrophic pathogens, the JA/ET signaling pathway is activated against necrotrophic pathogens [28]. So SA rely on living plant tissue for nutrients [34]; [79] and [13]. In contrast, plants produce jasmonic acid (JA) in response to wounding caused by insects and to necrotrophic microbes which obtain nutrients from dead host cells [13]; [118] and [102]. It can be said that the SA – mediated defenses have a major role in the basal resistance to the bacterial and oomycete pathogens, *Pseudomonas syringae* and *Peronospora parasitica*, respectively, turnip crinkle virus (TCV) and cucumber mosaic virus (CMV) [10]; [86]; [116]; [20] and [38]. In contrast, JA signaling has an important role in the basal resistance to the fungal pathogen *Botrytis cinerea* [13]. ET is a critical third player from the perspective of understanding how plants prioritize and tailor their responses to diverse attackers [1]. Also ET modulates SA related plant defense signaling both positively and negatively.

At the end of this section, it is important to point out that a complex network of cross – talk between the SA and JA pathways further fine tunes plant defense responses [10] and [11]. Most studies have identified antagonistic interactions between the SA – and JA – mediated signaling pathways [112]. But the relationship between SA and JA is not always antagonistic [101]. The following examples that are presented sequential help to clear this concept. The application of JA depressed the activation of the genes for the acidic PR proteins, [Pathogenesis – related protein(s)] which are SA dependent [112]. In rice is demonstrated that JA signalling positively regulates plant resistance to the biotrophic pathogen, *Xanthomonas oryzae* pv. *oryzae* (Xoo), [101**]** possibly due to a common defence system activated by both SA and JA [24].

II. BIOSYNTHESIS OF SALICYLIC ACID

As mentioned above Salicylic acid (SA) is an important signal molecule in plants.

Biochemical studies using isotope feeding have suggested that plants synthesize SA from cinnamate produced by phenylalanine ammonia lyase (PAL). PAL is a key regulator of the phenylpropanoid pathway and is induced under a variety of biotic and abiotic stress conditions. SA can be formed from cinnamate via *o* – coumarate or benzoate depending on whether the hydroxylation of the aromatic ring takes place before or after the chain – shortening reactions. In sunflower, potato and pea, isotope feeding indicated that SA was formed from benzoate, which is synthesized by cinnamate chain shortening reactions most likely through a β – oxidation process analogous to fatty acid β – oxidation. [35]. Feeding of 14 – labeled phenylalanine and cinnamate to young *Primula acaulis* and *Gaultheria procumbens* leaf segments indicated that SA was formed via *o* – coumarat [106]. In the same plants, labeled SA was also formed after treatment with 14C – labeled benzoate, [106] suggesting that these plants may use both pathways for SA synthesis. Likewise, in young tomato seedlings, SA appeared to be formed mostly from cinnamate via benzoate but after infection with *Agrobacterium tumefaciens*, 2 – hydroxylation of cinnamate to *o* – coumarate was favored [68]. In tobacco and rice, several lines of evidence suggest that SA is synthesized from cinnamate via benzoate [50]; [88] and [84]. First, infiltration of healthy tobacco leaf discs with 0.1 mM benzoate increased total SA level 14 fold after 18 hours [84]. Second, in TMV – infected tobacco, large increases in the levels of benzoate and SA were detected [84]. Third, in both TMV – infected tobacco leaves and rice seedlings, labeled benzoate and SA, but not *o* – coumarate, were detected after feeding with ^{14}C – labeled cinnamate [88] and [84] More label was incorporated into SA when ^{14}C – labeled benzoate was fed than when ^{14}C – labeled cinnamate was used, consistent with benzoic acid being the immediate precursor of [88] and [84]. Similar results were also obtained from the labeling experiments in potato and cucumber [87] and [57]. Furthermore, a benzoic acid 2 – hydroxylase (BA2H) activity was detected in plants including tobacco and rice. In tobacco, the BA2H activity was induced by TMV infection and was partially purified as a soluble 160 kDa protein that could be immunoprecipitated by antibodies against the soluble SU2 cytochrome P450 from *Streptomyces griseolus* [50]. Despite the extensive biochemical and molecular evidence, none of the enzymes required for the conversion of SA from cinnamate in the PAL pathway has been isolated from plants. Although partial purification and immunoprecipitation of a tobacco BA2H activity were reported in 1995, [50], there has been no further report on its purification or isolation of the corresponding gene(s).

III. BIOSYNTHESIS OF JASMONIC ACID

The synthesis of jasmonates and many other oxylipins[1] is initiated by lipoxygenases (LOXs), which catalyze the regio – and stereoselective dioxygenation of polyunsaturated fatty acids [26]; [44]; [33]; [29] and [15]. Linoleic acid (18:2) and linolenic acid (18:3) are oxygenated by specific LOXs at C9 or C13 to result in the corresponding (9S) – or (13S) – hydroperoxy – octadecadi(tri)enoic acids, which feed into at least seven alternative pathways resulting in the formation of a large variety of oxylipins [26] and [44]. The first committed step in the two parallel pathways for JA biosynthesis i.e. the octadecanoid pathway from 18:3 and the hexadecanoid pathway from 16:3 [41], is performed by allene oxide[2] synthase (AOS), an unusual cytochrome P450 which uses its hydroperoxide substrate as source for reducing equivalents and as oxygen donor, and is thus independent of molecular oxygen and NAD(P)H. AOS catalyzes the dehydration of 13(S) – hydroperoxy – octadecatrienoic acid (13 – HPOT) to form an unstable allene oxide, 12,13(S) – epoxy – octadecatrienoic acid (12,13 – EOT). In aqueous media, 12,13 – EOT rapidly decomposes to – and – ketols, or undergoes cyclization to form 12 – oxo – phytodienoic acid (OPDA). As opposed to spontaneous cyclization which results in a racemic mixture of OPDA enantiomers, allene oxide cyclase (AOC) ensures the formation of the optically pure 9S,13S enantiomer. Dinor – OPDA (dnOPDA) is generated in the parallel pathway from 16:3 The short half – life of 12,13 – EOT in aqueous media (26 s at 0◦C and pH 6.7, [81]; [60] and the optical purity of endogenous OPDA [22] suggest tight coupling of the AOS and AOC reactions in vivo. However, physical contact of AOS and AOC in an enzyme complex does not seem to be required for stereochemical control of the cyclization reaction [90]. Only 9S,13S – OPDA, i.e. one out of four possible OPDA stereoisomers, is a percursor for biologically active JA. AOC is thus crucially important to establish the enantiomeric structure of the cyclopentenone ring. The crystal structure of *Arabidopsis* AOC2 has recently been solved shedding light on how the enzyme exerts stereochemical control on the cyclization reaction [27]. Considering the fact that cyclization occurs spontaneously in aqueous solution, AOC2 does not

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[1]- constitute a family of oxygenated natural products which are formed from fatty acids by pathways involving at least one step of dioxygen-dependent [15].

Oxylipins are derived from polyunsaturated fatty acids (PUFAs) by COX enzymes (cyclooxygenases), by LOX enzymes (lipoxygenases), or by cytochrome P450 epoxygenase [115].

[2] - In organic chemistry, an allene (is a compound in which one carbon atom has double bonds with each of its two adjacent carbon centres.) oxide is an epoxide of an allene. The parent allene oxide is CH_2=C(O)CH_2 , a rare and reactive species of only theoretical interest. Typical allene oxides require steric protection for their isolation. Certain derivatives can be prepared by epoxidation of the allenes with peracetic acid. Allene oxides tend to rearrange to cyclopropanones [114].

Despite the esoteric character of synthetic allene oxides, allene oxides occur naturally. They are intermediates in the chemical defense of some plants against attack by herbivores. Specifically, a hydroperoxide of linolenic acid is the substrate for the enzyme allene-oxide synthase. The resulting allene oxide in turn is converted by allene oxide cyclase to jasmonic acid [2].

need to be much of a catalyst in terms of lowering the activation energy barrier. Indeed, binding of the substrate or the transition state does not involve any induced fit mechanism. The hydrophobic protein environment and very few ionic interactions with a glutamate residue (Glu23) and a tightly bound water molecule, ensure binding and correct positioning of the substrate 12,13 – EOT. Steric restrictions imposed by the protein environment enforce the necessary conformational changes of the substrate's hydrocarbon tail resulting in the absolute stereoselectivity of the AOC2 – mediated as opposed to the chemical cyclization reaction [27].

IV. Biosynthesis Of Ethylene

The biochemistry of ethylene biosynthesis has been a subject of intensive study in plant hormone physiology [36]. The discovery of ethylene as a plant growth regulator can be attributed to the work of the Russian scientist [23]. He reported that dark – grown pea seedlings showed a reduced hypocotyl growth in combination with an exaggerated hypocotyl bending when exposed to illumination gas [23]. Neljubov 1901 [23] could pinpoint ethylene gas as the active component that caused dark – grown pea seedlings to bend, by flowing the illumination gas over several filters prior to exposing the seedlings. This typical ethylene response of dark – grown seedlings was later defined as the triple response: (1) shortening of the hypocotyl and roots, (2) radial swelling of the hypocotyl, and (3) the exaggeration of the apical hook[3] [71].

Ethylene is formed from methionine via S – adenosyl – L – methionine (AdoMet) and the cyclic non – protein amino acid 1 – aminocyclopropane – 1 – carboxylic acid (ACC). ACC is formed from AdoMet by the action of ACC synthase (ACS) and the conversion of ACC to ethylene is carried out by ACC oxidase (ACO) [36]. In addition to ACC, ACS produces 5' methylthioadenosine, which is utilized for the synthesis of new methionine via a modified methionine cycle. This salvage pathway preserves the methylthio group through every revolution of the cycle at the cost of one molecule of ATP. Thus high rates of ethylene biosynthesis can be maintained even when the pool of free methionine is small [70].

Analysis of ACS gene induction in mutant fruit with disrupted ethylene signalling has been used to identify which ACS gene is ethylene – regulated. The Never ripe (Nr) mutant cannot perceive ethylene due to a mutation in the ethylene – binding domain of the NR ethylene receptor [73] and [53]. Fruit from the ripening inhibitor (rin) mutant do not show autocatalytic ethylene production [94]. and cannot transmit the ethylene signal downstream to ripening genes due to a mutation in the RIN transcription factor [52]. Nr and rin mutant fruit have shown that LEACS2 expression requires ethylene whereas LEACS1A and LEACS4 exhibited only slightly delayed expression in Nr indicating that ethylene is not responsible for regulation of these genes [19]. All four fruit ACS genes showed the same expression patterns in rin fruit as in mature green wild – type fruit, but did not show any ripening – related changes of expression [19]. Therefore, it has been proposed that LEACS1A and LEACS6 are involved in the production of system 1 ethylene in green fruit [19]. System 1 continues throughout fruit development until a competence to ripen is attained, where upon a transition period is reached, during which LEACS1A expression increases and LEACS4 is induced. During this transition period, system 2 ethylene synthesis (autocatalysis) is initiated and maintained by ethylene – dependent induction of LEACS2 [19]. Antisense inhibition of LEACS2, which also down – regulated LEACS4, reduced ripening – related synthesis of ethylene to 0.1% of control fruit. The antisense fruit displayed an abnormal pattern of ripening such as reduced lycopene accumulation, delayed softening and a much reduced climacteric peak [91].

V. The Role Of Salicylic Acid In Plant Defense

Salicyclic acid plays crucial role in various plant – pathogen interactions by activating defense responses. It is typically involved in defense against microbial biotrophs such as the bacteria *Pseudomonas syringae* [7]. SA biosynthetic pathway has been well characterized in plants. It includes two distinct enzymatic pathways, isochorismate synthase (ICS/SID2) – mediated isochorismate pathway and phenylalanine ammonia lyase (PAL) – mediated phenylalanine pathway, which has been reported to be required for both systemic and local acquired resistance as well as PCD [76]. Thus, SA is involved in inducing systemic acquired resistance (SAR) [108] and [67].

Stimulation of defense responses occurs not only at the recognition site of microbes, but also in distal parts of plants to protect undamaged tissues from subsequent microbial pathogen invasion for a long – lasting protection, commonly known as SAR [77]. Evidences from grafting experiments suggest that methyl salicylate (MeSA) is the critical mobile signal for SAR in tobacco plant [105]. SA signaling is activated to suppress viral, bacterial, and fungal pathogens invasion in many plant

[3] - Hook – like structure which develops at the apical part of the hypocotyl in dark – grown seedlings in dicots.

species through up – regulation of pathogenesis – related (*PR*) genes expression and the development of SAR [58]. The role of SA signaling in disease resistance was first illustrated by White and coworkers, who demonstrated that treatment of tobacco with SA, or its derivative aspirin significantly stimulated PR protein accumulation and enhanced plant resistance to tobacco mosaic virus (TMV) infection [97]. Increasing progress revealed that SA played an essential role in resistance against multiple pathogens [40]; [85] and [72]. A study conducted on wheat demonstrated that SA exerted direct and significant impact on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat; conidia germination and mycelial growth were remarkably inhibited at higher SA concentrations [85]. Exogenous application of SA induces *PR1*, *PR3* (chitinase), *PR5* (thaumatin – like), and *PR9* (peroxidase) gene expression as well as the resistance against bacterial pathogen *Pseudomonas syringae* pv. *syringae* in barley, which is characterized by a concomitant increase in endogenous SA levels [72]. Additionally, foliar application of SA can induce resistance against Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Phaseoli* in common bean which is accompanied with elevated contents of endogenous free and conjugated SA as well as activities of PAL and peroxidases [96]. In tomato, SA treatment induces expession of PR2 and PR3 proteins, activity of PAL, concentration of antioxidants as well as photosynthesis, thereby reducing the infections caused by *Potato virus X* [109]. Over – expression of SA biosynthetic genes or mutation in SA – related genes also affects plant disease resistance [76] and [75]. Over – expression of two bacterial SA biosynthetic genes in transgenic tobacco conferred highly increased SA accumulation accompanied with upregulated defense genes expression particularly those encoding PR proteins, and thus enhancing plant resistance to viral and fungal infection [75]. Transgenic tobacco plants with low or no salicylic acid biosynthesis capacity are defective to induce SAR [108]. Interestingly, exogenous SA application could establish SAR and enhance disease resistance to pathogens even in the SA biosynthetic mutants [76]. SA and benzothiadiazole (BTH, a SA analogue) treatments on perennial ryegrass (*Lolium perenne* L.) enhance expression levels of *PR* genes and deposition of callose, and thereby minimizing the incidence as well as severity of gray leaf spot disease (causal agent: *Magnaporthe oryzae*) [3]. However, plant responses to SA treatment are strictly dependent on developmental stage for a given pathogen. For instance, exogenous SA induces resistance to *Magnaporthe grisea* in mature rice plants, but not in young rice plants, which is accompanied with increased accumulation of SA and PR proteins, resulting in the formation of hypersensitive reaction lesions [110]. Environmental factors also stimulate SA accumulation and thus influence plant resistance. Cucumber plants exhibit increased resistance against *Sphaerotheca fuligineathan* under red light and this resistance is associated with enhanced SA – dependent signaling [40]. SA not only functions against biotrophs, but also activates plant resistance against the below – ground disease such as root – knot nematodes (RKN) [14] and [37]. RKN are sedentary endoparasite which feed cells for their nutrition and keep its sedentary life cycle [32]. The yield loss in agriculture caused by nematodes is devastating, which accounts for 5% to 12% of annual crop loss worldwide [69]. Branch *et al.* [14] demonstrated that SA was involved in *Mi – 1* – mediated defense response to RKN (*Meloidogyne incognita*) in tomato. SA induces glutathione status in tomato plant and imparts resistance against RKN *M. incognita* [42]. Foliar application of SA is able to trigger SAR response to RKN in tomato roots [99]. To date, numerous scientific studies indicate that activation of SA pathway is important for induced defense in plants against biotrophic pathogens, such as *Pseudomonas syringae* and *Golovinomyces cichoracearum* [103] and [31]. However, there are some exceptions that conflict with the current model. For instance, Novakova *et al.* [82] showed that SA was involved in the resistance against necrotrophic pathogen *Sclerotinia sclerotiorum (Lib.)* de *Bary*, which was previously known to be functioned by JA and ET signaling pathways. In spite of contradictory evidences, it is well accepted that SA is an important endogenous marker and crucial for plant disease resistance against biotrophic as well as necrotrophic pathogens. A number of studies demonstrate that invading pathogens or insects release effectors proteins that interfere or block the plant immune system for the benefit of its own establishment to cause disease development in the plant tissue [25]; [63] and [80]. Virulent *Pseudomonas syringae* produces the bacterial effector proteins virulence factor coronatine (COR), which is a phytotoxin that structurally and functionally mimic methyl jasmonate and suppresses SA – dependent immune defenses, thus the pathogen utilizes the hormone – regulated defense signaling network to promote susceptibility of the host [25] and [63]. Meanwhile, the fungal pathogen *Sclerotinia sclerotiorum* participates actively in the degradation of SA, and the presence of low levels of SA affect growth or oxalate production by *Sclerotinia sclerotiorum* [17]. *Hyaloperonospora arabidopsidis* (Hpa) causes downy mildew in *Arabidopsis thaliana*. During their interaction, Hpa effector suppresses SA – induced interaction and attenuates responses to SA [98]. Interestingly, mollusks secrete phytohormone – like substances into their mucus which contain significant amounts of SA and induce SA – responsive gene *PR1* expression in wounded leaves of *Arabidopsis thaliana*, suggesting the potential of mucus in regulating plant defense [49]. It is now clear that SA plays important role in activating plant defense mechanism and therefore application of SA could be considered as a feasible strategy to minimize biotic stress induced yield reduction in commercial agriculture.

VI. THE ROLE OF JASMONIC ACID IN PLANT DEFENSE

As previously mentioned Jasmonic acid and its metabolites, including methyl jasmonate (MeJA), are lipid – derived compounds originating from plastid membrane a – linolenic acid. They act as signals to mediate plant growth and developmental processes, as well as plant responses to biotic and abiotic stresses [2]. Numerous reports indicate that JA activates plant immune responses to necrotrophic pathogens, some phloem – feeding insects and chewing herbivores [48]. JA is also involved in other aspects of plant – pathogen interactions, including SAR. Application of JA to rice plants induces resistance against necrotrophs pathogen *Rhizoctonia solanivia* through the activation of phenylpropanoid pathway [89] Predominantly, JA responses are mediated through the coronatine insensitive 1 (COI1 Fbox protein and *coi1* mutants possess elevated SA levels and exert more resistance to bacterial pathogens [9]. On the contrary, *coi1* plants show greater susceptibility to *Botrytis cinerea* and *Alternaria brassicicola* [13]. Stotz *et al.* [39] unveiled JA – dependent and *COI1* – independent defense responses against *Sclerotinia sclerotiorum* in *Arabidopsis thaliana*; however, AUXIN RESPONSE FACTOR 2 (ARF2) acts as a negative regulator of defense responses against this pathogen. The environmental factors such as light quality can influence plant resistance via JA pathway. Low red/far – red ratios reduce *Arabidopsis* resistance to *Botrytis cinerea* and JA responses via a COI1 – JAZ10 – dependent and SA – independent mechanism [43]. Increasing evidences have proved that JA is critical for plant resistance against plant disease RKN [65] and [61]. After the foliar treatment with MeJA, the expression profiles of JA genes were induced resulting in highly enhanced resistance against RKN (*Meloidogyne incognita*) in tomato plants [111]. Transcript profiles from microarray analyses indicate that an intact JA signaling pathway is required for tomato susceptibility to RKN [66]. Several studies demonstrated that other hormones might function to suppress RKN through the crosstalk with JA pathway. Nahar *et al.* [62] reported that BRs suppressed the JA biosynthesis and signaling pathway under RKN infestation in rice and thus determined the outcome of the rice – RKN interaction through crosstalk between BR and JA pathway. Exogenous application of MeJA to the rice shoots also induces a strong systemic defense response in the roots, making the plant more resistant to nematodes infection, while ET activates the JA biosynthesis and signaling pathway in a facultative manner, indicating a pivotal role of JA pathway in systemically induced resistance against RKN in the root [61]. Environmental factors also play remarkable role in determining severity of RKN infection through the activation of JA pathway. Elevated CO2 changes the interactions between nematode and tomato genotypes differing in the JA pathway. CO2 enrichment possibly impairs the allocation of plant resources between growth and defense by affecting contents of secondary metabolites, volatile organic compounds and C: N ratio that eventually reduces plant resistance to RKN in tomato [121] and [122]. In addition to well established role in resistance against necrotrophic pathogens in various plant species, JA has also been reported to induce plant resistance against biotrophic pathogens [124] and [5]. Exogenous MeJA significantly induced nine PRs gene expression and enhanced the powdery mildew resistance in the susceptible varieties of wheat, suggesting that JAs play important role in defense against wheat powdery mildew infection and manipulation of JA pathway through breeding may improve powdery mildew resistance in wheat [124]. Exogenous application of JA can also enhance resistance to the bacterial blight and blast caused by *Xanthomonas oryzae* pv. *oryzae* [101] and *Magnaporthe oryzae*, respectively in rice [83]. It's worth mentioning that rice plants constitutively expressing the pathogen – responsive WRKY30 gene showed enhanced resistance against fungal pathogens *Rhizoctonia solani* and blast fungus *Magnaporthe grisea*, concomitantly with increased endogenous JA accumulation and induction of JA biosynthesis related genes expression [119].

VII. THE ROLE OF ETHYLENE IN PLANT DEFENSE

Plants have evolved sophisticated detection and defense systems to protect themselves from pathogen invasion. Ethylene seems to play an important role in various plant disease resistance pathways. However, depending on the type of pathogen and plant species, the role of ethylene can be dramatically different. Plants deficient in ethylene signaling may show either increased susceptibility or increased resistance. For example, in soybean, mutants with reducedethylene sensitivity produce less severe chlorotic symptoms when challenged with the virulent strains *Pseudomonas syringae* pv *glycinea* and *Phytophthora sojae*, whereas virulentstrains of the fungi *Septoria glycines* and *Rhizoctonia solani* cause more severe symptoms [113]. Simiarly, in *Arabidopsis*, the ethylene – insensitive mutant *ein2* develops only minimal disease symptoms as the result of enhanced disease tolerance when infected by virulent *P. syringae* pv *tomato* or *Xanthomonas campestris* pv *campestris* [8]. However, the *ein2* mutant also displays enhanced susceptibility to the necrotrophic fungus *Botrytis cinerea* [12]. On the basis of these observations, ethylene seems to inhibit symptom development in necrotrophic pathogen infection but enhances the cell death caused by other type of pathogen infection. In fact, *Arabidopsis* protoplasts isolated from the *etr1 – 1* mutant display reduced cell death from the fungal toxin fumonisin B1 [107 and presence of the *ein2* mutation reduces cell death in the accelerated *cell death 5* (*acd5*) mutant [59] supporting a role for ethylene in the regulation of programmed cell death.

Upon pathogen infection, the avirulence signal (*avr*) carried by pathogens is recognized by a specific plant resistance (*R*) gene product [64]. This avr/R interaction is called gene – for – gene resistance and often triggers a strong defense mechanism that includes the programmed cell death of plant cells at the site of infection (known as the hypersensitive response), resulting in efficient containment of the pathogen. In tomato, it has been demonstrated that a direct interaction between the *R* gene *Pto* and the avirulence gene *avrPto* in the *P. s. tomato* strain determines gene – for – gene specificity in this plant – pathogen interaction [104] and [95]. Recently, a transcription factor, Pti4, has been identified on the basis of its specific interaction with Pto [123]. Interestingly, this Pti4 protein shares extensive similarity with the amino acid sequences of EREBPs and can specifically bind the GCC – box cis element present in the promoter of many ethylene – regulated pathogen – related (*PR*) genes. Expression of *Pti4* in tomato leaves is rapidly induced by ethylene, and this induction precedes expression of GCC – box – containing *PR* genes. Moreover, phosphorylation of Pti4 by the Pto kinase enhances its binding to the GCC box. These results provide evidence that the ethylene response is linked to gene – for – gene resistance in tomato.

VIII. INTERACTION AMONG THE SALICYLIC ACID, JASMONIC ACID AND ETHYLENE IN PLANT DEFENSE

Activation of the hypersensitive response triggers a longlasting response known as systemic acquired resistance, which provides immunity against subsequent infections caused by a broad spectrum of pathogens [51]. In many cases, systemic acquired resistance is characterized by an increase in endogenous salicylic acid (SA) levels and expression of a subset of *PR* genes, as well as enhanced resistance to a broad spectrum of virulent pathogens. However, some pathogens can induce plant defense responses via activation of the ethylene and JA signal transduction pathways. *Arabidopsis* plants with defects in ethylene perception (*ein2*) or JA signaling (*coi1*) fail to induce a subset of *PR* gene expression, including the plant defensin gene *PDF1.2*, a basic chitinase (*PR-3*), and an acidic hevein – like protein (a lectin – like protein from *Hevea brasiliensis*) (*PR-4*), resulting in enhanced susceptibility toward certain pathogens [46]. Interestingly, the induction of *PDF1.2* requires both intact JA and ethylene signaling, whereas the majority of other responses mediated by these hormones are specific to only one of the signals. This suggests that the ethylene and JA pathways interact with each other, co – regulating expression of some genes involved in plant defense. Because only a small subset of genes is affected by both signals, the interaction between these two pathways is likely to be downstream, possibly at the level of the specific defense gene promoters. Nevertheless, ethylene and JA signaling may also function independently to regulate distinct processes in defense response. A recent study has shown that pathogen – or elicitor – induced accumulation of the defense compound 3 – indolylmethylglucosinolate is mediated by JA but not by ethylene or SA [30] indicating that ethylene and JA pathways may have different roles in disease resistance. Although SA – dependent and JA/ethylene – dependent pathways induce expression of different sets of *PR* genes and result in plant resistance to different pathogens, there appear to be considerable interactions between these two pathways in systemic acquired resistance. Here, use of the word "cross – talk" is reserved for communications between two separate, linear signal transduction pathways that are simultaneously activated in the same cell. Therefore, the components of the two signaling pathways have to be (1) shown to be expressed in the same cell and (2) demonstrated to physically interact under normal physiological conditions [100]. A recent survey of changes in the expression levels of 2375 selected genes upon pathogen infection or SA, JA, and ethylene treatment had revealed that although some genes are affected by one signal or another, many respond to two or more defense signals [92]. These results indicate the existence of a substantial network of regulatory interaction and coordination among different plant defense pathways. For example, two *Arabidopsis* mutants that constitutively express *PR* genes, *cpr5* and *cpr6*, express both *PR – 1* and *PDF1.2* genes in the absence of pathogen infection. Although the constitutive expression of *PR – 1* is dependent on SA, it is only partially suppressed by the *npr1* (for non – expressor of *PR – 1*) mutation, a gene that is required downstream of SA to activate PR – 1 gene expression, indicating the existence of a SA – mediated, NPR1 – independent response [55]. Only when ethylene signaling is also blocked by *ein2* in addition to *npr1* mutation in *cpr5* and *cpr6* mutants is *PR – 1* gene expression abolished completely. Furthermore, *ein2* potentiates SA accumulation in *cpr5* and dampens SA accumulation in *cpr6*. These results suggest the existence of interactions between ethylene – and SA – dependent signaling through an NPR1 – independent pathway. Interestingly, a suppressor of *npr1*, *ssi1*, which completely bypasses NPR1 function, constitutively expresses the JA/ethylene – dependent marker *PDF1.2* gene in an SA dependent manner, suggesting that SSI1, together with CPR5 and CPR6, may participate in the interactions between the SA – and JA/ethylene – dependent pathways. Recently, a null mutation in the *EDR1* gene has been shown to enhance resistance to *P. syringae* and *Erisyphe cichoracearum*, and causes rapid activation of defenserelated genes such as *PR – 1* [16]. This enhanced disease resistance depends on the SA – induced defense response pathway and is independent of the JA/ ethylene pathway. However, *PR – 1* gene expression, which is SA – dependent, is highly induced by ethylene treatment in*edr1* mutant plants, whereas it is almost undetectable in wild – type plants. This again suggests that there is significant interaction between the ethylene and SA – dependent pathways. In this case, ethylene potentiates SA – mediated *PR – 1* gene expression, and EDR1 negatively regulates this process. Removal of EDR1 produces a dramatic effect of ethylene on SA – dependent responses, resulting in enhanced disease resistance in *edr1* mutant plants. *EDR1* encodes aputative MAPKKK similar to CTR1, but unlike the *ctr1* mutant, *edr1* does not display ethylene response phenotypes. There are many other examples of similar interaction between the SA and JA/ethylene pathways. Perturbations in SA – dependent signaling have been reported to affect JA/ethylene-dependent signaling represented by *PDF1.2* expression [45] ; [46]; [54]; [55]; [47]; [117] and [79]. It has been noticed that there is a correlation between a decrease in SA levels and increased *PDF1.2* expression, indicating that SA may have an inhibitory effect on JA/ethylene biosynthesis or signaling [21]. Consistent with this observation, *PDF1.2* mRNA accumulates at higher levels in mutants defective in SA signaling compared with levels in the wild type after *B. cinerea* infections [56]. This may also explain why mutants that disrupt SA – mediated responses become sensitized for activation of the JA/ethylene pathway [54] ; [55] and [117]. On the other hand, JA/ethylene can also repress the expression of SA – induced genes by inhibiting SA accumulation. For example, the *mpk4* (for MAP kinase 4) mutant, which has elevated SA levels and constitutive activation of SA – dependent signaling, failed to induce the expression of *PDF1.2* gene upon JA treatment [78]. This could result from high SA levels antagonizing JA/ethylene signaling as described above. However, when the *mpk4* mutant is crossed to plants carrying the *nahG* transgene, which encodes an enzyme that degrades SA, activation of *PDF1.2* expression is still blocked in the *nahG mpk4* double mutant. These results suggest that block in JA/ethylene signaling relieves the suppression of SA signaling. Nevertheless, it should be pointed out that because both JA – and ethylene – dependent pathways are involved in regulating *PDF1.2,* changes in this gene expression may not always reflect an alteration in the ethylene – dependent pathway. In fact, although *mpk4* dwarfism was similar to that of the ethylene constitutive triple – response mutant *ctr1*, MPK4 does not act in the ethylene response pathway between CTR1 and EIN2 [78]. Recent studies of an ethylene pathway gene *ERF1* have shown that activation of ethylene responses by *ERF1* overexpression in *Arabidopsis* plants is sufficient to confer resistance to *B. cinerea* but reduces SA – mediated tolerance against *P. s. tomato* DC3000 [74] suggesting a negative regulation between ethylene and SA responses. Despite the above – mentioned antagonistic interactions, there are examples in which both ethylene – and SA – dependent pathways cooperate on defense – related responses. In *Arabidopsis*, both ethylene and SA signal transduction pathways are necessary to mount an effective defense response against *Plectosphaerella cucumerina* [74]. In tomato, the transgenic ethylene – underproducing ACC deaminase line (*ACD*) and the ethylene –insensitive mutant *Nr* show reduced accumulation of SA upon *X. campestris* pv *vesicatoria* infection, resulting in less severe disease symptoms [93]. Taken together, these results demonstrate that both positive and negative interactions between ethylene and SA pathways can be established, depending on the type of pathogen or specific defense responses. This is consistent with the results that *ein2* mutation increases SA accumulation in the *cpr5* mutant but decreases SA levels in the *cpr6* mutant, which represents a distinct resistance pathway regulated by CPR5 [55].

IX. CONCLUSION

As sessile organisms, plants are under frequent attack from a broad spectrum of microbial pathogens, including biotrophic and necrotrophic pathogens, namely biotrophs and necrotrophs respectively. SA is a crucial defense signal molecule against biotrophs. Also ethylene and jasmonate, play a major role in defense responses against necrotrophs. Although SA – dependent and JA/ethylene – dependent pathways induce expression of different sets of PR genes and result in plant resistance to different pathogens, there appear to be considerable interactions between these two pathways in systemic acquired resistance.

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Different land use systems improve soil fertility status of sandy soil and increase the yield of rice under rain-fed wet lowland tropical climatic conditions in Papua New Guinea

James Aipa[1], Patrick S. Michael[2]

[1]University of Natural Resources and Environment, Private Mail Bag, Kokopo, East New Britain Province, Papua New Guinea

[2]Department of Agriculture, PNG University of Technology, Lae, MP 411, Papua New Guinea.

Abstract— *The pratical use of different land use systems(LUS) as a management strategy and the effect of the LUSon soil proprties as an indicator of soil fertility status, and the understanding of the long-term effect of the LUS, are important to maintaining optimal soil fertility and yeild of crops. In the rain-fed wet lowlandtropical soils, studies related to rice production and the associated effectson soil properties are limited to a few studies. In this study, we investigated the effects of four LUS (crop rotation, continuous cropping, manure application and fallow) on soil properties that influence soil fertility status and yield of crops under a rain-fed wet lowland tropical sandy soil conditions.The data were compared with the natural soil data obtained prior to and at the end of the study. All the LUS had no to small effects on bulk density, moisture content, electrical conductivity and pH.Soil organic carbon, total nitrogen, available phosphorus, extractable potassium, and cation exchange capacity were all higher in all the LUS.Crop rotation increased soil organic carbon and cation exchange capacity, fallow increased total nitrogen, and manure application increased available phosphorus and extractable potassium contents, respectively.The LUS had no significant effects on particle composition except that small increases in the silt contentswere observed in the continuous, rotation and fallow systems. In almost all cases, soil organic carbon content influenced the fertility status of the sandy soil and yield of rice. Higher soil organic carbon contentresulted in higher available phosphorous and extarvale potassium,enhence reulted in higher yield of rice but decreased the total nitrogen content.Our results implied that the soil organic carbon content of sandy soils needs to be managed properly for optimal soil fertility and higher yieid.*

Keywords— land use systems, soil fertility, yield of rice, rain-fed, sandy soil.

I. INTRODUCTION

In agriculture, a good soil in terms of fertility is a soil which is able to deliver to the roots of crops nutrients that are needed for optimum growth and development. A fertile soil required for crop production is an attribute of the biophysical and biochemical composition of the soil [1; 2; 3]. Some of the important physical components of soils are particle size distribution, bulk density, field capacity, and soil color, whereas the chemical components include pH, electrical conductivity, organic matter, cation exchange capacity (CEC), and carbon to nitrogen (C: N) ratio. The third component of the soil system is the biological component, mostly all the living things.

The physical, chemical and biological components of the soil affect several processes important to crop production[4]. For instance, particle size distribution affects infiltration rate and CEC[5], soil pH and redox affect soil nutrient status and availability to crop plants[6; 7; 8].The biological component provides important ecological services in evolution of soil fertility and management of problem soils through decomposition of organic matter [9].In agricultural crop production, it is important properties are managed as their interactions that govern nutrient and availability to crops are affected by on the farm soil use systems [10] and affect farmers who depend on them [11; 12; 13].

The LUS is affected by the economic status of the farming community, the type of crop produced and thelevel of production. Farmers in the developed nations are able to afford farm machineries and farming technologies for intensive crop production. In the poor economies, affordability of farming equipment is a major concern, complicated by lack of investment and technical knowledge to intensify crop production. Regardless of where and how crop is produced, the increase in the human population and too many mouths to feed worldwide means more and more farm land and continuous crop production. In the tropics, continuous farming is solely monocropping of stables; sweet potato (*Ipomoea batatas*), taro (*Colocasia esculenta*), cassava (*Manihot esculenta*) and yam (*Discorea* sp.) in the tropical and rice (*Oryza sativum*),maize (*Zea mays*) and wheat (*Triticum assertivum*) in the temperate regions are dominating the farms.

Continuous farming of sole crops (monocropping) has advantages however loss of soil fertility and the implications on sustainable crop production is a common global issue, calling for a need to manage the soil (physical, chemical and the biological properties) to prevent decline in soil fertility and loss of crop productivity (yield). In PNG, the soils are strongly weathered and poor in soil fertility in some areas, making a few 'dominant crops'(sweet potato, taro, cassava, yam and Irish potato) to be widely grown. Among these staples are the recently introduced cereals – rice, sorghum and maize. Maize is widely cultivated by locals in food gardens, sorghum's potentials are yet to be realized and rice production is limited to a few places. In the light of these, rice feeds billions of people worldwide and in PNG is widely consumed in the major towns and cities. The main problems for large-scale production of some of these crops, despite the demand, are poor research for development and non-existence of extension services.

The global literature on soil fertility status, management and rice yield under different cropping systems that are region-specific, e.g. for tropical regions is limited, and where there is information available, cannot be widely adapted because of region-specific variations in climatic and soil conditions[14], local farmers inability to adapt [15] and use them [16]. Therefore, this study was conducted to evaluate the soil fertility status and yield of rice under different LUS in a sandy, mix isohyperthermic, TypicTropo fluvents soil (Soil Survey Staff, 2014) under rain-fed wet lowland tropical conditions.

II. MATERIAL AND METHODS

2.1 Study location

The study was conducted in the field at the PNG University of Technology Agriculture Farm, Lae, Morobe Province, PNG (Fig. 1) and completed by a battery of laboratory analysis. The farm (6°41'S, 146°98''E) is located at an altitude of 65 meters above sea level, with an annual rainfall of up to 3,800 mm, which is fairly distributed throughout the year. Average daily temperature is 26.3^{0}C (daily minimum of 22.9 ^{0}C and daily maximum of 29.7^{0}C). Annual evaporation (US Class A pan) is 2,139 mm and rainfall exceeds evaporation in each month. The climate is classified as Af (Koppen), i.e. a tropical rainy climate that exceeds 60 mm rain in the driest month. The soil at the experimental site is well drained and derived from alluvial deposits and classified as a sandy, mixed isohyperthermic, TypicTropofluents[17] or EutricFluvisol[18].

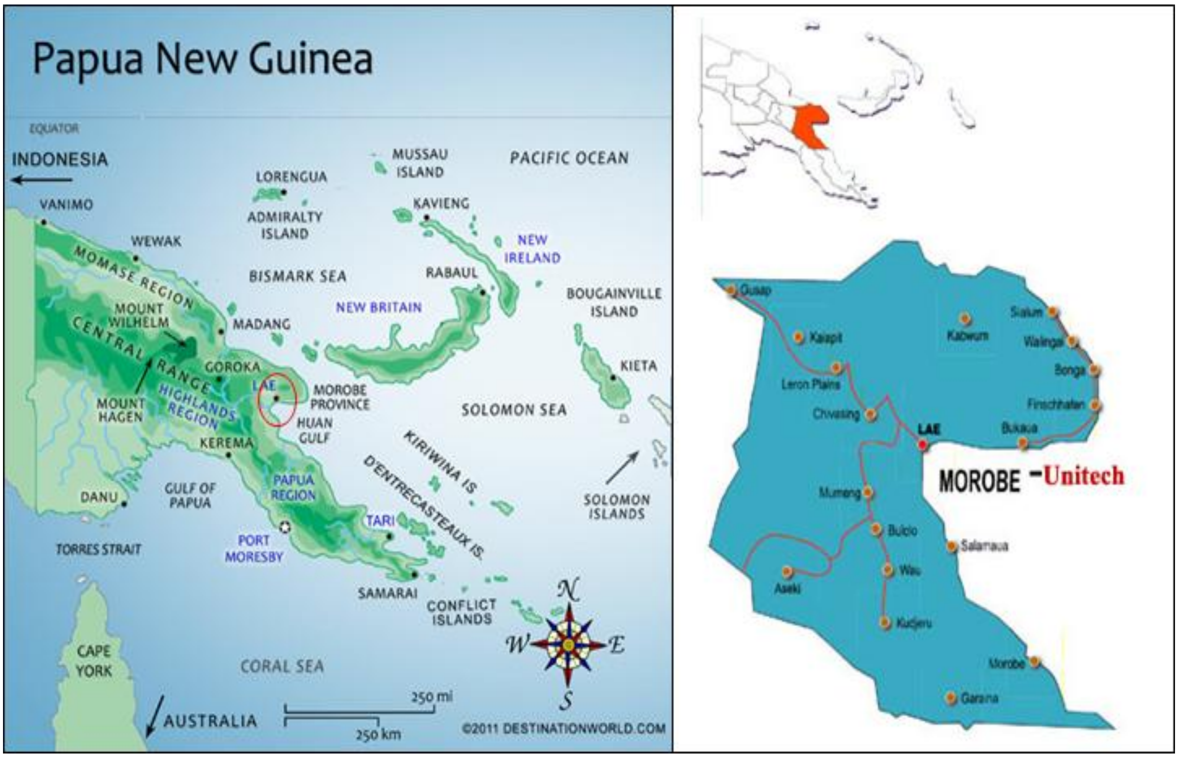

FIGURE 1. Locality of study site, Lae, Morobe Province, Papua New Guinea.

2.2 Treatments

The properties of the surface soil (0-15 cm depth) measured prior to and at the end of study are shown in Table 1. Prior to the study, the flat land on which the study was conducted was harrowed. Rice was used as the test crop and planted at a spacing of 30 cm x 30 cm in a plot size of 5m x 5m (25 m^2), giving a plant density of 289 hills per plot. The 4 LUS as treatments were replicated 4 times (n=4) and setup in a randomized complete block design. Data from only three replicates are presented. The LUS were "continuous rice cultivation", "rotation with maize", "deep litter poultry manure application (DLPM)" and "natural fallow".

In the continuous cropping treatment, rice was continuously grown to maturity for 3 consecutive cropping seasons. In the rotation treatment, maize was planted during the second cropping season after rice. In the DLPM treatment, rice was planted continuously for the 3 cropping seasons following the addition of 15 kg of DLPM per replicate per plot, which is equivalent to 6 tonnes per ha^{-1}. The DLPM was applied by broadcasting and mixed into soil by raking. In the fallow treatment, the plots were rested for 4 months. The duration of a cropping cycle was four months, and throughout the cycles, weeds were controlled manually using small handheld tools, and no chemical fertilizer was applied.

TABLE 1
SOIL PHYSICAL AND CHEMICAL PROPERTIES STATUS PRIOR TO AND AT THE END OF THE STUDY.

Soil properties	Unit	Average^A	Average^B
Particle size			
o Sand	%	84.7 ±0.0	83.30±0.3
o Silt	%	13.3 ±0.0	12.70±0.2
o Clay	%	2.0 ±0.0	4.00±0.1
Bulk density	g cm^{-3}	1.4 ±0.0	1.28±0.1
Water holding capacity	%	26 ±0.6	26.33±0.2
Electrical conductivity	dS m^{-1}	5.6 ±0.0	7.57±0.3
Soil pH	pH unit	6.0 ±0.0	5.9±0.1
Organic carbon	%	1.4 ±0.0	2.03±0.1
Total nitrogen	%	0.2 ±0.1	0.23±0.2
Available phosphorus	mg kg^{-1} soil	117 ±1.0	118.33±0.2
Extractable potassium	mg kg^{-1} soil	373 ±12	644.67±0.1
CEC mEq./100 g soil		25.4 ±2.1	29.73±0.1

The superscripts denote measurements of the natural control soil prior to (^{A}) and after (^{B}) the study. The values are average of three replicates ± standard error (s.e.).

2.3 Maturity and Harvesting

The maturity times of the rice plants per the three cropping season were 114, 112, and 110 days, respectively. During harvest, the tillers were cut using a sharp sickle. The cut tillers with grains attached were taken to a central location and threshed onto a canvas to remove the grains. The yield per plot on a fresh weight (kg) basis was taken by weighing. Data (fresh weight) from the three replicates were obtained in a similar manner, pooled, averages taken and kept as the final data. The average yield data were converted into maximum possible yields (tonnes ha^{-1}). Except for milled rice, a recovery rate of 60% was considered in determining the final yield of rice (t ha^{-1}) after determining the yield of the threshed rice. As an example, the total yield of threshed and milled rice from continuous cropping is: **(1)**

- Average yield of 4 plots = 70.96 kg
- Average yield of threshed rice per plot = 17.74 kg
- Total yield of threshed rice per ha = 7.10 tonnes
- Total yield of milled rice with 60% recovery = (60/100*7.10)

  = 4.26 tonnes

2.4 Measurements

Prior to the study, soil samples (gram, g) were taken from the "natural control soil", air dried and analysed. The data obtained are presented in Table 1. Data from subsequent sampling of the natural control soil at the end of each cropping season are presented in Table 2. There was no tillage in the natural control soil. The soil particle size distribution was determined using a modified hydrometer method of [19]. A sample of soil was pre-treated with 5 ml of 6% hydrogen peroxide to oxidise organic matter. This was transferred quantitatively to a measuring cylinder and 30 ml of 5% sodium hexameta phosphate

(Calgon) was added to promote dispersion. The content was thoroughly mixed with a plunger with gentle strides and hydrometer readings were taken at pre-determined time intervals of 5 minutes (sand) and 2 hours silt. Particle size was determined as:

$$\mathbf{S\ (\%) = [((R=R_L+ r) \div W)\ x100} \quad (2)$$

Where S is percentage of material in suspension, R is hydrometer reading, R_L is calibration correction, W is oven-dry weight (ODW) of soil samples (g), and r is a temperature correction factor.

Bulk density (BD) was measured using the soil core method of [20]. A cylindrical metallic core sampler was driven horizontally into the sharpened edges of the soils and carefully removed with a known volume of 100 cm^3 of soil (V, field volume of soil in cm^3). Extra soil extending from the edge of the cylinder was trimmed off to maintain the required volume. The core samples were oven dried in an oven at 105 ^{0}C for 24 hours and the ODW obtained by weighing. BD was measured as:

$$\mathbf{BD = (ODW \div V)} \quad (3)$$

Field capacity (FC) was measured by weighing 100 g of soil at 100% FC following saturation. These samples were weighed for the fresh weights (FW) and oven dried for 48 hours in an oven at 110^{o}C and reweighed for the ODW. The FC was determined on gravimetric basis as:

$$\mathbf{FC\ (\%) = [((FW\text{-}ODW) \div FW)\ x\ 100} \quad (4)$$

The electrical conductivity (EC) and pH were measured using standard dilution (conductivity meter and pH meter (1:5, soil: water w/v)) methods [e.g. 21], respectively. Salinity was measured by shaking the suspension in an end-over-end shaker for 1 hour and left overnight for 12 hours. The overnight suspensions were filtered through a Whatman No. 40 filter paper to obtain a soil: water extract. The extracts were agitated in an end-over-end shaker at room temperature for 1 hour and left to settle for another hour then EC measured in the supernatant of the extract (1:5 w/v).

Soil organic carbon (SOC), total nitrogen, available phosphorus, extractable potassium, and CEC were determined using air dried soil samples following the soil analytical methods of [22] as described in [23]. SOC was determined using the rapid wet oxidation method in which soil samples were oxidized in 0.5 M sodium chromate-sulfuric acid solution ($Na_2Cr_2O_7.2H_2O$–H_2SO_4) in an oil bath. Excess $Na_2Cr_2O_7$ was titrated with a 0.2 mol L^{-1} $FeSO_4$ solution. The soil particles were allowed to settle by cooling followed by calculation of the SOC from the amount of Cr^{3t} ion formed using a calometric procedure at 600 nm with sucrose as standards [24].The organic matter content was obtained by multiplying the carbon value by a factor of 1.72.

Total nitrogen was measured by the semimicro-Kjeldahl method after soil samples were digested with $HClO_4$ and HF. Finely ground soil samples were digested using sulfuric acid and sodium sulfate, using selenium as catalyst. The solution was allowed to settle for 30 minutes followed by an addition of 10 ml of H_3BO_3 and 25-30 ml of 60% NaOH solution. The solutions were diluted for 5 minutes at a rate of 8 ml per minute under a constant temperature of 40^{0}C. The distillate was titrated by adding 5-6 drops of Bromocresol Green-Methyl Red mix indicator with standard 0.01 M HCl. Ammonium N in the diluted Kjeldahl digest was determined using an automated isocyanurate calometric procedure similar to that of [25] as:

$$\mathbf{Total\ N\ (\%) = [(0.014\ X\ (V_1\text{-}V_2))]} \quad (5)$$

where V_1 is sample titration value (ml) and V_2 is blank titration value (ml).

Available phosphorus was extracted with a 0.5 mol L^{-1} $NaHCO_3$ solution and determined by molybdenum-blue colorimetry. This method is a modified method of the original bicarbonate procedure developed by [26]. The soil extracts were filtered with Whatman filter paper (P-free) followed by addition of 50 ml of deionised water and 2 ml of 1 M sulfuric acid to the filtered soil extracts and mixed. A further 5 ml of the 1 M sulfuric acid was added to the extract and left overnight to completely remove CO_2.The CO_2 free soil extracts were mixed again with 8 ml colour reagent, 8 ml ammonium molybdate-sulfuric acid-Sb solution, and 25 ml phosphorus as standard. The mixture was left for 30 minutes then a manual colometric finish procedure [27] was used to measure the absorbance at 882 nm. The bicarbonate-extractable phosphorus (P) was determined as:

$$\mathbf{P = (Sample\ value - Reagent\ blanks)\ mg\ kg^{-1}} \quad (6)$$

The first part of the K extraction procedure is similar to that of P described above. Extractable K was extracted with 0.5 mol L^{-1} NH_4OAc (pH 8.5) and then determined by flame photometry. The soil extracts were filtered with Whatman filter paper (K-free) followed by addition of 50 ml of deionised water and 2 ml of 1 M sulfuric acid to the filtered soil extracts and mixed. A further 5 ml of the 1 M sulfuric acid was added to the extract and left overnight to completely remove CO_2. The CO_2 free soil extracts were mixed again with 8 ml colour reagent, 8 ml ammonium molybdate-sulfuric acid-Sb solution, and 25 ml K as standard. The mixture was left for 30 minutes then the aliquot was determined by flame emission spectrometry (FES). The bicarbonate-extractable Kwas determined as:

$$\mathbf{K = (Sample\ value - Reagent\ blanks)\ mg\ kg^{-1}} \quad (7)$$

The CEC was measured using the 0.01 mol L^{-1} $(AgTU)^+$ method [28]and atomic absorption spectrometry(AAS). A soil/silver-thiourea solution (1:50 w/v) was shaken in an end-over-end shaker for 16 hours at room temperature. The solution was centrifuged until a clear supernatant was obtained. Some of the supernatant were diluted to a 1+9 with $Sr/Cs/(AgTu)^+$ solution. Same volume of both the supernatant (soil extract) and $(AgTu)^+$ standard were then diluted 80-fold with CsCl solution (1.0 g Cs L^{-1}). The CEC was determined by measuring the Ag^+ in the diluted standard and the soil extract by an ASS using an air/C_2H_2 flame and a 328.1 nm spectral line instrument. The CEC determined is based on CEC_{AgTu} (mEquiv./100 g) soil on an oven dry basis.

2.5 Statistical analysis

All the average data were analysed using SPSS 14 (SPSS Inc. ILL., Canada). The significance (*p<0.05*) of mean differences between rice yield and the soil properties between the LUS and the 3 cropping seasons was determined by One-way ANOVA followed by Tukey's Multiple Comparison Test.

III. RESULTS AND DISCUSSION

The statuses of the soil properties of the 'natural control soil' in which the study was conducted before and after are shown in Table 1. The changes in the soil chemical properties measured under the four different LUS are shown in Table 2. Compared to the natural control soil, there was no significant change in the bulk density, soil moisture content, electrical conductivity and pH. The bulk density was between 1.3–1.4 g cm^{-3} (Table 2) within the range of 1.2 –1.8 g cm^{-3} as reported for sandy soil by [29]. The total nitrogen contents of all the LUS were within the range of the natural control soil, except in the fallow soil where the content increased to 0.5%, consistent with the results of [30] where natural fallow increased the total nitrogen content in tropical Ghana. There is evidence too of increase in sandy soil nutrients,hence fertility with increasing fallow durations [31]. In a semi-arid area in Mexico, a land fallowed for 22 years resulted in total nitrogen recovery by 62% [32]. In Senegal, fallow did not significantly increase the nutrient content of sandy soils [33], similar to the small increase (0.5%) of this study.

TABLE 2
CHANGES IN SOIL PROPERTIES UNDER DIFFERENT LAND USE SYSTEMS.

Lands use system	Statistical parameter	D_b (g cm^{-3})	FMHC (%)	EC (dS m^{-1})	pH (1:5)	SOC (%)	Total N (%)	P_{av} (mg kg^{-1})	K_{ext} (mg kg^{-1})	CEC (mEq./100 g)
1	Mean	1.4	26	5.3	5.9	1.8*	0.19	136	410*	37*
	S.E	0.0	1.2	0.0	0.0	0.0	0.0	4.5	10.0	0.2
2	Mean	1.4	26	5.4	5.9	2.1*	0.2	143	302*	33*
	S.E	0.0	1.2	0.0	0.0	0.0	0.1	6.3	30.0	1.4
3	Mean	1.4	25	5.4	5.9	2.0*	0.2	184	509*	34*
	S.E	0.0	0.6	0.0	0.0	0.1	0.0	19.3	34.4	6.3
4	Mean	1.3	26	5.1	5.9	1.8*	0.5	145	504*	29*
	S.E	0.0	0.6	0.0	0.0	0.1	0.3	10.4	37.0	1.0
5	Mean	1.4	27	4.8	5.9	1.4*	0.1	126	437*	27*
	S.E	0.0	1.2	0.7	0.0	0.0	0.0	38	108.1	0.5

1 = Continuous, 2 = rotation, 3 = deep litre poultry manure, 4=fallow and 5 = natural control soil. An asterisk (*) indicates significant differences (p<0.05). D_b = bulk density, FMHC = field moisture holding capacity, EC = electrical conductivity, pH = soil pH, SOC = organic carbon, Total N = total nitrogen, P_{av} = available phosphorus, K_{ext} = extractable potassium, CEC = cation exchange capacity, and S.E = standard error. The changes in soil particle size composition are shown in Fig.2.

In sandy soils, phosphorus loss through leaching is possible [34] however in all the LUS the available phosphorus content increased, the highest amount measured in the DLPM treatment (Table 2). The available phosphorus content in the natural control soil measured at the end of the study was higher too, compared to the content measured prior to the study but the increase was small, 126 mg kg^{-1} soil (Table 2). The increase in available phosphorus content following addition of DLPM although are from a sandy soil in a rain-fed wet lowland tropical conditions are similar to the results of [35] where addition of cattle manure increased the phosphorus content by 35.4 mg kg^{-1} soil in a semiarid sandy soil in Brazil.

Changes in soil phosphorus content following addition of manure is widely reported in a range of soil types [e.g. 36; 37; 38; 39] but under tropical condition is not often reported. This study shows DLPM can help improve the fertility of sandy soil (that is increase the content of NPK) (Table 2) and increase the yield of crops like rice (Fig. 2). The potassium content was within the range of the natural control soil (Table 1), ranging from between 302 mg kg^{-1} soil in the rotation treatment to 509 mg kg^{-1} soil in the DLPM treatment. This indicates that the net change in the extractable potassium content was negligible. Contrastingly, [40] reported that addition of chicken manure to a semiarid sandy soil classified as LuvicCalcisol, FerralicArenosol, or VerticLuvisol[41] increased the potassium content in Botswana.

Soil pH and CEC are constraints to crop production, especially in sandy soil because the former affects nutrient availability and the latter provides a buffer against pH and influences nutrient retention [42]. The CEC was high in the continuous, rotation and DLPM, whereas in the fallow was within the range of the natural control soil. Under all the LUS, pH remained the same and EC rates increased. These results are consistent with the results of [43] where an increase in CEC rate resulted in no change in pHin a semiarid sandy loam soil. As widely known, a positive correlation between the SOC and CEC was found, an increase in SOC content resulted in higher CEC. For instance, when the SOC was 2.1%, the CEC was 34 mEq./100 g under rotation, and 1.43% SOC and 27 mEq./100 g CEC in the natural control soil, respectively (Table 2).

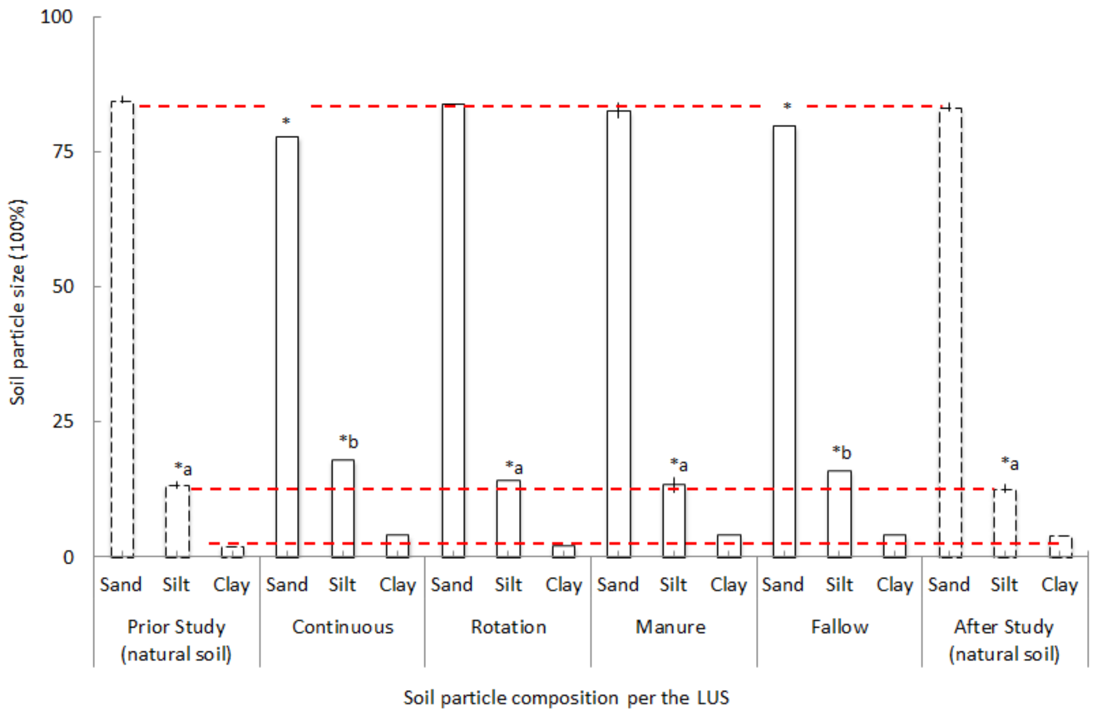

FIGURE 2. Soil particle composition of the four lus. The broken horizontal lines indicate the composition of the soil particles prior to and after the study of the natural control soil. The values are means ± standard error of three replicates (n=3). An asterisk indicates significant changes ($p<0.05$) between the LUS and the natural control soil. Columns with same letters are not significantly different from each other.

In the soil physical properties, there was no significant change in the bulk density and moisture content (Table 2). The soil particle size composition was fairly the same in all the LUS except a small decrease in the sand composition under the continuous and fallow soils (Fig. 2). Continuous cultivation and fallow increased the silt contents when compared to the natural control soil, measured prior to (natural soil) and at the time of crop harvest (natural soil) (Fig. 2). The clay composition was fairly the same in all the soils of the LUS, even if the content measured prior to the study was lower, comparatively. No significant change in particle size distribution in sandy soil under rice cultivation under tropical condition has been reported [44], indicating poor soil physical condition similar to ours as reported by [45].

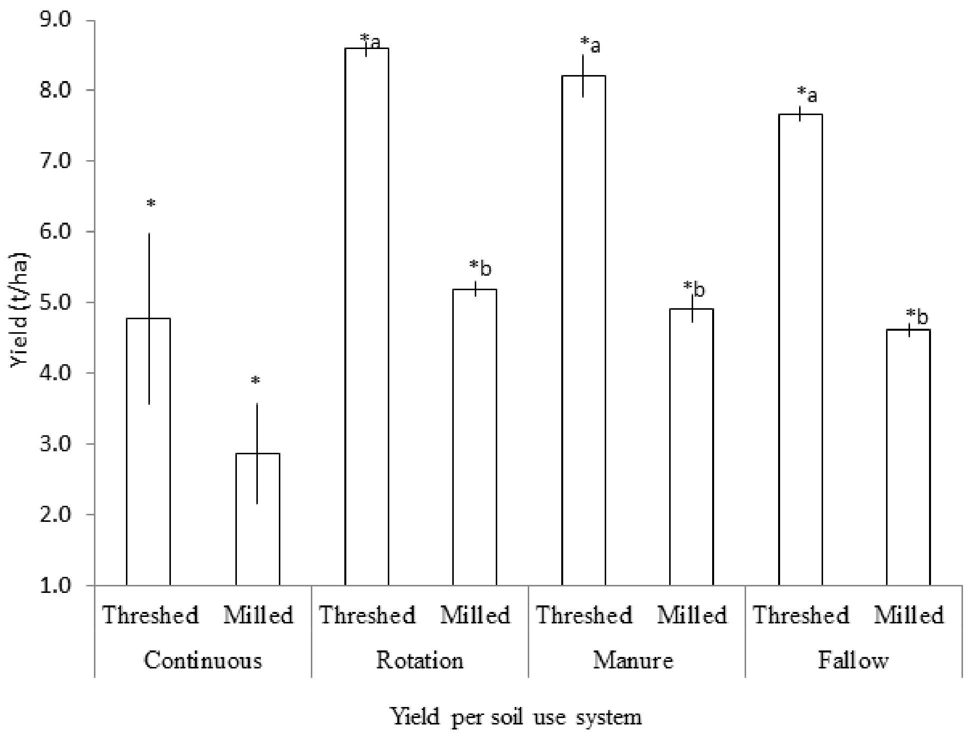

FIGURE 3. Yield of rice under the four different LUS. The values are means ± standard error of three replicates (n=3). An asterisk indicates significant difference ($p<0.05$) between the LUS. Columns with same letters are not significantly different from each land use system.

Figure 3 shows the threshed and milled rice yields obtained as per the calculation shown in Eq. 1. Among the LUS, continuous use of the soil resulted in low threshed yield. Similarly, the quantity of milled rice produced from continuous cropping was smaller than the amount of rice produced from the other LUS. These low yields under continuous cropping resulted from depletion of the SOC and total nitrogen (Table 2). Comparatively, again, rotation with maize slightly increased the amount of milled rice (Fig. 2) but poor yield was reported by others when rotated with barley [46]. Compared to our results, rice yield is reported to be low under other LUS in sandy soil [47]. As expected, addition of DLPM increased the SOC content and improved the nutrient status (NPK) (Table 2), hence the yield was higher from this LUS (Fig. 2).

Generally, fallow improves the soil nutrient status as a result of turnover of plant organic matter [48]. Therefore, the increased in SOC and in the nutrients of the sandy soil shown in Table 2 are not exceptional. Similarly, crop rotation, especially with legumes, improves the nutrient status of soils, again, because of organic matter turnover and the basic difference in soil use system created as a result of planting a different crop. Our data show improved SOC and NPK content of the sandy soil when rice was rotated with maize but the changes were small, similar to those in the fallow (Table 2). The main reason for these changes lies around the explanation that during the fallow period (4 months), the sandy soil was dominated by *Imperata cylindrical*, *Saccharum spontaneum* and *Rottboellia cochinchinesis*, and the crop rotated with rice was maize - all grass species of the Poaceae family. The presence of the grass species indicated poor soil quality associated with modifiable soil properties as pointed out by [49]. In PNG, it is a common knowledge that poorly weathered soils are dominated by these grasses, most often in areas where other vegetation types fail to colonise and establish.

IV. CONCLUSION

Crop rotation and addition of DLPM helped improve the poor fertility status of the sandy soil. Consequently, crop rotation and DLPM addition increased yields of rice (both threshed and milled) by a tonne, compared to the yield obtained under continuous cropping and fallow systems. The mechanisms for these appear to be addition of carbon and nutrients (NPK) to the sandy soil and these LUSs' potential to increase the CEC facilitated nutrients to be available to the rice plants. High SOC content resulted in low total nitrogen but higher available phosphorus and extractable potassium content. When rice cropping was rotated with peanut as a legume under the same conditions in a similar study, in almost all cases, yield of rice was smaller. These results imply that the main source of soil fertility, hence higher yield of rice on the sandy soil was SOC and not total nitrogen. These findings have implications for management and improvement of sandy soil fertility status to improve the yield of crops under rain-fed wet lowland tropical conditions.

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Study on financial management problems and countermeasures of public hospitals under the new reformation of the medical treatment

Zheng Dan

School of Economics and Management, Zhengzhou Normal University, Zhengzhou, China

Abstract— *The reform of financial management is the top task of the new health care reform in public hospitals, and is the cornerstone that new health care reform has succeeded. This paper clarifies two basic concepts which are "New medical reform" and "Financial management of public hospitals", and on this basis, it analyzes and studies the financial management of public hospitals, and puts forward some improvement measures, such as strengthening the construction of administrative management system and improving the mode of decision-making management; building a team of high quality financial management and deepening the comprehensive budget management; perfecting cost management and control system, standardizing medical service behavior, and strengthening the management of state-owned assets; establishing and improving the financial supervision and control mechanism of public hospitals; establishing the finance risk warning, prevention and management system of hospital.*

Keywords— new health care reform, public hospital, financial management, improvement measures.

I. CONCEPT DEFINITION

1.1 New health care reform

The plan of new health care reform which upholds the concept of "people-oriented" and a view combining theory with practice includes four principles. The first is ensuring the health rights and interests of the people. Second, we should establish a set of medical and health systems in line with China's national conditions. Third, government leading and market mechanism should be harmonized to combine fairness and efficiency. The last is a combination of problem solving and system improvement.

1.2 Financial management of public hospitals

The financial management of public hospitals refers to the financial activities and financial relationship which related to daily operations and business activities. In which that, the financial activities refer to the general term for all activities in the operation of funds and the financial relationship refers to the relationship between the participants in financial activities. The basic contents of financial management in public hospitals include financing management, financial budget management, revenue management, cost management, asset management, financial reporting and analysis, financial supervision and control. The goal of financial management of public hospitals is to maximize the balance of public hospitals, which on the basis of steadily increasing public hospital funds and the value of hospital assets. In other words, the essence of financial management of public hospitals is to obtain the maximum economic and social benefits with the least cost.

II. ANALYSIS OF THE EXISTING PROBLEMS IN FINANCIAL MANAGEMENT OF PUBLIC HOSPITALS IN CHINA

On the premise of defining the goal of financial management development of public hospitals in China, accurate concept definition is helpful for us to correctly grasp the development direction of financial management in public hospitals, to scientifically analyze the current situation and existing problems of financial management of public hospitals in China, to propose a targeted improvement. At present, the financial management of public hospitals in China is mainly reflected in the following aspects:

2.1 Budget Management

The reality that public hospitals do not pay enough attention to financial budget leads to the fact that comprehensive budget work cannot be carried out scientifically and effectively in public hospitals, so the role of comprehensive budget management in the financial management of public hospitals cannot be given full play. There are some specific manifestations as follows: First of all, financial staff in public hospitals do not pay enough attention to the role of budget management, or even grasp the basic method of comprehensive budget. Secondly, there are many problems in the selection of budgeting methods in public hospitals, such as the lack of effectiveness, single compilation methods and incomplete budget items. And then, most budget work of public hospitals is formal and not fully implemented, which makes budget management and hospital development strategy cannot be closely linked. Next, most of the financial budget work of public hospitals still stays in the traditional budget system of public institutions, and the new budget management mechanism of expenditure control and supervision has not been established. Finally, the performance appraisal system of public hospitals is not perfect enough to accurately reflect the income and expenditure status, business project completion, cost control and the realization of social and economic benefits of public hospitals.

2.2 Cost management

Hospital cost management refers to a management activity in which the hospital takes reducing medical costs as the goal, uses the method of cost accounting to analyze the cost expenditure, and controls the controllable part according to the analysis results. Although the new health care reform has broken a certain level of "supporting medicine with drugs", there are still problems with medical costs that are difficult to quantify. For example, different doctors may also give different treatments to the same patients, resulting in different medical costs and specific project costs incurred by inpatients due to specific needs of the individual, etc. All of these would lead to differences in the final cost of treatment. In addition, the non-standard management of fixed assets is another important problem of cost management, because the total fixed assets account for a large proportion in the total assets, which is the basis of all the operation services of the hospital. Once there are problems in fixed assets management, such as the waste of idle equipment, unreasonable medical facilities scrapping procedures, etc. All of it would lead to the loss of state - owned fixed assets

2.3 Financial risk management

Public hospitals are faced with inevitable financial risks in the process of operation, so how to minimize risks and reduce losses has become one of the most urgent problems for public hospitals to solve. The financial risks faced by public hospitals are mainly reflected in the following aspects: First, the increasingly complex external environment, such as economy, law and culture, makes the financial management of public hospitals more difficult. We should not only consider the internal management needs of the hospital, but also adapt to the changes in the external environment, keep abreast of the latest market trends, and adjust the financial management deployment. Second, the management team of public hospitals lacks risk awareness and a sound financial risk prevention mechanism, making it difficult for the financial department to provide leaders with effective information and reports on financial risks in a timely manner. Third, public hospitals continue to use the traditional decision-making mode, which leads to the financial decision-making risks caused by decision-making mistakes. Fourth, the internal control system of public hospitals is not perfect and lacks power balance.

III. MEASURES TO IMPROVE THE FINANCIAL MANAGEMENT OF PUBLIC HOSPITALS IN CHINA

3.1 Strengthen the construction of administrative management system and improve the decision-making management mode

The realization of financial management goals of public hospitals is inseparable from the effective implementation of scientific administrative management system. The new health care reform has made it clear that we should actively promote the reform of the public hospital's inner workings, strengthen asset management, strict internal and external financial management, and establish a scientific decision-making system. Therefore, it is imperative to organize the leading team and financial staff of public hospitals to study legal norms and establish an effective administrative decision-making system.

3.2 Build a high-quality financial management talent team and deepen comprehensive budget management

The comprehensive quality of financial management staff in public hospitals has a direct impact on whether their financial management can develop into strategic, informatization, humanization and innovation. The hospital financial system issued by the government in 2010 provides detailed Regulations for the public hospital budget management system, which suggests that financial staff is no longer just a "accounting" type talents, and should be a wider range of knowledge, higher professional quality and moral standards better and interdisciplinary talents with innovative ability.

3.3 Perfect the cost management control system, standardize medical service, strengthen the management of state-owned assets

First of all, the measures to improve the cost management and control system mainly include improving the cost management and control system of public hospitals, effectively controlling drug costs, medical costs and other cost expenditure items, detailing each cost into each department, establishing a comprehensive bidding system to ensure the reasonable quality and price of purchased goods, strengthening fund raising and usage management to improve the utilization efficiency of capital and reduce operating cost. Secondly, In order to regulate medical service behavior, the medical institutions and the health supervision department should strengthen supervision of hospital services, strictly regulate the behavior of doctors during the diagnosis and treatment, and avoid the occurrence of over-medication, repeated examination, etc. in violation of doctors' professional ethics. Finally, strengthening the management of fixed assets in public hospitals is an important requirement of the new health care reform. It should improve the utilization efficiency of fixed assets in public hospitals, ensure the maintenance and appreciation of state-owned assets, and thus avoid the purchase of unnecessary large medical equipment which is not suitable for the development of hospitals, thus avoiding the waste of funds.

3.4 Establish and improve the financial supervision and control mechanism of public hospitals

Whether the financial supervision system of public hospitals is sound or not, which has a direct impact on the normal operation of hospital finance and the orderly conduct of various financial activities. Therefore, to establish and perfect the system of financial supervision is the key content of financial management, For example, we should deepen the supervision and management of financial budget, deepen the supervision and management of financial operation, deepen the supervision and management of costs and expenses, and so on. The effect of financial control is directly related to the effectiveness of financial management. Therefore, the public hospitals should establish and improve their own financial control system on the basis of the existing national system, strengthen the responsibility system of the implementation of financial control system, achieve clear power and responsibility, clear division of labor, and link all departments together and restrict each other, which should be good for the public hospital's financial control system.

3.5 Establish a hospital financial risk alert system

The financial risk early warning system adopts the comprehensive risk early warning method, which is an important part of the hospital financial management. It is a tool to monitor financial risk by analyzing the financial activities of the unit and the potential financial risk forecasting activities of the financial environment. If we are able to alert the risk signal accurately and promptly and take appropriate measures, then the loss caused by the financial crisis can be mitigated. Therefore, the financial personnel should combine the actual situation, establish financial monitoring indicators, and implement monitoring. If the financial indicators are found to be beyond the warning line of financial risks, the reasons should be timely analyzed, countermeasures should be found and reported to the leadership team, so that the leadership team could make accurate judgments and decisions, and minimize the losses caused by the financial crisis.

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Preliminary Study on Investigation of Turf Fungi Disease in Hailing District, Taizhou City, Jiangsu Province China

Zhang Cheng-xia[1], Wu Hong[2], Wang Rui[3]

Jiangsu Agri-Animal Husbandry Vocational College, Taizhou, 225300, China

Abstract— *In order to find out the disease of turf fungi and the damage of the disease to the lawn in Hailing District of Taizhou, this paper investigates fungal diseases of lawn and grassland, such as Festuca arundinacea, Cynodon dactylon, Zoysia matrella, Lolium perenne, Trifolium repens, in major parks and urban green spaces in Hailing District of Taizhou City. Then this paper separated and identified pathogenic fungi, mainly there are Alternaria alternate, Bipolaris sorokinianum, Drechslaris poae and Curvularia lunata, etc (8 kinds in all), and then basically defined the species, distributions and damage of turfgrass fungal diseases in Hailing District, Taizhou. In the meantime, this paper put forward specific proposals for the prevention and control of lawn diseases.*

Keywords— Taizhou city, turfgrass, fungal diseases, control.

I. INTRODUCTION

In recent years, As China's economy grows, people's requirements for the ecological environment are constantly improving. The development speed of lawn industry in our country is very fast. Especially in recent years, the evaluation activities of civilized cities and livable cities all over the country have made the lawn green area of large and medium-sized cities increase rapidly. According to incomplete statistics, in nearly 500 cities in the country, the lawn area is nearly 70,000 hectares, and the lawn area in Beijing, Shanghai, Dalian is increasing at the rate of 1.50,2.3 and 2.4 million square meters each year [1]. With the enlargement of lawn planting area, lawn diseases also increase. In less severe cases, this makes the lawn greenish and yellowing, forming exsciccation patches, affecting the appreciation and use of lawns. In severe cases, this will kill the lawn quickly. Diseases not only affect the ornamental value of the lawn, but also affect the commercial operation and environmental beautification of the lawn [2,3]. Therefore, the investigation and control of lawn diseases have become an indispensable part of the healthy development of the lawn industry. Taizhou belongs to the Lixia River region. The climate is north subtropics humid type, and it has four distinct seasons. It is hot and rainy in summer, mild and less rainy in winter. The annual average temperature is about 15℃, and the annual average rainfall is about 1000 mm. The abundant rainfall and warm temperature provide natural conditions for lawn planting. At the same time, the high temperature in summer provides unique natural conditions for lawn planting. Both high humidity and high humidity provide favorable environmental conditions for the occurrence of fungal diseases in turfgrass. In this study, the disease of lawn grass in the main urban area of Taizhou City was investigated for the first time. The purpose was to find out the types and distribution of the disease, and to provide the basis and reference value for scientific management of lawn and formulate disease control measures in this area.

II. MATERIALS & METHODS

2.1 Place of investigation

This study investigated the turf grass diseases in Tiande Lake Park, Taizhou People's Park, Taishan Park, Fenghuang Road Campus of Jiangsu Animal Husbandry & Veterinary College, and Municipal Government Square and other urban green space. The turf grasses collected were mainly *Festuca arundinacea*, *Cynodon dactylon*, *Zoysia matrella*, *Lolium perenne* and *Trifolium repens*.

2.2 Methods of investigation and identification of indoor pathogens

From July to October 2015, we investigated and collected lawn grass disease specimens in the sampling spot designed for the experiment by the method of making an on-the-spot survey. The symptoms of the disease were observed and recorded in detail. After the specimens are taken back to the laboratory, the diseased and health border tissues were isolated and purifying-cultured. The culture medium was potato agar medium (PDA). The isolated pathogens were identified by referring to the "Manual of Fungi Identification", " Illustrated Genera of Imperfect Fungi Barnett "[5] and other materials.

III. RESULTS

3.1 Main diseases

From Table1, it can be inferred that 8 kinds of Pathogenic fungi like *Alternaria alternate, Bipolaris sorokinianum, Drechslaris poae, Rhizoctonia solani, Curvularia lunata, Fusarium equiseti, Fusarium poae* and *Pythium aphanidermatum,* are separated from the turfgrass specimens, which were collected from Taizhou Tiande Lake Park, Taizhou People's Park, Taishan Park, Jiangsu Agri-animal Husbandry Vocational College Fenghuang Road Campus, City Government Square and other urban green space. And these pathogenic bacteria results in turfgrass disease like leaf spot, brown spot, black spot and fusarium wilt of *Festuca arundinacea, Cynodon dactylon, Zoysia matrella* and *Lolium perenne.* (Table1)

Table 1
Species and distribution of main fungal diseases on lawn in Hailing District, Taizhou

Host Plant	Disease Name	Pathogenic Bacteria	Distribution
Festuca arundinacea	Black spot	*Alternaria alternate*	Taizhou people's Park City government square
	Leaf spot	*Bipolaris sorokinianum*	
Zoysia matrella	Black spot	*Alternaria alternate*	Taizhou people's Park
	Leaf spot	*Drechslaris poae*	Jiangsu Agri-animal Husbandry Vocational College
	Cercospora leaf spot	*Rhizoctonia solani*	Taishan Park, Tiande Lake Park
Lolium perenne	Leaf spot	*Bipolaris sorokinianum*	Taishan Park City Government Square
	Black spot	*Alternaria alternate*	
		Curvularia lunata	
Cynodon dactylon	Leaf spot	*Drechslaris poae*	Jiangsu Agri-animal Husbandry Vocational College
		Fusarium equiseti	Tiande Lake Park, Jiangsu Agri-animal Husbandry Vocational College
		Bipolaris sorokinianum	Tiande Lake Park, City Government Square
	Black spot	*Alternaria alternate*	Jiangsu Agri-animal Husbandry Vocational College, Tiande Lake Park
		Curvularia lunata	Jiangsu Agri-animal Husbandry Vocational College Fenghuang Road Campus
	Cercospora leaf spot	*Rhizoctonia solani*	Taizhou people's Park Tiande Lake Park
	Fusarium wilt	*Fusarium poae*	Tiande Lake Park
		Pythium aphanidermatum)	
Trifolium repens	Leaf spot	*Curvularia lunata*	Tiande Lake Park ,Taizhou people's Park,Jiangsu Agri-animal Husbandry Vocational College

3.2 The main symptoms, pathogenesis and preventions of major diseases

3.2.1 Black Spot

The study collected *Festuca arundinacea* and *Zoysia matrella* from Taizhou People's Park, *Cynodon dactylon* from Tiande Lake Park and Jiangsu Agri-animal Husbandry Vocational College Fenghuang Road Campus, *Lolium perenne* from Taishan Park, *Festuca arundinacea, Lolium perenne* and *Cynodon dactylon* from City Government Square, *Alternaria alternate* and *Alternaria alternate* were separated from them, which caused Black spot.

When the disease occurs, the plants in the grass patch are dwarf, grey and dead. Red and brown edges are formed between the healthy tissue and sometimes brown spots on the affected leaves. *Alternaria alternate* and *Alternaria alternate* can both infect common lawn grass species, badly in high temperature, mainly occurs in around 30℃ with high humidity, especially in Spring when the temperature rises, they infect reviving plants and spread with wind and rain, causes reinfection and happens continuously in Summer and Autumn. The disease can occur when the environment is humid and too much nitrogen fertilizer is applied. Therefore, reasonable pruning, timely removal of grass debris and withered layer, and more full-price fertilizer are used to improve the resistance of lawn grass and control the occurrence of black spot.

3.2.2 Cercospora Leaf Spot

Cynodon dactylon collected from Taizhou People's Park and Tiande Lake Park, *Zoysia matrella* collected from Taishan Park and Tiande Lake Park, *Rhizoctonia Solani* was separated from them. At the early stage of the disease, the lesions showed a bluish gray water immersion, reddish brown border, and brown spots at the later stage. In severe cases, the lesion spread around the stem, causing the stem and neck base to become brown, rotten or yellow. If the environmental conditions are suitable for the rapid development of the disease, the diameter of the withered zone from a few centimeters quickly expanded to about 2 meters, because the center of the withered spot after the restoration of the plant color than the edge of the disease, so that the withered spot presents a ring or "frog eye" shape.

Brown spot is a highly prevalent disease, and is one of the most widely distributed diseases in turfgrass diseases. Sclerotium has a strong ability to withstand high and low temperatures, but the optimum temperature for infection and onset is 21-32%. Therefore, in turf maintenance and management should be reasonable fertilization, appropriate increase of phosphorus and potassium fertilizer to improve plant resistance, can effectively control the occurrence of disease; summer cannot be too low pruning, too dense turf should be properly perforated and sparse the lawn. At present, fungicides, such as Chlorothalonil and Methyl Toprazine, are good fungicides for controlling brown spot.

3.2.3 Leaf Spot

The collected turfgrass *Cynodon dactylon, Festuca arundinacea, Lolium perenne, Zoysia matrella, Trifolium repens* from Tiande Lake Park, Jiangsu Agri-animal Husbandry Vocational College Fenghuang Road Campus, City Government Square, Taishan Park and Taizhou People's Park, *Drechslaris poae, Fusarium equiseti, Bipolaris sorokinianum* and *Curvularia lunata* were separated from them. At the beginning of the disease, a lot of small oval waterlogging spots appeared. The color became dark evenly, showing reddish-brown to purple-black. Many lesions healed to form large necrotic spots. The size of the lesions ranged from a few centimeters to several meters. The severely diseased turf died with the drying of the diseased leaves, and the turf became sparse and premature senescence.

Leaf spot disease is most suitable for infection at about 20 C. The main epidemic period of leaf spot disease is spring with warm temperature and autumn with high temperature and rainy weather. In addition to the most basic lawn maintenance and management measures, cannot be irrigated at night, timely removal of grass debris, withered grass layer after cutting; at the same time, the choice of disease-resistant varieties. The new turf can be mixed with 25% Triadimefon wet-table powder or 50% TMTD wet-table powder by seed weight of 0.2%-0.3%. Spraying fungicides at the initial stage of turf disease can better control the disease development, such as 50% TMTD wet-table powder, 70% manganese zinc wet-table powder and so on.

3.2.4 Fusarium Wilt

Fusarium poae and *Pythium aphanidermatum* were separated from *Cynodon dactylon* collected from Taindehu park, they can infect the stems and leaves of turfgrass, causing rot and collapse of stems and leaves. At the onset of the disease from the tip of the leaf down or from the base of the sheath up a waterlogged wilt, the late edge of the lesion brown red. High temperature and humidity are the most suitable conditions for the occurrence of Fusarium wilt. In general, the highest temperature in the daytime is above 30℃, the lowest temperature is above 20℃ at night, and the relative humidity is above 90% and lasts more than 12 hours. Fusarium wilt can occur in a large area. Round yellow-brown spot with a diameter of 2-5 cm appears on the lawn.

Establishing good site conditions is the key measure to control Fusarium oxysporum and Fusarium oxysporum wilt, avoiding irrigation in the evening or at night. When the thickness of the grass layer is over 2cm, it should be removed in time. In summer, fungicides should be used to control diseases in high temperature and humidity season in South China, such as protective fungicides TMTD, manganese zinc, inhalant fungicide metalaxyl, metalaxyl and so on.

IV. DISCUSSION

More than 80% of the turf grass diseases are caused by fungi[2]. The pathogen passed through the winter in the roots and dead stems and leaves of the plant.. When the external environment conditions are suitable, it will harm the turf through the spread of soil, air and water, seriously affecting the use and ornamental value of the turf. In this study, eight pathogenic fungi, including *Alternaria alternate*, *Bipolaris sorokinianum* and *Drechslaris poae*, were isolated and purified from turfgrass samples collected from the main parks and urban greenbelts in Hailing District of Taizhou City. They mainly caused Black spot disease, Brown Patch disease, Leaf spot disease and blight of *Cynodon dactylon*, *Zoysia matrella*, *Festuca arundinacea*, *Lolium perenne* and *Trifolium repens*. These diseases are more common in cool-and-warm-season type turfgrass. Black spot disease, Fusarium wilt disease, Brown Patch disease and Pythium disease of turf grass have a strong possibility of transmission in China, and their adaptability is strong, which has a serious impact on urban environmental greening [3].

Black spot is a kind of high temperature disease, which mainly infects cold and warm seasonal type turfgrass, such as *Festuca arundinacea* and *Cynodon dactylon* that are experiencing high temperature stress or high temperature growth cessation. The occurrence of the disease on turfgrass was reported in Gansu, Inner Mongolia and Hainan.[6-8]. Brown Patch disease is a common disease on *Festuca arundinacea*, *Festuca arundinacea* and *Zoysia matrella* and other turfgrass. It also occurs on lawns in Gansu, Nanjing and Chongqing of Jiangsu Province[6,9-10]. Leaf spot disease is one of the most common diseases on *Festuca arundinacea* roots in warm-season type turf. Some scholars reported that the disease was isolated from *Festuca arundinacea* [11]. Turfgrass wilt occurred in Gansu, Inner Mongolia, Chongqing and other places[6-7,10]. High temperature and humidity are the main conditions for turf fungal diseases. Taizhou is located in the south-central part of Jiangsu Province in the middle and lower reaches of Changjiang River. It belongs to the north subtropical humid climate zone. The high temperature and humidity in summer provide a more suitable environment for the occurrence of turf fungal diseases. Therefore, in order to prolong the service life of the turf and improve the ornamental value of the turf, the daily maintenance management for the turf is particularly important.

V. CONCLUSION

The occurrence of turf diseases is the result of the interaction of environmental factors (such as temperature and humidity), susceptible plants and pathogenic bacteria. Therefore, in the process of turf planting and maintenance, we should create the environment conditions which are beneficial to the growth of turf plants but not conducive to the storage of pathogens, and it should adapt to local conditions and we should comprehensively and systematically apply various preventive measures to coordinate the ecosystem composed of turf plant, disease and environment, and control the disease damage to the lowest level, so as to achieve the best economic, ecological and social benefits. The prevention and cure of lawn diseases should follow the principle: main prevention and comprehensive cure. Specific control measures are: first, the selection of disease-resistant varieties, balanced and appropriate fertilizer, moderate increase of P, K fertilizer can improve the disease resistance of lawn grass; second, scientific maintenance and management, such as timely pruning and clearance of grass debris and withered layer, and the summer pruning can not be too low, and when the resistance of the plant is low, we should improve the height of pruning, Timely removal of disease plants, no evening or night irrigation, etc. Third, for the newly-planted turf, we adopt mix seeds with pesticide. We can use protective fungicides (Thiram, Capeton, etc.) and inhalant fungicides (metalaxyl, Benomyl, thiophonate-methyl, etc.) in advance to prevent the occurrence of turf diseases to a minimum to extend the service life and use effect of lawn.

In short, through this study, we preliminarily understand the main species distribution, pathogenic pathogens, symptoms of turf grass fungal diseases in Hailing District of Taizhou City, providing some basic information for turf scientific conservation and management.

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