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CONTENTS

EFFICACY OF FIVE INSECTICIDES AGAINST *ORIBIUS INIMICUS* MARSHALL AND *O. DESTRUCTOR* MARSHALL (COLEOPTERA: CURCULIONIDAE)

Pus Wesis[1], Benjamin Niangu[1], Mark Ero[2&3], David Elmouttie[3] and Anthony R. Clarke[3]

ABSTRACT

Oribius inimicus *and* O. destructor *are serious horticultural pests of the Highlands region of Papua New Guinea. Adult weevils feed externally on all non-woody parts of a wide range of agricultural and amenity plants. No insecticides currently registered for use in PNG have been formally tested against these insects. In this study we used laboratory bioassays to evaluate the efficacy of five insecticides (Karate® [Lambda-cyhalothrin], Malathion, Chlopyrifos, Target® [Primiphos-Methyl Permethrin] and Orthene® [Acephate]), all of which are commercially available and commonly used by Highlands' farmers. Follow-up laboratory and field trials of the two most promising chemicals tested efficacy at one-quarter, one-half and 1 x manufacturers' label dilution rates. Of the insecticides tested, Karate®, Target® and Chlophyrifos were found to be the most effective in terms of both total knockdown and time to knockdown. In laboratory bioassays, 100% mortality was reached within 20 minutes for these chemicals when insecticide was applied topically to individuals. In field studies, plots treated with Karate® yielded a significantly greater weight and number of fruit than plots treated with Target®, but different dosage rates (one-quarter, one-half or full manufacturers' recommended concentrations) within insecticide type did not alter yield. We recommend Karate® as the chemical of preference for Highlands' growers for* Oribius *control because of its significantly lower cost and better field control.*

Keywords. *Grey weevils, pest management, efficacy, Papua New Guinea*

INTRODUCTION

Weevils of the genus *Oribius* (Marshall) (Coleoptera: Curculionidae) are abundant throughout Papua New Guinea (PNG) and West Papua, Indonesia. Belonging to a group of closely related, small flightless weevils known regionally as "grey weevils" (which also includes, for example, the genera *Apirocalus* Pascoe and *Hypotactus* Marshall [Moxon 1992]), *Oribius* spp are restricted to the island of New Guinea (Thomas & Verloop 1962) and the northern tip of Cape York, Australia (Zimmerman 1991). While the genus is considered to contain over 50 species (Marshall 1956), only eight of these are recognised as pestiferous in PNG (authors' unpublished data): no pest species are known to occur outside the island of New Guinea.

In common with other grey weevils, *Oribius* species are flightless and the adults walk onto their host plants: larvae are soil dwelling and pupation occurs in the soil. Adults are the damaging stage, feeding on growing tips, soft shoots, green stems, flower buds and developing fruit of their host plants (Thistleton 1984). In the Highlands region of PNG, a very wide range of agricultural crops are attacked, from leafy greens through to introduced orchard trees, such as apples and oranges, and field crops such as coffee (Marshall 1957, 1959; Szent-Ivany 1959; Szent-Ivany & Stevens 1966; Greve & Ismay 1983; Thistleton 1984; Waterhouse 1997). *Oribius* spp are also recorded as forestry pests (Gray & Wylie 1974) and they are an abundant component of the PNG rainforest insect fauna (Novotny *et al.* 2002). Feeding by oribius weevils causes significant loss of growth, yield decline, downgrade of crop marketability and, in severe cases, tree and seedling mortality.

Although recognised as important pests since at least the 1950's, there has been very little formal

[1]National Agricultural Research Institute, Main Highlands Programme-Aiyura, P.O. Box 384, Kainantu, Eastern Highlands Province, Papua New Guinea
[2]National Agricultural Research Institute, National Agricultural Insect Collection, P.O. Box 1691, Boroko, National Capital District, Papua New Guinea
[3]School of Natural Resource Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Qld 4001, Australia
Corresponding Author, Clarke, A.R., School of Natural Resource Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland, Australia, 4001, Phone: + 61 7 3864 5023, Fax: + 61 7 3864 1535, Email: a.clarke@qut.edu.au

study of the ecology or management of oribius weevils (references cited herein cover the available literature). Currently, there is little control of *Oribius* species within Papua New Guinea's agricultural sector, despite crop yields increasing 100-200% when weevils are managed (authors' unpublished data). While some farmers do try to manage oribius using insecticides, the efficacy of the different chemicals available and their most efficient and cost effective application rates have not been evaluated. Because of the highly significant yield gains which can be made when weevils are managed, it is imperative that research be undertaken which supplies easily implemented recommendations for weevil control. This paper represents the outcome of some of this research, on laboratory and field evaluation of five, locally available insecticides. We deliberately chose locally available chemicals as previous pest management research in PNG had demonstrated that trialling non-available chemicals (for example those registered in Australia but not sold in PNG) led to significant grower dissatisfaction when they could not immediately implement the benefits of trials in which they had participated or observed. Studies were conducted on two pest *Oribius* species, *O. inimicus* Marshall and *O. destructor* Marshall, which occur sympatrically in the Highlands' cropping districts.

MATERIALS AND METHODS

Study Site

Both laboratory and field trials were conducted at the [PNG] National Agricultural Research Institute (NARI) Main Highlands Program, Aiyura Valley (6°20'23" S 145°54'18"E), via Kainantu, Eastern Highlands Province, PNG. Laboratory trials were conducted under ambient conditions: mean temperature and relative humidity were 20.5°C and 90.0% respectively.

Insects and Laboratory Trials

Adult *Oribius inimicus* and *O. destructor* were bulk collected from various host plants around the Aiyura valley in the week preceding their use in laboratory trials. Insects were held in an outdoor insectary containing a mix of known host plants until used. Insects used in trials were thus of unknown age and maturity, although they could be, and were, sexed.

Five insecticides were used for the trials: Karate® [Lambda-cyhalothrin, 25g/L active ingredient], Malathion [500g/L active ingredient], Chlopyrifos [500g/L active ingredient], Target® [Pirimiphos-Methyl/Permethrin, 5 + 95g/L permethrin pirimiphos-methyl] and Orthene® [Acephate, 75%w/v active ingredient]. Insecticides were chosen based on their ready availability for Highlands' growers, rather than any preconceptions about preferred mode of action, etc. For each insecticide three treatments and a control were applied which manipulated the insecticide's application mode:

(i) Filter paper dipped into the insecticide and then allowed to air dry before being added as a lining to a petri dish;
(ii) Insecticide misted onto weevils until droplets appeared (topical application);
(iii) Host plant (Thickhead, *Crassocephalum crepidioides*) leaf material immersed in insecticide dilution, allowed to air dry, and then placed with weevils into a petri dish.
(iv) Control – Water sprayed on individuals.

The basic replicate, 10 for each treatment, consisted of a 90mm diameter disposable petri dish, lined with filter paper, containing five male and five female weevils. Recordings of weevil morbidity were made at 5, 15, 30, 60, 360 and 1440 minutes after exposure, and then as required once every 24 hours until 100% mortality was reached. Treatments were abandoned after 336 hours. Insecticides were applied at a rate equivalent to manufacturers' label instructions for general horticultural use. These were: Karate® 0.9ml/L water; Malathion 2.5ml/L water; Chlopyrifos 4ml/L water; Target® 5ml/L water; Orthene® 0.24g/L water.

The three most efficacious insecticides were then further tested at lower concentrations (one-quarter and one-half of full dosage) using the most effective application methods. Each insecticide was tested using the same protocols as for the initial trials.

Field Trials

Based on lab results, two of the three most efficacious insecticides (Karate® and Target®, see results) were subsequently field validated at full (Karate®, 1ml/L; Target® 5ml/L), one-half and one-quarter concentrations. Three replicate field plots were planted on-station for each of the two insecticides tested. Each replicate plot consisting of 80 capsicum plants, separated into four quadrants, each quadrant of which was treated with the same insecticide at the three concentrations and a control (no insecticide application). Capsicums were chosen as they are readily available and commonly grown in the Highlands, have a rapid growth rate and are known hosts of *Oribius* (authors' unpublished data).

Trials were planted at Aiyura on 19.04.2005 and fruits were progressively harvested and weighed as they reached maturity (last harvest date 29.07.2005), in line with normal local farming practice. Insecticide application was conducted on a "as need" basis (based on observations from full concentration plots): this resulted in three sprays being applied on 23.05.2005, 22.06.2005 and 29.07.2005. On completion of the crop cycle, cumulative weights, the total number of fruits harvested and the total number of damaged fruits were calculated for each insecticide/concentration replicate.

Cost/benefit of control

A simple cost/benefit analysis was undertaken on the results obtained from the field trial. Costings were based on average local market value of capsicum for 2004-2005 (Kina 3.52/kg) and average local store costs of insecticides (Karate® K29.03/L, Target® K72.30/200ml, Wetting agent - Holimpas K16.00/5L). Labour costs were not included as we were targeting individual grower gardens where owner-farmers carry out all work. For the same reason we kept the results of the analysis at a small plot size, as against, for example, converting it to a per hectare figure, as horticultural crops in the Highlands are very rarely grown in large, contiguous fields.

RESULTS

Laboratory Results

Combining the different insecticides, application method significantly affected efficacy ($F=33.434$, $df = 2$, $p < 0.001$), with topical application of insecticide being the most effective exposure technique: this overall result was consistent regardless of the insecticide or *Oribius* species tested (Table 1). Independent of exposure type, Chlophyrifos, Target® and Karate® were significantly more effective than the other two chemicals against both *O. inimicus* and *O. destructor* ($F_{inimicus} = 31.376$, $df = 4$, $p < 0.001$; $F_{destructor} = 24.886$, $df = 4$, $p < 0.001$). While the different *Oribius* species responded slightly differently to the different insecticides, with the most obvious difference being the much greater efficacy of malathion against *O. inimicus* than *O. destructor*, there was a generally consistent pattern in the response of both species to the different insecticides (Table 1).

Chlophyrifos, Target® and Karate® were further tested at reduced concentrations. For all insecticides combined, concentration had a significant effect on mortality ($F_{inimicus} = 6.489$, $df = 2$, $p < 0.001$; $F_{destructor} = 14.375$, $df = 2$, $p < 0.001$), with full strength applications being significantly more efficacious in seven of the twelve comparisons (Table 2). The efficacy of Chlophyrifos was most affected by a reduction in concentration, with time to 100% mortality for *O. destructor* being 12 times slower at one-quarter manufacturer's recommended dose than at full strength. Excluding this exception, all other reduced rate insecticides still caused 100% mortality within a maximum of 40 minutes, and on average within 22 minutes (Table 2).

Table 1: **Mean time (minutes) to 100% mortality for *Oribius destructor* and *Oribius inimicus*, using five insecticides and three exposure treatments**

Insecticide	*Oribius* spp.	Treatment			
		Topical	Substrate	To food	Control
Karate®	*O. destructor*	18.5 ± 2.7	330 ±3 0	360 ± 0.00	> 20160
Target®	*O. destructor*	12 ± 1.5	468 ± 108	360 ± 0.00	> 20160
Chlophyrifos	*O. destructor*	14.5 ± 2.2	1440 ± 0.0	1440 ± 0.0	> 20160
Malathion	*O. destructor*	160 ± 54	> 20160	> 20160	> 20160
Orthene®	*O. destructor*	330 ± 30	> 20160	1152 ± 256	> 20160
Karate®	*O. inimicus*	7 ± 1.3	108 ± 42	360 ± 0.00	> 20160
Target®	*O. inimicus*	8 ± 1.5	10092 ± 2915	6288 ± 3029	> 20160
Chlophyrifos	*O. inimicus*	16.5 ± 1.5	900 ± 180	1322 ± 108	> 20160
Malathion	*O. inimicus*	16.5 ± 3.2	15300 ± 2195	17568 ± 1319	> 20160
Orthene®	*O. inimicus*	2052 ± 364	13248 ± 2369	2448 ±5 28	> 20160

(Sample size for each treatment equals 10 replicates of 10 insects each).

Field Results

In the field validation studies for Karate® and Target®, both insecticides led to greater harvest yields than unsprayed control plots. However, insecticide type significantly influenced yield regardless of dosage treatment: plots treated with Karate® had a significantly greater harvestable weight of capsicums ($t = 2.65$, $df = 70$, $p = 0.01$) and number of fruit ($t = 2.286$, $df = 70$, $p = 0.025$) than those treated with Target® (Table 3). There was no significant effect of changing dosage rate within an insecticide. Insecticide type did not effect the number of damaged fruit ($t = 1.637$, $df = 70$, $p = 0.106$), which was high in all treatments (Table 3).

Cost/benefit of control

Results of the cost-benefit analysis showed that treatments to control oribius weevils were highly profitable, with one-quarter strength Karate® control yielding a K66.40 increase in return over not controlling weevils (Table 4).

DISCUSSION

This study indicates that use of selected insecticides, such as Karate® and Target®, can be used to reduce damage and increase productivity in crops that are attacked by oribius weevils. Further, the cost/benefit analysis demonstrated that significant profit is to be gained with only a few spray applications during a cropping cycle. Because of its extra efficacy in the field (Table 3) and lower cost (Table 4), we recommend Karate® as the chemical of choice for *Oribius* control.

Yield benefits were recorded despite a relatively high level of damage still being experienced in

***Table 2*: Mean time (minutes) (± 1 S.E) to 100% mortality for *Oribius destructor* and *O. inimicus* using three insecticides at one-quarter, one-half and full manufacturers' recommended concentration.**

Insecticide	Species	Treatment		
		¼ Concentration	½ Concentration	Full Concentration
Karate	*O. destructor*	40.5 ± 5.5*	18 ± 5.9	18.5 ± 2.7
Target	*O. destructor*	27.5 ± 4.6*	14.5 ± 3.6	12 ± 1.5
Chlophyrifos	*O. destructor*	363 ± 47*	38 ± 3.6*	14.5 ± 2.16
Karate	*O. inimicus*	22.5 ± 2.5*	27 ± 2.0*	7 ± 1.3
Target	*O. inimicus*	14 ± 1.0*	9.0 ± 1.63	8 ± 1.5
Chlophyrifos	*O. inimicus*	14 ± 1.0	15.5 ± 3.45	16.5 ± 1.5

* indicates significant difference at $p<0.05$ from full concentration trial

***Table 3*: Mean (± 1 S.E.) number of undamaged fruit, damaged fruit, and harvestable weight of capsicum fruit grown under different insecticide regimes at Aiyura, Eastern Highlands Province, PNG.**

Insecticide	Mean treatment yield (± 1 S.E.)			
	Control	¼ dose	½ dose	Full dose
Karate				
Number undamaged fruit	27.3 ± 5.9a	44.1 ± 10.1b	37.1 ± 8.7b	42.1 ± 11.9b
Number damaged fruit	25.1 ± 5.6a	33.7 ± 7.9b	30.8 ± 7.6b	31.5 ± 9.5b
Fruit weight (Kg)	23.77 ± 4.6a	42.5 ± 9.0b	39 ± 9.3b	39.6 ± 10.1b
Target				
Number undamaged fruit	19.3 ± 4.6	25.0 ± 7.6	28.0 ± 8.1	25.7 ± 6.8
Number damaged fruit	18.4 ± 4.5	22.7 ± 7.0	25.1 ± 7.9	22.8 ± 6.4
Fruit weight (Kg)	16.9 ± 4.5	24.6 ± 7.7	22.2 ± 6.6	25 ± 6.4

Each treatment consisted of three replicated, 20 plant plots.

Table 4: **Cost/benefit analysis for control of *Oribius* species on 60 capsicum plants using three insecticide concentration rates for Karate® (active ingredient Lambda-cyhalothrin) and Target® (active ingredient Pirimiphos-Methyl/Permethrin).**

Parameter	Treatment			
	Unsprayed	One-quarter manufacturer's recommended rate	One-half manufacturer's recommended rate	Full manufacturer's recommended rate
KARATE®				
Treatment cost	0.00	0.16	0.23	0.38
Market Value (K/kg)	3.52	3.52	3.52	3.52
Productivity (Kg)	23.7	42.6	39.0	39.7
Profit (Kina)	83.42	149.79	137.05	139.36
TARGET®				
Treatment cost	0	3.7	7.31	14.54
Market Value (K/kg)	3.52	3.52	3.52	3.52
Productivity (Kg)	16.7	24.6	22.2	25.0
Profit (Kina)	58.78	82.89	70.83	73.46

Calculations based on the Papua New Guinea Kina (K) and figures are based on local store costs in Eastern Highlands Province, PNG. Profit is based on average local market value and total production for each treatment. Three spray applications were made during trial.

our treatment plots. In a separate trial (authors' unpublished data), fortnightly calendar spraying with Karate® did lead to reduced levels of damage in comparison to unsprayed controls, but did not negate all damage. Along with unpublished observational studies of the weevils, we explain the high level of damage in insecticide treated plots as being caused by: (i) the mobility of the weevils, which walk on and off plants twice daily; and (ii) the cryptic nature of surface feeding damage, which on young fruit may go unnoticed, but which becomes progressively more noticeable as the fruit grows. In this latter case it may imply that the three "as needed" spray applications in our field trials were either insufficient, or badly timed. Given these problems, particularly when garden plots are surrounded by non-treated weed vegetation (as is common in the Highlands), insecticide would need to be applied with a very high frequency to eliminate all damage. We do not endorse such an approach, however, given the well documented negative impacts which follow high level insecticide use.

Although a topical application is recommended (based on lab results), the efficacy of Karate® relative to Target® in the field may be linked to the chemical's persistence and mode of action. Lambda-cyhalothrin is considered to have a half life on the leaf-surface of five days (National Pesticide Telecommunications Network) and, in addition to its direct mortality effects, has a repellency action against insects (Tomlin 1997). This persistence on the substrate and repellency action may help it control weevils which walk onto the plant after application.

The efficacy of Target® and Karate® at reduced concentrations has significant implications for management. Although time to kill was lengthened in the laboratory environment, efficacy at one-quarter and one-half recommended concentrations did not lower yields within field trials. Using insecticides at one-quarter concentration reduces insecticide cost by 75%, a significant benefit in a cash poor society (Benjamin *et al.* 2001), and may make the difference as to

whether control is applied or not. The benefits of a lower concentration application may also be magnified as, although reduced concentrations still provide a lethal dose to *Oribius* weevils, the general principles of Integrated Pest Management (IPM) would predict that impacts on potential natural enemies and pollinators may be reduced.

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ASSESSMENT OF KAVALACTONES IN KAVA, *PIPER METHYSTICUM* FORST. F., CULTIVARS OF PAPUA NEW GUINEA

Michael Dom

ABSTRACT

Assessment has been made of the kavalactones in eight cultivated varieties of kava, Piper methysticum, *grown in three locations in Papua New Guinea. A total of forty-seven samples of dried root, stem and peeling from Lae, Madang and the Keravat Germplasm Collection of the PNG National Agricultural Research Institute representing eight cultivars were analyzed by HPLC methods to determine the kavalactones. Three chemotypes are indicated, two identified by Lebot et al (1999) and another that is similar to that in* P. wichmannii. *Kavain, dihydrokavain and dihydromethysticin were found to be the predominate components. The Total KL (%) however is lower than found in kava from other South Pacific countries.*

Keywords: *Kava,* Piper methysticum, Piper wichmannii, *kavalactone, kavain, dihydrokavain, dihydromethysticin, methysticin, yangonin, desmethoxyyangonin.*

INTRODUCTION

Kava is a plant from the Piperaceae family and consists of two botanically differentiated species, *Piper wichmannii* C. DC. and *P. methysticum* Forst. f. The latter has been cultivated by people in Oceania for some 3000 years (Lebot 1997) and was first described in 1786 by a botanist traveling with Captain James Cook. Evidence has shown that *P. methysticum* is the sterile, cultivated form while *P. wichmannii* forms the fertile wild population (Lebot and Levesque 1996). New Guinea, Vanuatu and the Solomon Islands are thought to be the centers of origin of the kava plant, where the natural occurrence of *P. wichmannii* and other closely related species is isolated (Lebot *et al.* 1991).

Kava is a cash crop of several Pacific island nations. Kava die-back disease led exporters to search for alternative sources for production, cultivation and export. This led to an increased interest in kava cultivars grown in PNG. The PNG National Agricultural Research Institute (NARI), as the leading agency for research in agricultural crops, has produced information on the horticultural and agronomic aspects of kava cultivation. However, there had been limited information on the range of cultivars in PNG in terms of chemical components. As well as defining the chemical composition, an additional aim of the paper was to suggest the cause of strength of brew ('kick') reportedly found in the PNG Madang Short kava drink.

Kavalactones (a-pyrones and 5,6–substituted dihydro-a-pyrones) are psychoactive compounds released in a brew made by soaking the masticated or powdered root. The lactones are also present in the stump, stem, peelings and leaves. There are six major kavalactones each having different physiological effects. Studies into the pharmacology of these six kavalactones have been summarized by Hansel (1968) as narcotic (intensifying barbiturate narcosis), analgesic, local anaesthetic, contraction inhibiting and anticonvulsant, antispasmolytic and fungistatic towards select species of fungi and streptomyces. Kretzschmar's studies (1970; cited by Lebot *et al.* 1999) show the psychopharmacology of kavain is characterized by emotional and muscular relaxation, stabilization of the feelings and stimulation of the ability to think and act.

The work here is based on analysis of 47 samples that were received by the NARI Chemistry Laboratory from Keravat, Lae and Madang. Complete descriptions of the growth sites, soil conditions and morphotypes were not available at that time. This study is based on the observed chemotypic variation. Such data is essential for facilitating the selection of superior or commercially desirable cultivars (Lebot *et al.* 1991).

PNG National Agricultural Research Institute PO Box 4415 Lae 411, Morobe Province Tel: (675) 4751444/1445 Fax: (675) 475 1450 Email: Michael.dom@nari.org.pg Website: www.nari.org

MATERIALS AND METHODS

The samples were received in four accessions from Keravat (Lon.4° 20' N; Lat.152° 92' E), Lae (Lon.7° N Lat.146° 30' E) and Madang (Lon.6° 30'N; Lat.145° 30' E). The varieties were given local names: Madang Short (MS var.), Madang Tall (MT var.), Manus Green (ManG var.), Manus Pink (ManP var.), Manus Tall (ManT var.), Daru Tall (DT var.), West New Britain (WNB var.) and Kavieng (KAV var.). Samples were received as dry material but were further dried in a forced air draft oven at 105°C. The phytochemical analysis was made by High Performance Liquid Chromatography (HPLC) following the INA Method 101.002 first developed by Shao *et al.* (1998).

About 750 mg (±0.1mg) of finely ground stem, peeling, or root material was placed into a 50-ml volumetric flask along with 40 ml of methanol/ water (70/30) and sonicated for 60 minutes at room temperature. The flask was allowed to cool and contents diluted to volume with methanol/water (70/30). About 5ml was then filtered through 0.45mm nylon micro filters (Whatman) into a HPLC vial and capped. Kavain was used as the stock standard prepared as for the samples. The literature response factors were used to quantify the other five components. System suitability was ensured for the standards 0.01, 0.04, 0.10 and 1.00 mg/ml having a linear coefficient of 0.9999 and a resolution between desmethoxyyangonin and yangonin of 5.7. Analysis was carried out on a Varian Star Chromatography System Series 9000 consisting of a 9012 SDS, 9100 AutoSampler and a 9065 UV-Vis. A suitable analytical column, Microsorb MV, 5 mm (C-18), 4.6 ' 250 mm substituted the reported YMCbasic 5 mm (C8). A temperature of 32-34°C was maintained in a water bath in which the column was immersed. Mobile Phase was isocratic acetonitrile: methanol: water: acetic acid (20: 20: 60: 0.1 v/v) at a flow rate of 1.0 ml/min. Injections of 10ml were made using a Valco valve and sample detection was made at 220 nm. Total run time was 45 minutes. Calculation of the components was made as follows:

$$\% \text{ Individual Kavalactone} = \frac{(A)(SR)(FV)(D)(F)(100)}{(W)}$$

Where: A = Peak area response of the kavalactone in the sample.

SR = The response of the corresponding kavalactone reference standard (slope of the calibration plot)

FV = The final volume of the sample preparation (ml).

D = The dilution factor of the sample preparation.

F = The correction factor for quantitation against kavain

W = The sample weight (mg).

HPLC analysis allowed separation of six major kavalactone (KL) components; in order of elution (reverse phase) they are: methysticin (M), dihydromethysticin (DHM), kavain (K), dihydrokavain (DHK), desmethoxyyangonin (DMY) and yangonin (Y). Chromatograph and retention times and response factors used to calculate the percent compositions are in Table 1. Dry extracts were not made as the method assays directly from solution.

Figure 1. **A sample chromatogram of kavalactones.**

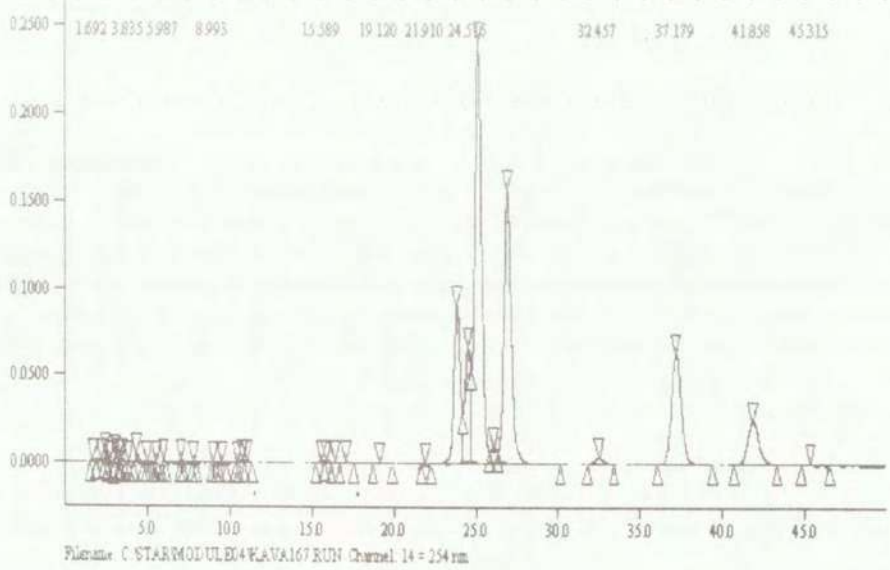

Table 1. **Retention Times for HPLC analysis above.**

Kavalactone	Retention Times (minutes)	Response Factors
Methysticin (M)	23.847	0.6602
Dihydromethysticin (DHM)	24.516	1.638
Kavain (K)	25.117	1.000
Dihydrokavain (DHIL)	26.892	1.641
Desmethoxyyangonin (DMY)	37.179	0.9329
Yangonin (Y)	41.858	0.9669

* From INA Method 101.002 (Institute for Nutraceutical Advancement 2000)

RESULTS AND DISCUSSION

There were a total of 47 samples; 15 root, 20 stem and 8 peelings. An additional four samples were received in powdered form (labeled 'unknown'). The results of HPLC analysis and the chemical coding and chemotypes are presented in Table 2. Descriptive Statistics were applied to root and stem samples (Tables 3 and 4). Table 5 shows the results of simple linear correlation analysis for the roots and stems. Total Kavalactone (KL) percent variation in the 15 root and 20 stem samples was very large, coefficient of variance of 50.522 and 59.200 respectively. Desmethoxyyangonin had the greatest variance in both root and stem samples and yangonin had the least variance. High DMY content was observed in ManT and ManG and WNB varieties where they account for 43 – 60% of the total KL. Chemotypes of these three cultivars are characteristically low in dihydrokavain and yangonin. In Madang Short root, stem and peelings, kavain and dihydrokavain are almost equally expressed. In general, tested varieties showed higher levels of kavain, dihydrokavain and desmethoxyyangonin reflected in the chemotype codes. Lebot *et al.* (1999) found that environmental factors play an important role in the formation of kavalactones. Such factors include shading (negatively) and high levels of fertilization (positively). Similar correlations were not possible for this work. However, the samples originated from two distinct geographic locations. Lae and Madang are in mainland New Guinea and Keravat is in the Islands region. Keravat in East New Britain is known to have soil of volcanic origin whereas the Lae and Madang soil is alluvial. All locations have high rainfall (>2500mm annually) but wet periods are more regular in Lae and Madang. The plantings were well established in all three locations for five to ten years or longer. Madang samples had three of the highest total KL percent from the peelings. Madang sites grew only MS varieties but these showed two distinct chemotypes (codes: 245-163/613 and 425-163/613). Samples from Lae were of MT and MS. Lae kava displayed only chemotype C for both varieties grown, MS and MT. There were four samples of unknown origin and two of these were of a different chemotype (code: 425-163/613). Keravat germplasm consisted of all eight varieties and KL percent in these differed significantly at the 95% level. Keravat kava presented three chemotypes.

Overall there are three chemotypes observed here. Chemotype C: 245, 254 and F: 421, 425 have been assigned according to Lebot and Levesque (1989). Chemotype arbitrarily designated Q for codes: 142, 145, 146 are indicative of Vanuatu and PNG collections (Lebot and Levesque 1989). In Lebot *et al.* (1991), Table 1 of *P. wichmannii* and *P. methysticum* germplasms, lists PNG as having 23 wild forms, 4 cultivars and chemotypes; B (521634), C (254613), D (643251) and F (426315). Lebot *et al* (1999) found that KL percent 'decreases from the root to the stump, the basal stems and the leaves exhibit lower concentrations'. The group also found that 'peelings of the bark had a higher kavalactone content' (Lebot *et al.* 1999). The findings are similar for this work.

In general the appreciated effect [of kava brew] is correlated with high percentages of kavain and lower dihydromethysticin and dihydrokavain (Lebot *et al.* 1999). Chemotype F (426315) is the most appreciated by kava drinkers (Lebot 1990). PNG kava varieties showed high levels of kavain and dihydrokavain almost equally expressed and accounting for up to 78 percent of the Total KL percent Dihydromethysticin was also prominent and there were lower methysticin and yangonin. Kavain, DHK and DHM are of particular interest. Kavain and DHK both have local anaesthetic properties and DHM has a stronger narcotic effect (Hansel 1968). DHK has a relative analgesic effect stronger by dosage than that of acetylsalicylic acid (aspirin) (Bruggeman and Meyer 1963 in Hansel

1968). The predominance of these three kavalactones may be the underlying cause of the strength ('kick') appreciated by PNG kava consumers. PNG kava varieties however, showed lower Total KL percent content compared to that found in other kava producing countries.

Table 2. **Kavalactone HPLC analysis results of (47) Kava samples displaying chemotypes at three locations.**

Lab No.	Variety	Location	Section	Totals (%)	M	DHM	K	DHK	DMY	Y	Code	Chem otype
0143(1)	Madang short	Lae	stem	0.39	6.77	19.28	26.94	33.84	8.61	4.57	245163	C
0143(2)	Madang short	Lae	peelings	2.29	7.71	17.67	26.59	32.30	8.43	7.30	245163	C
0143(3)	Madang short	Lae	roots	6.55	7.10	11.30	31.47	34.50	10.75	4.88	245163	C
0143(4)	Madang short	Lae	peelings	4.69	7.36	13.64	29.68	34.02	9.74	5.57	245163	C
0143(5)	Madang short	Lae	stem	5.40	7.38	11.47	32.72	34.35	9.68	4.40	245163	C
0144(1)	Madang tall	Lae	stem	1.73	8.26	20.07	25.56	33.05	6.76	6.29	254613	C
0144(2)	Madang tall	Lae	stem	0.81	5.07	22.00	18.08	43.31	6.32	5.21	254136	C
0144(3)	Madang tall	Lae	peelings	3.54	8.84	19.07	26.05	30.27	7.33	8.44	245631	C
0144(4)	Madang tall	Lae	peelings	2.96	5.77	23.56	17.10	39.99	6.46	7.12	254316	C
0144(5)	Madang tall	Lae	roots	5.40	7.38	11.47	32.72	34.35	9.68	4.40	245163	C
0145	Madang	Lae	unknown	6.39	5.93	13.95	29.08	41.06	6.38	3.60	245163	C
0403	Madang short	Lae	unknown	3.74	8.33	11.26	37.71	26.33	8.57	7.79	425163	F
0507	Madang short	Lae	unknown	3.03	8.01	12.12	35.03	32.57	7.72	4.56	425613	F
0135 (a)	Madang short	Madang	roots	2.87	6.13	13.10	28.13	38.12	9.15	5.38	245163	C
0135 (b)	Madang short	Madang	stem	1.46	6.87	19.73	23.95	37.98	5.80	5.67	245613	C
0135 (c)	Madang short	Madang	peelings	2.45	6.93	20.11	23.43	35.28	7.11	7.14	245316	C
0139(1)	Madang short	Madang	roots	5.87	8.32	9.22	40.35	29.30	7.92	4.90	425613	F
0139(2)	Madang short	Madang	peelings	9.20	6.50	10.02	35.92	35.12	8.38	4.06	425163	F
0139(3)	Madang short	Madang	stem	2.79	8.08	14.21	34.48	33.22	5.45	4.57	425613	F
0139(4)	Madang short	Madang	stem	3.29	7.99	14.07	34.92	32.67	5.39	4.96	425613	F
0140(1)	Madang short	Madang	stem	2.98	7.50	13.83	34.04	34.26	5.75	4.63	245613	C
0140(2)	Madang short	Madang	peelings	10.47	6.30	9.80	36.21	34.88	8.63	4.19	425163	F
0140(3)	Madang short	Madang	stem	2.62	7.05	15.23	31.61	34.10	6.75	5.27	245613	C
0140(4)	Madang short	Madang	roots	4.30	8.38	9.40	39.79	27.75	8.61	6.08	425163	F
0141(1)	Madang short	Madang	peelings	11.88	6.58	11.06	33.30	35.85	8.28	4.94	245163	C
0141(2)	Madang short	Madang	stem	2.84	8.77	13.71	35.49	30.35	6.30	5.37	425613	F
0141(3)	Madang short	Madang	roots	3.59	9.41	12.68	36.37	30.01	6.52	5.01	425613	F
0141(4)	Madang short	Madang	stem	2.63	8.79	16.69	30.71	32.70	5.32	5.79	245631	C
0142(1)	Madang short	Madang	unknown	2.58	8.28	24.31	23.56	30.56	6.20	7.09	254631	C
0142(2)	Madang short	Madang	stem	3.22	8.84	22.10	23.13	31.50	6.21	8.22	245631	C
0142(3)	Madang short	Madang	roots	4.86	9.17	13.19	32.51	29.23	8.36	7.53	425613	F
3738	Madang short	Keravat	roots	3.86	7.94	9.81	38.00	27.82	10.36	6.06	421563	F
3739	Madang short	Keravat	stem	2.61	8.81	15.59	33.26	30.30	6.00	6.04	425631	F
3740	Manus pink	Keravat	roots	3.95	6.01	9.12	36.69	31.60	9.98	6.60	421536	F
3741	Manus pink	Keravat	stem	1.91	5.78	17.33	26.98	37.36	6.67	5.89	245136	C
3742	Manus tall	Keravat	stem	0.80	12.07	10.36	15.31	7.13	50.42	4.70	146523	Q
3743	Manus tall	Keravat	roots	0.48	11.18	10.84	12.30	7.92	52.38	5.38	146523	Q
3744	Manus green	Keravat	roots	0.64	11.92	8.10	10.59	4.76	60.28	4.36	164523	Q
3745	Manus green	Keravat	stem	0.56	11.45	12.13	21.74	7.13	43.01	4.54	145623	Q
3746	Kavieng	Keravat	roots	3.01	7.84	13.94	29.77	32.44	9.36	6.65	245163	C
3747	Kavieng	Keravat	stem	0.86	7.55	20.58	24.43	35.44	5.94	6.06	245631	C
3748	Madang tall	Keravat	roots	4.93	8.25	9.42	39.94	26.63	10.05	5.70	421563	F
3749	Madang tall	Keravat	stem	2.30	8.45	15.96	32.81	31.46	5.97	5.36	425613	F
3750	WNB	Keravat	roots	1.01	11.86	11.70	14.40	11.63	44.42	6.00	146523	Q
3751	WNB	Keravat	stem	0.72	10.60	12.13	22.16	14.95	34.49	5.67	142563	Q
3752	Daru tall	Keravat	roots	6.00	7.42	11.37	35.52	27.24	11.94	6.52	421563	F
3753	Daru tall	Keravat	stem	3.90	6.04	20.06	24.47	34.28	8.43	6.71	245136	C

Measured at 0.100AUFS

* Lebot et al, 1999 Chemotype coding.1 = DMY; 2 = DHK; 3 = Y; 4 = K; 5 = DHM; 6 = M. These are not the order of elution by RP- HPLC.

Table 3. **Descriptive Statistics Resulting from the analysis of 15 Root samples.**

	KL %	DMY (1)	DHK (2)	Y (3)	K (4)	DHM (5)	M (6)
Mean	3.822	17.982	26.220	5.696	30.570	10.979	8.554
Minimum	0.484	6.517	4.756	4.357	10.595	8.097	6.013
Maximum	6.551	60.276	38.115	7.534	40.348	13.942	11.918
Standard Deviation	1.931	18.085	9.969	0.909	10.104	1.758	1.859
Coeff. Of Variation	50.522	100.572	38.020	15.967	33.053	16.010	21.736

Table 4. **Descriptive Statistics Resulting from the analysis of 20 Stem samples.**

	KL %	DMY (1)	DHK (2)	Y (3)	K (4)	DHM (5)	M (6)
Mean	2.191	11.964	30.468	5.496	27.640	16.326	8.106
Minimum	0.385	5.316	7.126	4.400	15.310	10.365	5.071
Maximum	5.396	50.425	43.312	8.218	35.492	22.101	12.066
Standard Deviation	1.297	13.520	9.510	0.920	5.980	3.641	1.773
Coeff. Of Variation	59.200	113.00	31.213	16.749	21.634	22.304	21.873

Table 5. **Simple Linear Correlation analysis of Kavalactones in Root (15 samples) and Stem (20 samples).**

Roots Parameter				Stems Parameters			
X	Y	Corr. Coef.	Significance	X	Y	Corr. Coef.	Significance
DMY	DHK	-0.95	**	DMY	DHK	-0.94	**
	Y	-0.30	ns		Y	-0.29	ns
	K	-0.93	**		K	-0.60	**
	DHM	-0.30	ns		DHM	-0.57	**
	M	0.83	**		M	0.78	**
	Totals_%	-0.81	**		Totals_%	-0.46	*
DHK	Y	0.18	ns	DHK	Y	0.22	ns
	K	0.79	**		K	0.39	ns
	DHM	0.40	ns		DHM	0.65	**
	M	-0.91	**		M	-0.92	**
	Totals_%	0.75	**		Totals_%	0.36	ns
Y	K	0.25	ns	Y	K	-0.22	ns
	DHM	0.33	ns		DHM	0.65	**
	M	-0.18	ns		M	-0.11	ns
	Totals_%	0.09	ns		Totals_%	0.12	ns
K	DHM	-0.03	ns	K	DHM	-0.26	ns
	M	-0.73	**		M	-0.21	ns
	Totals_%	0.83	**		Totals_%	0.62	**
DHM	M	-0.15	ns	DHM	M	-0.63	**
	Totals_%	0.03	ns		Totals_%	-0.15	ns
M	Totals_%	-0.69	**	M	Totals_%	-0.25	ns

Table values are; Root, 0.6411 and 0.5139:Stem, 0.5614 and 0.4438; for 1% and 5% significance levels
** Significant at 1% and 5% level; ns, not significant

CONCLUSION

Kava, *P. methysticum* cultivated in Lae, Madang and Keravat today display three chemotypes C, F and Q. The last is likely to be a chemotype of wild forms from West New Britain and Manus. There are eight (8) cultivars that are locally identified in the Keravat germplasm collection. Of these cultivars Madang Short variety has been the most successfully produced and enjoyed kava brew. The major psychoactive components indicated for varieties having consumer preference are kavain, dihydrokavain and dihydromethisticin. Although the latter two compounds are considerably more potent, the lower Total (KL%) may be reducing their effect or there may be a modifying or synergetic effect with kavain, changing their physiological actions. This work presents a glimpse of the chemical variety of PNG kava that is now in cultivation but more work needs to be done to further the development of this crop.

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SELECTION OF RICE (*ORYZA SATIVA L*) VARIETIES FOR LOWLANDS RAIN-FED CONDITIONS IN PAPUA NEW GUINEA.

Stanis Malangen and Birte Komolong

ABSTRACT

Four rice (Oryza sativa L.) varieties, NR 1, NR 9, NR 15 and NR 16 were provisionally released in 2003 by the National Agricultural Research Institute (NARI) for the lowland areas ranging from 0-1200 meters altitude. They were selected on the basis of grain yield, eating quality and pest and disease resistance from the first season of trials.. The varieties were tested by NARI for another two seasons at five different sites in the Madang and Morobe Province. The superior performance of the four NR varieties over the standard varieties TCS 10 and FB91 was confirmed in both seasons. The varieties NR 1, NR 9, NR 15 and NR 16 are recommended for final release to farmers in the Lowlands of PNG based on yield performance and taste.

Keywords: *Varieties, released, National Agricultural Reseach Institute, seasons, performance.*

INTRODUCTION

Rice (*Oryza sativa L*) has many advantages over most of the traditional food crops of Papua New Guinea (PNG). Rice is easy to store, does not perish during transportation to distant locations, consumption of small volumes is enough to satisfy the human appetite compared to the traditional root crops and is considered a novel food. Unfortunately the crop has not integrated well into the farming systems of PNG. Non-availability of suitable varieties (with superior agro botanical and physiochemical traits) and lack of agronomic information have been reported to be the two of the major constraints for rice production in PNG (Sajjad 1995; Sajjad 1998).

Numerous efforts to evaluate introduced varieties suitable for PNG conditions have been accomplished in the past, but only a few of these have been published (Wohuinangu and Kapp 1982). The most predominant work was done by the Department of Agriculture and Livestock at Bubia Agricultural Research Centre, where a limited number of varieties imported from IRRI by the end of 1960, were evaluated across seven sites ranging from Dumpu in the Ramu Valley to Munum in the lower Markham valley. The results of the studies were non conclusive due to lack of information for optimum planting dates for the sites, soil conditions, erratic rainfall, Rice bugs (*Leptocorisa* spp.) and unsuitable varieties (Sumbak 1977).

Superior and modern varieties with ideal agro-botanical and physico-chemical traits and agronomic information are pre-requisites to developing domestic rice production (Ten 1974). Several promising varieties were identified and recommended for cultivation in PNG by various researchers in the past (Sajjad 1995; Sajjad 1996a; Sajjad 1996b; Sumbak 1977; Wohuinangu & Kapp 1982). These varieties, however, were recommended for release prior to investigating the stability of their performance across varying environments.

A study was conducted to evaluate adaptability of promising rice varieties under different environments in PNG. Based on the results of the first season of trials, four rice varieties were provisionally released for upland cultivation in areas ranging from 0-1200 meters altitudes. The four varieties NR 1, NR 9, NR 15 and NR 16 were selected on the basis of grain yield, eating quality and pest and disease resistance (Wamala 2003 and Waramboi *et al* 2003). Results of the second season of trials were discussed in Okpul *et al.* 2005. This paper reports on the third seasons of multi-location trials and compares performance of promising varieties across all three seasons of multi-location trials.

National Agricultural Research Institute, Sir Alkan Tololo Research Centre, P.O Box 1639, Lae 411, Morobe Province, Papua New Guinea

MATERIALS AND METHOD

Location and Methods

A series of multi-location trials was conducted between 2001 and 2005 at different sites in Madang and Morobe Provinces to evaluate the performance of upland rice varieties across different envioroments. Trial 1 was conducted at Intoap (Mutzing), Gusap (Ramu Sugar), Usino, Wareo (Finchafen), and Garaina while Trials 2 and 3 were conducted at Intoap, Gusap, Usino, Boana and Dagging. Table 1 shows the soil and agro-ecological features of all the sites used during the series of trials.

Soils at the trial sites were moderately fertile with acid to moderately acid soil pH, low organic matter and total nitrogen and phosphorous low but medium to high ratings for Mg and K at most sites. Data for Wareo are not available (Appendix Table A1).

The rice varieties were among 1250 accessions introduced from the International Rice Research Institute (IRRI) since 1960 (Sumbak 1977). From previous trials conducted between 1991 and 1996 initial eight upland rice varieties were selected for the season of multi-location trials. The number of varieties was increased to 10 varieties for seasons 2 and 3 (Table2).

***Table 1*. Experimental sites and their agro-ecological features**

Sites	Province	Average annual Rainfall (mm)	Seasonality of rainfall (mm)	Average Temperature (°C)	Average Humidity (%)	Soil texture	Altitude (m.a.s.l)
Intoap [1,2]*	Morobe	1500-2300	100 - 350	34	84	Sandy loam	200
Gusap[1,2]*	Madang	1500-2300	100 - 200	32	83	Clay loam	300
Saussi[1,2]*	Madang	3500-4000	200 - 350	33	92	Loamy	300
Boana[2]	Morobe	2000-2500	100 - 200	27	90	Silty clay loam	940
Dagging[2]	Morobe	4000-5000	200-350	29	94	Clay loam	600
Garina[1]	Morobe	1500-2700	100-250	30	85	Clay loam	<600
Wareo[1]	Morobe	2400-3700	200-300	29	94	Clay loam	>600

[1]Trial sites in season 1 (2001/2002).
[2]Trial sites in season 2 and 3 (2003/2004, 2004/2005)
*= Sites common for all three seasons

***Table 2*. Rice varieties evaluated at five different sites in Papua New Guinea**

NARI codes[3]	Source	Donor name/ No.	Origin	Plant height
NR 1 [1,2]*	IRRI[4]	IR 19961-23-2-2	Philippines	Short
NR 2[1]	IRRI	Ayung	Indonesia	Short
NR 4 [1,2]*	IRRI	BG 379-2	Sri Lanka	Short
NR 9 [1,2]*	NARI[5]	N6-94	PNG	Medium
NR 11[2]	IRRI	IR 841-1-1-2	Philippines	Medium
NR 13[2]	IRRI	IRAT 104	Ivory Coast	Short
NR 14[2]	IRRI	IRAT 170	Ivory Coast	Short
NR 15 [1,2]*	IRRI	Salumpikit	Philippines	Tall
NR 16 [1,2]*	IRRI	Azucena	Philippines	Tall
FB 91 [1,2]*	Unknown	Finsch Brown 91 (standard)	Finschhafen	Tall
TCS10 [1,2]*	TATM[6]	Taichung Sen 10 (standard)	Taiwan	Medium

[1]Varieties evaluated in trial 1
[2]Varieties evaluated in Trial 2 and 3
*Varieties evaluated in all 3 trials
[4]International Rice Research Institute; [5]National Agricultural Research Institute;
[6]Taiwanese Agricultural and Technical Mission

Trial design and maintenance

The trial at each site was planted using a randomized complete block design and replicated five times. Planting was done in December and harvested the following year following the normal rainy season (figure 1). About five seeds were sown per hole at a plant spacing of 20 x 20 cm between rows and plants. The plot size was 15 m ^{2}. At each site inorganic fertilizer was applied at the rate of 100: 50: 50 Kg-ha $^{-1}$ N, P and K. All P, K and 40 % of N were applied at the time of planting. The two top dressings were applied with N (30 % each) in the form of urea (46 % N) at 25 and 45 days after the planting date. Hand weeding was done twice prior to fertilizer application. The series of trials was harvested at four months after planting. TC Seng 10 (TCS 10) and Finch Brown (FB 91) were used as control varieties.

Data collection and Statistical Analysis

Data were collected from 25 randomly selected hills from each plot representing 1m^{2} after removing the guard rows. Various plant growth parameters were measured including yield, plant height, panicle length, % spikelet fertility and Thousand Kernel Weight (TKW). Grain yield per plot was measured at 14 % seed moisture content in g and converted to t/ha. Incidence of pest and diseases on different varieties was recorded at each site and assessed using the Standard Evaluation System provided by IRRI (IRRI, 2002a).

First, the results of the multi-location trials of the third season were considered. Data of all sites were then pooled and ANOVA was used to quantify sources of variation in the data set. The second part of the analysis considered a data set of common sites and varieties across all three seasons. ANOVA was applied to compare mean yield data of seven varieties at three sites across three seasons.

ANOVA was performed using software Genstat® version 3.2 for each site and means were separated using Least Significant Difference (LSD) and pooled for the combined results across the five sites.

RESULTS

Third season (2004-2005).

The results for mean grain yield (t/ha) of the ten rice varieties tested in five locations in the third season of multi-location trials are presented in Table 3. A summary of results for the other parameters measured is presented in Table A2 in the Appendix. There were significant differences ($P<0.01$) between varieties as well as between sites. There was also a significant Site x Variety interaction (Table 4). The mean yield over the ten varieties ranged from 3.62 t/ha (FB 91) to 6.31 t/ha (NR 9) (Table 3). Average yield of the ten varieties was 5.48 t/ha. All NARI varieties performed significantly better compared to TCS 10 and FB 91. There were significant differences between NR varieties in the mean yield data across all sites (Table 3).

Table 3. **Mean grain yield (t/ha) of ten rice varieties tested in five locations in Papua New Guinea for the third season (2004-2005)**

Variety	Boana	Dagging[1]	Intoap	Gusap	Sausi	Mean
NR 9	7.07^{c2}	7.58^{cd}	8.84^{c}	3.26^{c}	4.81^{ab}	**6.31^{e}**
NR 14	7.17^{c}	7.64^{cd}	7.85^{c}	1.89^{a}	6.10^{b}	**6.14^{de}**
NR 16	7.44^{c}	6.48^{bc}	7.32^{bc}	3.06^{c}	6.31^{b}	**6.12^{cde}**
NR 4	7.19^{c}	6.75^{bcd}	N/A	3.12^{c}	5.71^{b}	**5.93^{cd}**
NR 15	4.17^{ab}	8.25^{d}	7.66^{c}	3.5^{c}	5.37^{b}	**5.79^{cd}**
NR 1	6.90^{c}	6.55^{bcd}	7.2^{bc}	3.45^{c}	5.59^{b}	**5.94^{cd}**
NR 13	7.07^{c}	6.48^{bc}	4.95^{ab}	3.08^{c}	5.41^{b}	**5.40^{c}**
NR 11	5.99^{bc}	6.91^{bcd}	4.93^{a}	2.81^{bc}	4.99^{b}	**5.13^{c}**
TCS 10	6.71^{c}	5.50^{b}	N/A	3.14^{c}	3.30^{a}	**4.92^{b}**
FB 91	3.72^{a}	3.52^{a}	3.21^{a}	2.09^{ab}	5.56^{b}	**3.62^{a}**
Mean	**6.32**	**6.57**	**6.50**	**2.94**	**5.32**	**5.49**
% CV	**23.9**	**20.7**	**23.7**	**22.3**	**24.8**	

[1]The local standard variety at Dagging was Finch Brown while at other sites TCS 10 was used.
[2]Numbers followed by the same letter are not significantly different at $p<0.05$ using Least Significant Differences

Table 4. **Pooled analysis of variance of grain yield (t/ha) of the ten rice varieties tested at the five sites in Papua New Guinea.**

Source of Variation	Degrees of Freedom	MS
Variety (V)	9	15.85**
Site (S)	4	114.19**
S x V	34	5.50**
Residual	172	1.97

** = Significant at the 1% probability level.

Among sites, all varieties at Gusap performed significantly lower than other sites. In individual sites ranking of varieties according to yields was different causing the significant (SxV) interaction, but among the NARI varieties only NR 15 was significantly different to other NARI varieties in Boana and Dagging (lowest and highest) and NR 14 at Gusap. In almost all sites FB 91 (local landrace) had the lowest yield except at Sausi where TC Seng 10 had the lowest yield. At Intoap the variety NR 04 could not be harvested due to heavy lodging and spikelets of TCS 10 had 100% sterility as a result of drought conditions during the reproductive phase. This variety had to be replanted twice as a result of poor germination rates.

Incidences of pests and diseases at trial sites were variable across sites (data not shown). Intoap was free of pests and diseases while Usino showed the highest incidence of pests. Most varieties were affected by the rice bug (*Leptocorisa oratorius*) with more severe infestation on NR 01, 11 and 09, stem borer (*Chilo sp.*) and leaffolder (*Cnaphalocrocis medianalis*). Dagging had the highest incidence of diseases among sites where brown spot (*Bipolaris oryzae*) and sheath blight (*Rhizoctonia solani*) were observed on a number of varieties, in particular NR 01, 04 and 09. Brown plant hopper *(Nilaparvata lugens)* and false smut (*Ustilaginoidea virens*) occurred in low incidence on a few varieties at different sites.

Comparison of results across three seasons

In order to establish how the rice varieties performed across different seasons the yield results of all common sites and varieties across the three seasons were pooled and subjected to statistical analysis. Comparisons across seasons showed that all five NARI varieties performed significantly better compared to the control varieties TCS 10 and FB 91. NR 15 performed consistently well across seasons. The yields for 2003/2004 seasons were the lowest for most varieties (Table 5).

Table 6 shows the mean data across three seasons for other plant growth parameters measured during the series of multi-location trials. Most parameter show significant differences between varieties except for Spikelet fertility (SF). SF ranged between 72.5% in TCS 10 to 83.5% in NR16. NR01 and NR 04 are the shortest varieties while FB 91 grows tall with 151.9 cm. There was little variation among varieties for the Number of Productive Tillers and Panicle Length. FB 91 produced significantly less number of tillers but had the longest panicle among varieties. It also produced the highest Thousand Kernel weight

Table 5. **Mean yield (t/ha) of seven rice varieties evaluated at three sites across three seasons in the Lowlands of Morobe and Madang Provinces from 2001-2005.**

Variety	Mean yield (t/ha) 2001-2002*	Mean yield (t/ha) 2003-2004*	Mean yield (t/ha) 2004-2005*	Mean*	Ranking
NR 15	6.29^{e}	4.27^{ab}	5.52^{c}	5.36^{d}	*1*
NR 1	5.53^{d}	4.45^{b}	5.43^{c}	5.14^{cd}	*2*
NR 9	5.29^{cd}	3.92^{ab}	5.63^{c}	4.95^{c}	*3*
NR 16	5.05^{bcd}	4.13^{ab}	5.56^{c}	4.91^{c}	*4*
NR 4	4.74^{bc}	4.67^{b}	5.25^{c}	4.89^{c}	*5*
TCS-10	4.50^{ab}	3.73^{ab}	4.2^{b}	4.14^{b}	*6*
FB 91	3.97^{a}	3.03^{a}	3.62^{a}	3.54^{a}	*7*
Mean	**5.05**	**4.03**	**5.03**	**4.70**	

*Numbers followed by the same letters are not significantly different at p<0.05 using Least Significant Differences.

followed by NR 15 and NR 16. The number of Days to Maturity was only available from the 2001/2002 seasons. NR 15 reached maturity already after on average 104 days while FB 91 had the longest growth period of 145 days.

DISCUSSION

The last series of multi location trials and the comparisons of yield performance of rice varieties have confirmed that the varieties released provisionally by NARI in 2003 are superior in regards to yield performance compared to the widely distributed variety TCS10 and the local landrace FB 91. Additionally variety NR 4 has emerged as another variety that can be considered for release to farmers.

Consistent with trials in previous seasons (Wamala, 2003; Okpul *et al.* 2005) all NARI varieties produced significantly higher yields compared to the control varieties TCS 10 and FB 91 in 2004/2005. This is confirmed by the mean yield results of the varieties tested in all three seasons (Table 5). Discrepancies in yield potentials of varieties at different locations and in different seasons may be due to genotype x environment interaction caused for example by different times of planting, rainfall or soil types (Sajjad 1995).

The variety FB 91 was the lowest yielding variety throughout the three seasons. The origin of this variety is not known but it is considered a landrace as it has been commonly grown in Finschhafen for a long time. There are three major types of rice recognized worldwide. They are the Indica, Javanica and Japonica types, each differing in morpho-ecological characteristics. FB 91 shows typical characteristics of a Javanica type rice with low tillering rate, long panicles, tall plant stature and high Thousand Kernel Weights (Table 6) (IRRI 2002b). Sumbak (1977) reported that Javanica type varieties were tall and susceptible to lodging which results in lower yields. Sajjad (1995) also reported a similar trend when comparing FB 91 against Niupela and IAC 165, two Indica types, where the yield of FB 91 was lower.

Among the trial sites, the site at Gusap produced the lowest yields in the 2003/2004 and 2004/2005 season, while the yields at other sites were not much different to each other (Okpul *et al.* 2005, Table 3). Soil characteristics at Gusap are fairly similar to other sites (Table 1) with medium soil pH, medium to high content of extractable bases, cation exchange capacity, low organic matter and available P and N. Low organic matter was also reported in a study by Harteminck (2000) on land management in Ramu Sugar Plantations. Low rainfall at grain filling stage may have affected the yield in the 2003/2004 season at Gusap as reported by (Nass-Komolong 2005) (Figure 1). More rainfall was experienced in the 2004/2005 seasons during the reproductive phase at Gusap (Figure 1). However, as there are no weather data available for other sites it is not possible to draw further conclusions regarding the influence of rainfall on yield performance. Basic weather data should be recorded at all trial sites in future trials.

Pests and diseases are other important factors that may influence yield performance of rice varieties. This has not been studied in detail for the varieties in question. During the trials incidence of pests

Table 6. **Mean data for six-plant growth parameters of seven rice varieties evaluated in three locations in Morobe and Madang Provinces from 2001-2005**

Variety	PH[1] (cm)	No. of PT[2]	PL (cm)[3]	SF (%)[4]	TKW[5] (g)	D to M[6,7]
FB 91	152[d,8]	8.7[a]	29.9[c]	80.6[9]	34.3[c]	145
NR 01	83[a]	18.3[d]	22.5[ab]	77.3	25.0[a]	132
NR 04	82[a]	15.4[cd]	22.1[ab]	76.2	23.1[a]	134
NR 09	105[bc]	11.8[ab]	21.8[a]	74.4	23.5[a]	135
NR 15	113[c]	14.2[bc]	22.2[ab]	75.0	29.6[b]	104
NR 16	111[c]	12.9[bc]	22.6[ab]	83.5	28.0[b]	113
TCS 10	94[ab]	14.2[bc]	23.9[b]	72.5	25.1[a]	133

[1]PH – Plant Height; [2]PT – Number of Productive Tillers; [3]PL – Panicle Length; [4]SF – Spikelet Fertility; [5]TKW – Thousand Kernel Weight; [6]D to M – Days to Maturity

[7]Days to Maturity data are only available from the first season in 2001/2002

[8]Numbers followed by the same letter are not significantly different at $p<0.05$ using Least Significant Differences

[9]not significant at $p<0.05$

and diseases was recorded but infestation levels varied from site to site and from season to season. Infestation with the Rice bug (*Leptocorisa oratorius*) affected a number of varieties such as NR 01, NR 09 and NR 11, which may explain their relatively lower yield at Gusap compared to other sites in the 2004/2005 seasons.

Stability performance established by Okpul *et al* 2005 found that NR 11, NR 13 and NR 14 were responsive to favorable environments. This was confirmed in 2004/2005 when NR 14 was among the highest in fertile sites (Boana) but lower in poor site (Ramu). These varieties could be used by semi commercial farmers who can afford to produce rice using higher inputs such as the use of fertilizer.

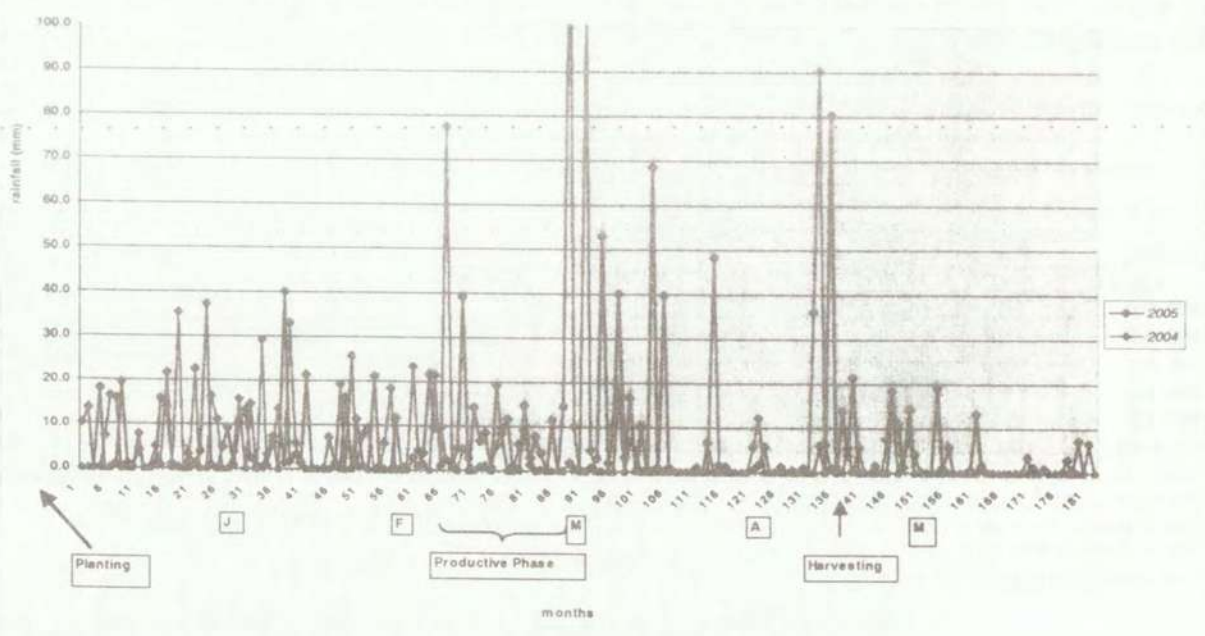
Comparison of rice varieties across three seasons showed the superior performance of NR varieties compared to control varieties FB 91 and TCS 10 in regards to yield. In the 1990s the Department of Agriculture and Livestock (DAL) screened about 1,149 exotic genotypes introduced from IRRI as part of a Rice and Grain Research and Development Project among them the varieties NR 13 (IRAT 104), NR 14 (IRAT 13), NR 15 (Salumpikit) and NR 16 (Azucena) (Sajjad 1994a, Sajjad 1994b, Sajjad 1995). Varieties tested in those trials showed generally lower yields. However, Sajjad (1995) reported that the trials were grown under minimum input conditions with no or only one basal fertilizer application and reduced weeding. The varieties NR 15 and NR 16 were either considered for release or for advanced elite selection (Sajjad 1994a; Sajjad 1994b).

Eating quality, milling and physico-chemical characteristics are important factors to be considered before variety release. Sumbak (1977) stated that a lot of varieties tested in the past with high yields were superseded or discarded because of inferior cooking qualities. Waramboi *et al.* (2003) studied the eating quality of the rice varieties used in the first season of multi-location trials. Results showed that among NR varieties, NR 01, NR 16 and NR 09 were preferred over NR 15 and NR 4.

Resistance to major pests and diseases of varieties evaluated in this series of multi-location trials is another important consideration for variety release. However, little is known about levels of resistance among these rice varieties. Previous data are not available and during the multi-location trials varieties were not sufficiently exposed to various pest and diseases for any conclusions on varietal resistance. A small study on resistance of NR 01, 04, 09, 15, 16 and TCS 10 against the Brown Plant Hopper is in the planning stage at the NARI Wet Lowlands Mainland Programme.

In conclusion, the four varieties that were released on a provisional basis in 2003 have consistently produced higher yields compared to the control varieties TCS 10 and FB 91 across different environments. The varieties show a good mixture

Figure 1 Rainfall (mm) in Ramu Sugar during the month of January to May 2004 and 2005



of different maturity times with two early maturing varieties (NR 15 and 16) and varieties with variable plant heights. NR 01 and 09 are short varieties that may give advantages in areas with higher risks of lodging. Consumer preference studies (Waramboi *et al*. 2003) showed that the varieties are well liked by consumers. Based on this information they should be formally released. NR 04 is a short variety similar to NR 01 and 09 in its maturity time and other features. It also produces superior yields but is not as well liked by consumers. This variety may be considered for release if it shows to be superior in other traits such as resistance to pest and diseases. This work is still in progress at the National Agricultural Research Institute.

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APPENDIX

The Dagging and Gusap sites were tested in (2002-2003) season while the Boana, Intoap and Sausi site sample were tested for the (2004-2005) season.

Table 1. Soil Analysis Results

Location	pH	Extractable Bases				CEC	Olsen P	Organic	Total	Particle size %		
		Ca	Mg (me %)	K	Na	(me%)	(mg/kg)	C (%)	N (%)	sand	silt	clay
Boana	4.3*	13.9	0.9	0.05	0.09	29.7	4.3	7.6	0.4	29	27	44
Dagging	5.8	16.5	7.21	0.21	0.15	34.8	5.9	2.73	0.29	7	39	54
Intoap	6.3	17.9	9.88	0.73	0.35	18.7	25.4	1.52	0.14	36	39	25
Gusap	6.0	15.5	5.24	0.87	0.14	23	20	2.54	0.2	21	30	49
Sausi	5.4	12	9.7	0.14	0.05	44.2	1.6	2.38	0.2	31	29	40
Critical values	<5.5	<5.0	<1.0	<0.3	>0.7	<6.0	<5.0	<3.0	<0.3			

* Values lower than the others.
Soil samples were analyzed by National Agricultural Chemistry Laboratory (KilaKila) Using methods NACL-Standard Methods

Table 2. **Means of various growth parameters of ten varieties across five sites in 2004/2005 season**

Variety	PH[1] (cm)	No. of PT[2]	SF (%)[3]	FLA (cm^2)[4]	TKW[5]
NR 1	86.5	21.06	69.47	33.3	N/a[6]
NR 4	84.1	17.08	74.15	32.5	N/a
NR 9	121.8	13.44	76.94	69.2	N/a
NR 11	76.2	16.14	78.69	39.6	N/a
NR 13	129.0	9.00	77.67	83.1	N/a
NR 14	65.56	18.06	72.51	36.6	N/a
NR 15	122.3	16.52	75.76	51.5	N/a
NR 16	117.3	12.82	85.10	55.7	N/a
TCS 10	98.6	15.10	65.88	45.4	N/a
FB 91	167.9	8.74	83.79	83.1	N/a

[1] PH – Plant Height; [2]PT – Number of Productive Tillers; [3]SF – Spikelet Fertility;
[4] FLA – Flag leaf area; [5]TKW – Thousand Kernel Weight; [6]not available – data not collected in 2004/2005 season

PREVALENCE OF ANTIBODIES TO LEPTOSPIRAL SEROVARS IN FARM RUMINANT ANIMALS IN THE MARKHAM VALLEY, PAPUA NEW GUINEA

Andy K. Yombo[1], M. A. Samad[2] and S. Reid[3]

ABSTRACT

A study on the prevalence of antibodies to leptospiral serovars in adult farm animals was carried out in vaccinated (n = 47) and non-vaccinated (n = 47) cattle, and non-vaccinated sheep (n = 21) and goats (n = 39) in the Markham valley of Papua New Guinea (PNG) during the period between June and July 2006. Sera were separated from all the randomly selected animals and subjected to the Microscopic Agglutination Test (MAT) against a reference panel of 22 live leptospira serovars. A titer of (³1:400) was considered as positive and accordingly 12.76% vaccinated as well as 17.02% non-vaccinated cattle had positive titer to leptospira infection. This indicates leptospirosis is prevalent in PNG and vaccination has limited role in immune response. This study also confirms hardjo, tarassovi and topaz as the predominantly occurring leptospiral serovars in ruminant food animal populations with topaz as a serovar never previously recorded in PNG. The seroresults of the tested sheep and goats showed none had positive titer (³ 1: 400) to leptospirosis. This indicates small ruminants currently may not be important host of leptospirosis in PNG. It may be concluded from the results of this study that leptospirosis is an important disease in ruminant farm animal herds in the Markham valley of PNG which needs attention for further study on it's zoonotic aspects and control in human and animal populations.

Keywords: *Ruminant, leptospirosis, serovars, Microscopic Agglutination Test (MAT)*

INTRODUCTION

Leptospirosis is a worldwide bacterial zoonotic disease, caused by Spirochetes of the genus *Leptospira* that affects humans and a wide range of animals, including mammals, birds and reptiles. All the pathogenic leptospiras were formerly classified as members of the species *Leptospira interrogans*, however the genus has recently being recognized and pathogenic leptospiras are now identified in several species of leptospira (Yasuda *et al.* 1987; Ramadass *et al.* 1992). Internationally there are more than 200 distinct leptospiral serovars recognized within the seven species of pathogenic leptospira (Marshall and Manktelow, 2002) and these are arranged in 23 sero-groups (Veloso *et al.* 2000). Serovars are identified based on antigens on the surface of the organism (Bolin 2003).

A recent study in Trukai farm has confirmed the occurrence of 15 leptospiral serovars and established that hardjo as the dominant serovar maintained in beef herds in Papua New Guinea (PNG) (Wai'in *et al.* 2003). Sub-optimal fertility is an ongoing problem in beef herds and the role of leptospirosis in sub-fertility is not clear in PNG. Although Trukai uses a bivalent vaccine regularly containing the serovars hardjo and pomona as antigens, the problems often persist, which might be due to either leptospirosis or other factors. Diagnosis of leptospirosis can be broadly divided into those that detect bacteria, their antigens or genomic material and those that detect antibody of the infecting bacteria. A variety of serological techniques are available for diagnosis of leptospirosis like Microscopic agglutination test (MAT), Enzyme linked immunosorbent assay (ELISA) and the fluorescent antibody test (FA). Among the DNA based assays the polymerase chain reaction (PCR) test is used to detect leptospiral DNA from tissues or body fluids in clinical cases. In general the PCR test has specificity and reliability but can not determine the infecting serovar and the process can be exquisitely sensitive to contamination with exogenous leptospiral DNA and therefore maybe prone to false-positive reactions. The MAT therefore; remains the only serological assay

[1] Ex-PGD Student, Department of Agriculture, University of Technology, Lae 411 Morobe Province PNG
Present Address: Numundo Plantation P.O Box 26 Kimbe WNBP PNG email: **kimbevet@online.net.pg**
[2] Professor, Department of Veterinary Medicine Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh.
[3] Senior Research Fellow, School of Veterinary and Biomedical Sciences, Murdoch University, WA 6150 Australia

widely accepted as capable of producing serovar-specific results (Smith *et al.* 1994; Cumberland *et al.* 1999; O'Keefe 2002). This paper describes the sero status of leptospiral serovars in vaccinated and non-vaccinated beef cattle and non-vaccinated small ruminant farm animals in PNG.

MATERIALS AND METHODS

Cattle (cows) destined for the slaughter house from Trukai farm (vaccinated against leptospirosis with serovar hardjo and pomona) and Markham farm (non-vaccinated) were randomly selected for this study. Forty seven cows from each farm were selected for blood collection. The sheep (n=21) and goats (n=39) were also randomly selected from the Department of Agriculture and Livestock demonstration flock at Erap for this study. All the selected small ruminant animals were adults and sex was not considered. Blood samples of at least 10mls in sheep and goats were collected from the jugular vein with vacutainers and a similar volume of blood was collected from cows slaughtered at the abattoir. Sera were separated from blood clots by centrifugation as per conventional method and stored in sterile vials at –20 °C until shifted to the testing laboratory. The samples were then packed and sent to the WHO / FAO / OIE Leptospirosis Reference Laboratory in Brisbane for the microscopic agglutination test (MAT). Each serum sample was tested for antibodies to 22 live leptospira serovars as described by Stallman (1984) with some modifications. The serovars included: (1) Pomona, (2) Tarassovi, (3) Celledoni, (4) Australis, (5) Robinsoni, (6) Kremastos, (7) Medanensis, (8) Cynopteri, (9) Bataviae, (10) Javanica, (11) Shermani, (12) Hardjo, (13) Grippotyphosa, (14) Copenhageni,(15) Zanoni,(16) Canicola, (17) Szwajizak, (18) Bulgarica, (19) Aborea, (20) Djasiman, (21) Panama and (22) Topaz.

MODIFIED TEST PROCEDURE

Doubling dilutions of the sera from 1: 50 to 1: 6400 were prepared in 96 well microtiter plates using phosphate buffered saline (PBS) pH 7.4. An equal volume of live leptospira culture containing approximately 2-4 x 10^8 cells / ml was added to each dilution. After 90 minutes at 30 °C, the trays were examined by dark filled microscopy for agglutination of leptospira cells. The degree of agglutination was assessed in terms of the proportion of free leptospires. The reported titer was the reciprocal of the highest dilution of serum that agglutinated at least 50% of cells for each serovar used; as compared with the control culture diluted at 1: 2 PBS. A single titer of ^{3} 1: 400 was accepted as the cut off value for this study and was considered as positive. The results were analyzed in terms of percentage, and the difference in leptospira infection between vaccinated and non-vaccinated cattle and small ruminants was analyzed.

RESULTS AND DISCUSSION

The present study on the comparative leptospiral antibody prevalence in vaccinated and non-vaccinated cattle and small ruminants was carried out for the first time in PNG. Wai'in *et al.* (2003) reported the prevalence of leptospiral serovars in only non-vaccinated cattle from PNG. The overall prevalence of leptospirosis in ruminant farm animals is presented in Table 1.

It appears that there is no significant difference on the overall sero-prevalence of leptospira infection between Trukai and Markham farms. Of the cattle tested 25.53 % of cows from Trukai and 27.66% from Markham farm did not demonstrated any antibodies. There was however; a slightly high number of suspect (1:50-1:200) animals on Trukai

Table 1. **Seroprevalence of leptospirosis in adult farm ruminant animals**

MAT titer	Interpretation	Sero-results in different farms, No. (%)			
		Trukai farm	Markham farm	DAL Erap Farm	
		Cattle (n = 47)*	Cattle (n = 47)	**Sheep (n = 21)	**Goats (n = 39)**
< 1: 50	Negative	12 (25.53)	13 (27.66)	12 (57.14)	35 (89.74)
1:50-1:200	Suspect	29 (61.70)	26 (55.32)	09 (42.86)	04 (10.26)
? 1: 400	Positive	06 (12.76)	08 (17.02)	0	0

farm (61.70%) compared to Markham farm (55.53%). The elevated titers from Trukai may be the result of the vaccination program under taken in the farm and a valid analysis was not possible in this study. However, the Markham farm had a high number of positive (e"1:400) animals (17.02%) compared to Trukai farm (12.76%). In general high titers are suggestive of recent clinical disease and this work indicates Markham farm may have problems. Between the small ruminants, goats (89.74%) had negative titers for leptospira antibodies compared to sheep (57.14%). However, leptospira is widespread in sheep and goats with suspect antibody titers of (42.86%) and (10.26%) respectively. There is no evidence to suggest sheep and goats are reservoir hosts for hardjo and tarassovi, the main serovars responsible for clinical leptospirosis in cattle.

The results on the prevalence of antibodies to leptospiral serovars in vaccinated and non-vaccinated cattle is shown in Table 2. It appears that the PNG cattle had leptospira antibody titer at different levels against 12 serovars. However, the positive level of antibodies was only found against hardjo (8.51%), and tarassovi (2.13%) and topaz (2.13%) serovars respectively in vaccinated cattle, whereas non-vaccinated cattle had positive titer only against hardjo (14.89%) and tarassovi (2.13%). The dominance of hardjo, szwajizak and tarassovi in PNG cattle has been reported by Wai'in *et al.* (2003). Of the dominant serovars, L. interrogans topaz has not been screened before.

It is possible for cross reactions to occur among serovars with similar antigenic component at higher titers but this study was not able to determine cross reactivity. A positive result indicates exposure of animals in the herd to infection, but there are no published data available at present, which allow correlation of antibody level with the probability of active versus chronic infection. Nevertheless the results presented here confirm that exposure to infection is widespread in the unvaccinated cattle herds in PNG cattle. The Trukai cattle were vaccinated against hardjo and pomona serovars but the antibody detected only against hardjo, not against pomona serovar. This may be due to several factors including negligible immune response and the varying effect on the host with the inoculated antigen which occurs due to relatively low initial development of agglutinating antibody titers. The low immune response however does not mean the absence of the antibodies but the serological test employed merely could not detect the pomona antibodies. Although, a high cut off titer (e"1:400) was used for this study and Trukai vaccinated against leptospirosis, antibodies to prominently occurring serovars in the two cattle farms are essentially the same.

Table 2. **Prevalence of Leptospira serovars in vaccinated (n = 47) and non-vaccinated (n = 47) beef cattle detected by MAT**

Serovars	MAT titer with No. of cases												Total positives (≥ 1: 400) No. (%)	
	1: 50		1: 100		1: 200		1: 400		1: 800		1: 1600			
	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV
Hardjo	9	3	8	7	2	7	3	5	1	2	-	-	04 (8.51)	7 (14.89)
Szwajizak	9	13	6	10	5	-	-	-	-	-	-	-	0	0
Arborea	4	2	1	-	-	-	-	-	-	-	-	-	0	0
Topaz	7	8	8	4	3	-	-	1	-	-	-	-	1 (2.13)	0
Kremastos	1	6	5	3	-	-	-	-	-	-	-	-	0	0
Tarassovi	8	5	3	6	-	2	-	1	-	-	1	-	1 (2.13)	1 (2.13)
Medanensis	7	5	2	3	3	2	-	-	-	-	-	-	0	0
Pomona	4	-	-	2	-	-	-	-	-	-	-	-	0	0
Bataviae	2	1	1	-	-	-	-	-	-	-	-	-	0	0
Australis	-	1	1	-	-	-	-	-	-	-	-	-	0	0
Shermani	2	-	-	-	-	-	-	-	-	-	-	-	0	0
Panama	-	-	-	2	-	-	-	-	-	-	-	-	0	0
Total													6 (12.76)	8 (17.02)

V = Vaccinated with serovars hardjo and pomona, NV = Non-vaccinated, - = Negative

The prevalence of antibodies to leptospiral serovars in small ruminant animal species is presented in Table 3. Although none of the sheep and goats showed positive level of antibody titer (≥1: 400) against all tested serovars, the suspected level of antibodies (1: 50 - 1: 200) was detected against arborea and topaz serovars. The result shows that three (14.29%) and six (28.57%) sheep had antibodies to arborea and topaz, respectively, whereas only topaz serovar was recorded in four (10.26%) goats (Table 3).

Although more works needs to be done topaz seemed to be a dominant serovar maintained in ruminant farm animals in PNG.

This result could not be compared due to lack of similar inland reports on leptospirosis in small ruminants from PNG. Elsewhere however, Batra *et al.* (1990); observed pomona as a dominant serovar in Haryana. Ciceroni *et al.* (2000) documented catellonis as the highest serovar in South Tyrol and in an earlier study in South–East Bolivia Ciceroni (1992) reported poi as the dominant serovar in sheep and goats. A recent review by Levett (2001) observed pomona and hardjo as dominant serovars in sheep.

This variation in dominance might be due to the varying epidemiological andmaintenance-host relationship; characteristic of the agro ecological systems in different parts of the world. Data on clinical leptospirosis in food animals and its significance on productivity is scarce in PNG. However, this work confirms earlier studies by Wai'in *et al.* (2003) that leptospirosis is prevalent in PNG beef cattle herds. There are 15 (Wai'in, *et al.* 2003) leptospira serovars known to be prevalent in cattle. In addition this work confirms L. interrogans topaz as a new serovar never previously recorded in PNG. The results also indicate small ruminants may not be important hosts of hardjo and tarassovi the main serovars responsible for clinical leptospirosis in PNG. There is a significantly high proportion of cattle with active infection in Markham farm compared to Trukai farm.

Further work is required to determine the source and extend of leptospira infection in animal and human populations and to determine the role of vaccine in the control of leptospirosis in experimental and field conditions in PNG.

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Table 3. **Leptospira serovar status in small ruminants**

Serovars (n = 22)	Animals species	No. of animals tested	No. of animals with antibody titer		
			1: 50	1: 100	Total , No. (%)
Arborea	Sheep	21	2	1	3 (14.29)
	Goats	39	0	0	0
Topaz	Sheep	21	5	1	6 (28.57)
	Goats	39	4	0	4 (10.26)

n= No. of Serovars tested

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YIELD OF FOUR VARIETIES OF RICE (*Oryza Sativa* L.) IN TWO SOIL TYPES AND CONTRASTING AGROECOLOGICAL ZONES

Dr. Muhammad Muneer

ABSTRACT

Field experiments were conducted to evaluate soil properties and the yield performance of rice in two agro-ecological zones of Morobe province, Papua New Guinea. Study show that with the current practice of low input and subsistence farming of the province, soil nutrients was not a major impediment for rice cultivation. However, yield had been affected by the moisture supply as a result of different mechanical and physical properties of soils in these two agro-ecological zones. The average grain yield of 2.8- 4.7 t/ha suggests that both regions have the potential to grow rice and is capable to meet the current demand of the province.

Keywords: *Rice, soil types, agro-ecological, zones, Markham valley.*

INTRODUCTION

Morobe province is one of the twenty provinces of Papua New Guinea (PNG) that occupies 33,525 km^2 in the north of the country (Fig. 1). Population of the province is 3, 07, 000 where banana (*Musa* cultivars), sweet potato (*Ipomoea batatas*), and taro (*Colocassia*) are the major staples. While these are the most important crops consumed, rice has quickly become popular for many people, both in the rural and urban areas of the province. Despite the demand, very little rice is produced locally and almost all the rice consumed is imported from overseas, resulting in the spending of large amounts of foreign exchange by the government (Manning 1998). Therefore, government of PNG and many non-governmental organizations are promoting domestic production of rice in order to become self reliant and to ensure food security for the households. This has achieved limited success and has never exceeded 2% of the country's requirements (FAO 2000).

In the province two kinds of cropping systems are practiced, namely the cash crops like coffee (*Coffea spp.*), cocoa (*Theobroma cacao*), coconut palm (*Cocos nuerfera*), and the subsistence agriculture which produce staple crops. Almost all of the 25% of the arable land of the province is being used to produce both the cash and traditional food crops where yield is depended on the inherent soil fertility and available moisture supply (Hanson *et al.* 2001). With this system of agriculture, however, yield is low. Therefore, to increase yield, since 1950, much of the research carried out was focused on the land use and nutrient status of cash crops (Baseden 1960; van Wijk 1959; Fahmy 1977). Significant research on nutrient deficiencies in traditional food crops started only in the 1970s (Hartemink and Bourke 2000; Hartemink *et al.* 2000). As rice was not considered a major staple it received little or no attention and is cultivated only in a few isolated locations.

It is well known that crop growth and yield would largely be determined by the climate, biological, and edaphic factors of the region. This study investigated soil properties, particularly soil chemical properties on the yield performance of four rice cultivars under low input conditions in two agro-ecological zones in Morobe province. Soil properties have been used to provide an overall assessment of soil fertility, noting any apparent deficiency of nutrient elements that might affect crop growth and yield. In evaluating rice yield, rainfall which is one of the dominant characterists of the region also was taken into consideration. It is hoped that findings of this investigation would entail the land manager's, particularly smallholder farmers, to understand the soil's potential and limitations, and the role of climate on rice yield.

MATERIALS AND METHODS

Climate of the experimental sites

Two sites were selected for the study where agriculture is dominated by the traditional food crops. One is at the University of Technology (Unitech) experimental farm (6°39¢ S, 147°00¢E and

Department of Agriculture, University of Technology, Lae, Papua New Guinea.

65 m.a.s.l), Lae the capital of Morobe province and the other in Trukai experimental farm at Markham valley (6°31¢ S, 146°29¢ E and 112 m.a.s.l.) which is about 60 km North West of Lae (Fig. 1). Unitech represents wet coastal areas and Markham valley represents the drier interior valley of the province.

The climate of both experimental sites is tropical humid and classified broadly as Koeppen Af (Ford 1974) i.e. rainy tropical climate. It has two main rainy seasons with short but important transition periods between them. The north-west season, influenced by the perturbation belt and occurring from December to late March. The south-east season, which occurs from May to October, when south-east trade winds dominate the weather.

Unitech receive rainfall in south-east season and have the greatest incidence of long wet spells unlike other wet places in the province. Whereas, Markham valley receives most of its rainfall during north-west season. The annual rainfall at Unitech and at Markham valley is about 3452 mm and 1139 mm respectively (five year average) (Figs. 2 and 3). Annual evaporation (US Class A pan) is 2139 mm at Unitech and 2200 mm at Markham valley. At Unitech rainfall exceeds evaporation whereas at Markham valley evaporation exceeds rainfall. Temperature at both experimental sites are constantly high, with mean maximum readings of 28-34 °C and mean minimum readings of 20-25 °C. Temperature range throughout the year is usually small, 1-4 °C for the mean and 3-9 °C for the extreme of monthly maxima and minima. Daily range is usually at least double that of the average monthly range (Bleeker 1983a).

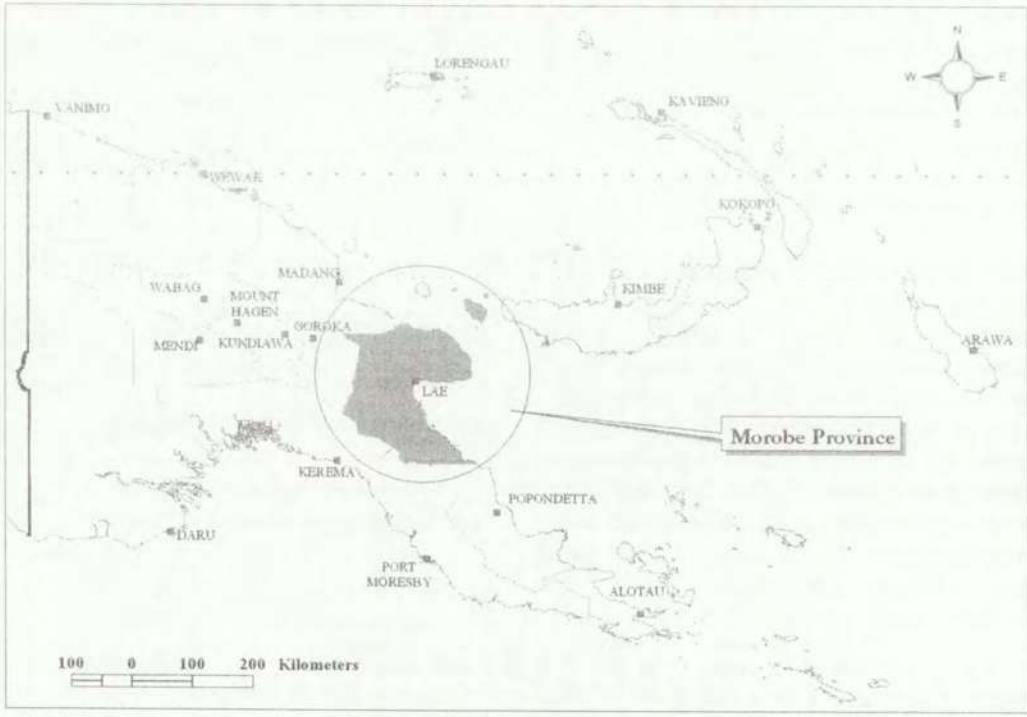
Soil and land use of the experimental sites

Soil at Unitech is derived from alluvial deposits whereas at Markham valley it is derived from colluvial and alluvial deposits (Bleeker 1983b). According to USDA Soil Taxonomy (1999) and FAO/UNESCO (1988) Unitech soil is classified as *Typic Tropofluvents* and *Eutric Fluvisol* whereas Markham valley soil is classified as *Typic Troporthents* and *Haplic Phaeozems* respectively. Soil at Unitech is well drained and has moderate slope 10-20°, whereas, soil at Markham valley is moderately to poorly drain and is steeply sloping > 20°. The natural vegetation at Unitech farm is mainly savanna grassland (*Imperata cylindrica*) with scattered shrubs while Markham valley has predominantly grassland vegetation (*Themeda austrailis*) with scattered trees. Taro is the dominant crop grown at the farm, whereas, banana and sweet potatoes are common at Markham valley.

Field Establishment and Maintenance

A total of four experiments were conducted over 3 year period having two at each location. At Unitech, experiments were conducted during May to October in 2001 and 2002 whereas at Markham valley during December to May in 2002 and 2003. Land was kept fallow between the trials. Four rice cultivars were used. These are Taichung Sen-10

Figure1. **Location of Morobe Province in Papua New Guinea**



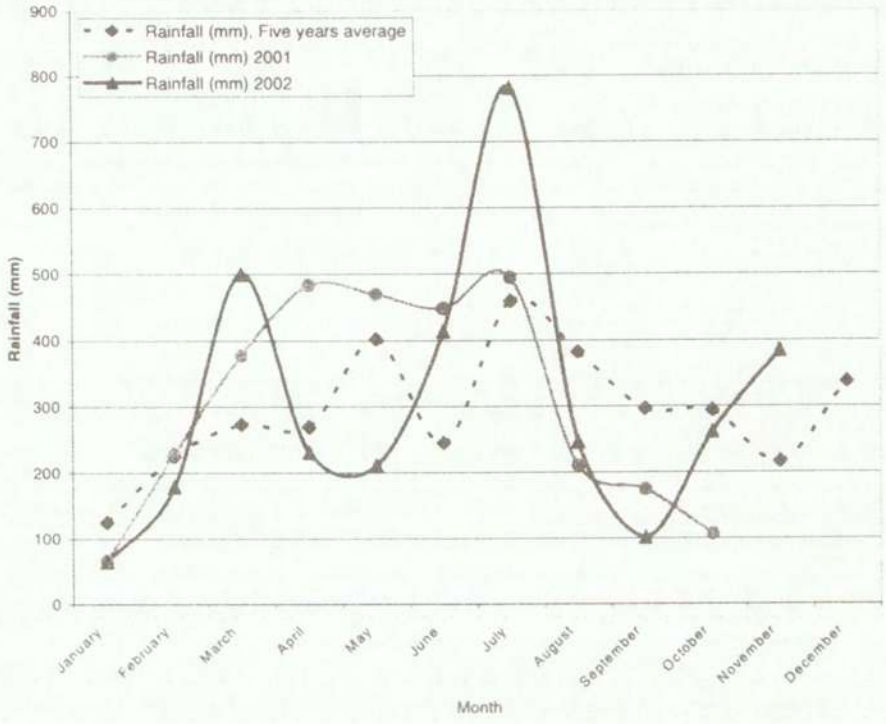


Figure.2. Rainfall at the University of Technology agriculture farm in 2001, 2002 and five year's average

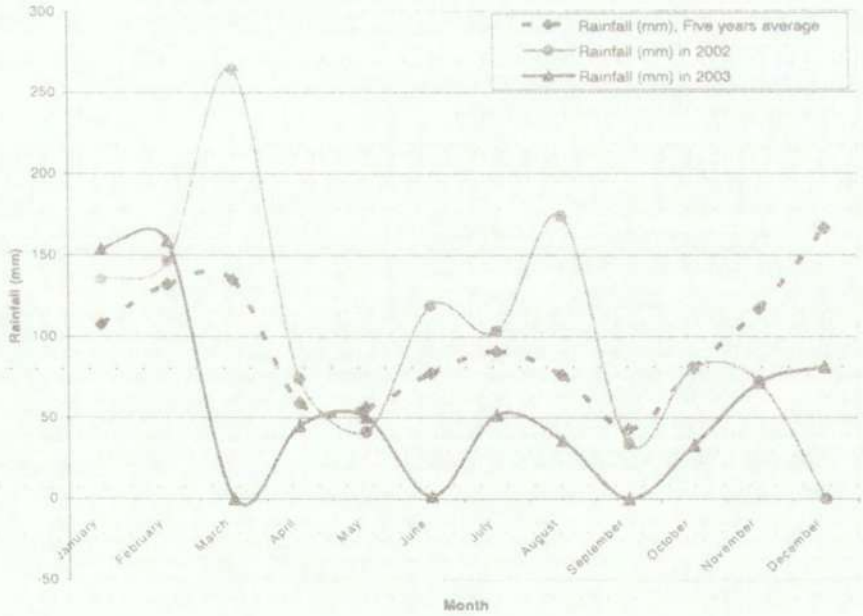


Figure 3. Rainfall at Markham valley experimental site in 2002, 2003 and five year's average

(TCS 10), IR 19661-23-3-2-2 (IR 19661), BG 379-2, and Finsch. Finsch is known to exist in PNG for over one hundred years while other three are exotic varieties imported from overseas. At the time of land preparation, Glyphosate (Round up) a pre-planting herbicide was applied (4L/ha). Seedbed was established by ploughing and harrowing twice to incorporate any primary growth/ stubble. The trial was laid out in a Randomized Complete Block Design, with four replications. The plot size was 3m x 5m with one meter clearance between them. Seeds were planted by dibble stick method. In this method a pointed stick was pushed to the ground to make a hole for 3-5 seeds at a depth of about 4 cm. The holes were spaced about 25 cm apart. Experimental field received 50 kgs of NPK (12:5:17) per hectare as a basal dose. Additional nitrogen fertilizer (20 kg/ha) was applied as urea in two splits during early and late tillering stages. Fertilizer was broadcast manually. At Unitech three manual weeding was done at seedling, early and late tillering stages. There was very little infestation of weeds at Markham valley which were weeded manually at seedling stage only. Harvesting was carried out at Unitech site in October 2001, 2002 and at Markham valley in April 2002, April-May 2003 for the first and second trial respectively. Harvesting was done manually with a knife (sickle). After harvest each of the treatment plots was threshed separately by beating on a wooden block. Grains were cleaned, sun dried and weighted along with the moisture content (Satake Moisture, Model SS-5). The yield data was adjusted to 14% moisture content. The above treatments and management practices remained the same in all trials.

Soil properties measurement

Measurement of soil physical properties included particle size distribution, bulk density, field moisture capacity, and saturated hydraulic conductivity. Soil particle size distribution was determined by hydrometer method (Gee and Bauder 1986). Soil bulk density was determined using the core method (Blake and Hartge 1986). Field moisture capacity (*in situ*) was determined by the method as described by Cassel and Nielsen (1986). Soil saturated hydraulic conductivity *(Ks)* measurements were carried out by ring infiltration method (Rowell 1994). At the beginning of each trial three soil samples were taken from each site for chemical analysis. Each soil sample consisted of a mixture of four subsamples taken from the top 20cm depth of the soil. Soil samples collected were air dried and then ground lightly with a mortar and pestle to pass through a 2 mm round-hole sieve. Soil chemical analysis was carried out by the following methods as described by Rayment and Higginson (1992). The procedures were as follows. Total Nitogen-semimicro Kjeldahl, steam distillation (reference: 7A1); Organic carbon-Walkley and Black (reference: 6A1); Exchangeable bases and CEC-1M ammonium acetate at pH 7.0 (reference: 15D1); Olsen-extractable phosphorus (reference: 9C1). The data presented here is the average of three measurements.

Statistical Analysis

The data were analyzed using the standard procedure of randomized block design to compare means for grain yield. Analysis of variance (ANOVA) was used to test the difference in yield. Treatment means were compared using the least significant difference (LSD) procedure and Duncan Multiple Test Range (DMRT) at the 5% probability level. Yield among and between the varieties and the yields obtained in two trials were computed.

RESULTS AND DISCUSSION

Rice yield obtained at Unitech and Markham valley trials are given in Table 1. In the first trial at Unitech highest yield obtained was in TCS 10 (3.8 t/ha) and at Markham valley it was in IR 19661 (5.6 t/ ha). In both experimental sites BG 379-2 have produced the lowest yield. The yield obtained in BG 379-2 was 1.9 t/ha and 3.3 t/ha at Unitech and Markham valley respectively.

The results of selected chemical and physical analysis of Unitech and Markham valley soils are

Table 1. **Rice yield (t/ha) at the Unitech in 2001 and 2002, and at Markham valley in 2002 and 2003 trials**

	Unitech agriculture farm		Markham valley	
Rice variety	2001	2002	2002	2003
TCS 10	3.81a	3.21b	5.63ab	5.89ab
Finsch	3.08b	2.76c	5.03bc	3.82c
BG 379-2	1.96c	1.68b	3.38d	2.75d
IR 19661	3.17b	3.04b	6.77a	4.87bc

Means followed by the same letter in the vertical column are not significantly different according to Duncan's Multiple Range Test (pd" 0.5).

given in Table 2. Analysis of soil collected on the onset of first trial show that the contents of nutrients at Markham valley and Unitech soil were not different significantly except organic carbon, available P and CEC. At Markham valley the content of organic carbon was 2.24% whereas it was 1.6% at Unitech. At Markham valley the levels of available P (Olsen- P) was 24 mg/kg compared to 11 mg/kg at Unitech soil. Soil CEC was 30 coml. /kg and 49 cmol. /kg at Unitech and Markham valley respectively. It is postulated that yield difference between these two sites was primarily due to the differences in nutrient contents particularly in organic carbon, available P and CEC (Ponnamperuma *et al.* 1981; Yamaguchi 1997; Pandey 1998). However, yield difference among the varieties was expected as cultivars respond differently to the available water and nutrients because of genetic variations (Hedley *et al.* 1994; Ladha *et al* 1998; Wade *et al* 1999).

In the second trial at Unitech yield has declined significantly ($p < 0.05$) in TCS 10, Finsch, and BG 379-2 varieties. Whereas, there was no significant difference in soil nutrient contents between the trials (Table 2). However, in the second trial at Markham valley there was a significant loss of organic carbon and available phosphorus but yield has declined in IR 19661 only. Therefore, it is assumed that apart from plant nutrients other factors might have involved in the variation of yield at both experimental sites. The loss of organic carbon at Markham valley may decrease biomass, organic matter turnover, and increase mineralization of added organic matter (Juo and Lal 1977, 1979; Jaiyeoba 2003). Organic matter affects CEC (Bernal *et al.* 1992; Tisdale *et al.* 1993). Even though there was a significant loss of organic matter at Markham valley soil, but it did not show any effect on CEC probably due to the valley charged clay particles (Ca^{2+}, Mg^{2+}, K^{+} etc.). The decrease in available P in the valley soil after the cropping was expected (Parfitt and Thomas 1975; Parfitt and Mavo 1975).

In rainfed rice ecosystems apart from plant nutrients the availability of water is considered to be very important to the productivity of rice (IRRI 1993). The total rainfall at Unitech 2001, 2002 trials was 3057 mm and 3828 mm, and at Markham valley 2002, 2003 trials it was 1612 mm and 898 mm respectively (Fig. 2 and 3). Rainfall data show that the amount of rainfall that Unitech have received in both trials was more than double than that of Markham valley. But in two consecutive trials all the variety have produced higher yield at Markham valley (Table 1). Mechanical analysis show that Unitech soil was a loamy sand, whereas, Markham valley was a clay loam (Table 2). The hydraulic conductivity (*Ks*) and field moisture capacity at Unitech soil was 45 mm/h and 22% and at Markham valley it was 15 mm/h and 52% respectively. It is postulated that due to the high hydraulic conductivity and low field moisture capacity of Unitech soil, plants could not use moisture and nutrients efficiently and hence low in yield (Lal 1979; Singh *et al.* 2000; Saleh *et al.* 2000; Oberthur and Kam 2000). In each experimental site the yield difference between the trials is presumed due to the pattern of rainfall of the site. In both experimental sites the pattern of rainfall was very erratic. Therefore, it is assumed

Table 2. **Some chemical and physical properties of Unitech and Markham valley soil.**

	Unitech		Markham valley	
	2001	2002	2002	2003
pH (1:5 w/v)	6.5	6.5	6.8	6.7
Organic Carbon (g/kg)	16.0±0.33	13.0±0.23	22.4±0.24	13.9±0.23*
Total Nitrogen (g/kg)	2.0±0.02	1.9±0.01	2.1±0.01	1.8±0.02
Available P Olsen (mg/kg)	11.0±2.65	11.0±3.35	24.0±6.51	10.0±7.33*
CEC pH7 NH_4OAc (cmol/kg)	29.0±3.00	30.0±3.51	49.0±4.58	5 3.0±1.00
Exchangeable cations (cmol/kg)				
Ca	21±2.73	19±1.00	57±6.63	56±5.77
Mg	3±0.90	3±0.76	9±1.35	9±1.32
K	0.91±0.19	0.7±0.29	2±0.31	2±0.29
Sand content (g/kg)	840	-	380	-
Silt content (g/kg)	140	-	340	-
Clay content (g/kg)	20	-	280	-
Bulk density (Mg/m3)	1.26	-	1.14	-
Field moisture capacity (g/kg)	260	-	530	-
Ks (mm/h)	45	-	15	-

(-) not determined

* indicates significance at $p<0.05$

that it is not the amount of rainfall but the extreme variability both within and between the years have affected rice yield (Craig and Pisone 1985; Patil *et al* 1998; Fukai *et al*. 2000).

CONCLUSIONS

The average grain yield obtained at Unitech 2001 and 2002 trials was 3 t/ha and 2.6 t/ha and at Markham valley 2002 and 2003 trials it was 5.2 t/ha and 4.3 t/ha respectively. In rainfed rice cropping system the global average yield of rice is 1-3 t/ha (Dobermann and Fairhurst, 2000). This suggests that under current practice of low input and low intensity agriculture of the province soil nutrients was not a major limiting factor for rice cultivation. However, hydraulic conductivity and moisture holding capacity of soil could influence the available moisture to plants and hence yield. It was also observed that there was no simple relationship between rainfall and grain yield.

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Short Communication

VOLATILE CHEMICAL CONSTITUENTS OF PATCHOULI (*POGOSTEMON CABLIN* (BLANCO) BENTH.: LABIATAE) FROM THREE LOCALITIES IN PAPUA NEW GUINEA

Stewart W Wossa[1], Topul Rali[2] and David N Leach[3]

ABSTRACT

Fresh aerial parts of patchouli (Pogostemon cablin) *were obtained from Rabaul, Port Moresby and Inawabui in the Mekeo area of the Central Province. The volatile oil constituents were extracted by exhaustive hydrodistillation where each of the patchouli samples afforded oil yields of about 0.1 percent. Detailed chemical investigations of the oils indicated patchouli alcohol to be the main constituent in the oils from the three localities. It was noted that the patchouli alcohol composition in the cultivar from Rabaul, Port Moresby and Mekeo were 71.8, 71.7 and 43.7 percent compositions respectively, suggesting that the patchouli oil from PNG can attract a ready market on the basis of its high patchouli alcohol contents in the patchouli oil as opposed to between 30 – 40 percent composition from other patchouli oil producing countries.*

Keywords: Pogostemon cablin, *Labiatae, Patchouli, Essential oil composition, Patchouli alcohol, Sesquiterpene.*

INTRODUCTION

Patchouli oil is obtained from *Pogostemon cablin* (Blanco) Benth., a member of the plant family Labiatae. It is a predominantly tropical herbaceous and perennial plant species with wide distribution in most South East Asian nations where it has also been documented as having many significant uses in traditional medicine and agriculture (Guo 2001). The leaves and stem contain a yellowish and viscous oil that has a unique and intense camphoraceous odour with many useful applications, hence high market value in the perfumery industry.

On the basis of its traditional uses as alternative medicine, agricultural pest control agent and applications in the perfume industry, detailed phytochemical studies were pursued to identify the chemical constituents responsible for the perceived activities. In one such study, the acetone extracts of the leaves were found to contain sesquiterpene hydroperoxide, which showed significant trypanocidal activities (Kiuchi *et al.* 2004). Another study also identified the cytotoxic Licochalcone A, Ombuim and 5,7-dihydroxy-3',4'-dimethoxyflavanone as the main chemical compounds from the aerial parts (Park *et al.* 1998). The hexane extracts were also noted to contain patchouli alcohol, pogostol, stigmast-4-en-3-one, retusin and pachypodol, which showed antiemetic activities (Yang *et al.* 1999).

The study of the chemical components within the essential oil extracts were found to be made up of patchouli alcohol, delta-guaiene, alpha-guaiene, seychellene, alpha-patchoulene, aciphyllene, trans-caryophyllene (Feng *et al.* 1999; Zhao *et al.* 2005; Guan *et al.* 1994). Luo and co-workers (1999) investigated the patchouli oil from the Gaoyao County, China and noted the stem to contain high pogostone content while the leaves contained high patchouli alcohol contents. While the chemical components were noted to be the same from different regions studied, the compositions were found to vary between regions as influenced by various environmental factors (Singh *et al.* 2002; Yan *et al.* 2002). Furthermore, the oil composition from different cultivating locations and different harvesting times were obviously different (Luo *et al.* 2002), suggesting that detailed chemical study was required to establish the reasons for such differences. Such studies revealed that there were two main chemotypes in patchouli; one being the pogostone-

[1] Faculty of Science, University of Goroka, PO Box 1078, Goroka, Eastern Highlands Province, PNG.
[2] Chemistry Department, University of Papua New Guinea.
[3] Centre for Phytochemistry and Pharmacology, Southern Cross University, Australia.

type and the other being the patchouliol-type on the basis of the molecular evidences (Liu *et al.* 2002). The pogostone-type contained rich oxygenated components especially pogostone in the volatile oil while the patchouliol-type contain rich patchouliol, delta-guaiene, alpha-guaiene and other non-oxygenated components (Luo *et al.* 2003).

Morphological studies of the leaves of patchouli also indicated the leaves to contain external trichomes as well as specialized internal accumulatory cells where concentrations of the sesquiterpenes were noted to be exceptionally higher than other parts of the plant (Henderson *et al.* 1970). Further biosynthetic studies revealed high concentrations of the enzyme sesquiterpene cyclase (patchoulol synthase) in the leaves, which was postulated to be responsible for the conversion of the farnesyl pyrophosphate, through biosynthetic mechanisms, to the cyclic sesquiterpenes (alpha and beta-patchoulene, alpha-bulnesene and alpha-guaiene) found in patchouli oil (Munck and Croteau 1990; Croteau *et al.* 1987). Another study of the leaf extracts revealed the presence of patchoulan-1,12-diol, which was also postulated to be the main precursor of nor-patchoulenol (Trifilieff 1980).

Interesting biological activities have been reported for the extracts of patchouli by various authors. The patchouli oil has been reported to show activity against three strains of methicillin-resistant *Staphyloccocus aures* (Edwards-Jones *et al.* 2004), cytotoxic (Park *et al.* 1998), bactericidal activity against *Campylobacter jejuni* and *Listeria monocytogenes* (Friedman *et al.* 2002), antibacterial and antifungal activities (Pattraik *et al.* 1996; Osawa *et al.* 1990), unusual tissue destructive effects on the exoskeletons of Formosan subterranean termites, *Coptotermes formosanus* Shiraki (Zhu *et al.* 2003), effective mosquito repellency effects (Trongtokit *et al.* 2005), showed promise as alternative preservative of perishable foods (Holley and Patel 2005), antiemetic activities (Yang *et al.* 1999) and trypanocidal activities (Kiuchi *et al.* 2004). Such interesting biological activities led to a number of synthetic studies to attempt their synthesis on a laboratory scale (Magee *et al.* 1995; Niwa *et al.* 1984; Niwa *et al.* 1987; Cory *et al.* 1990) including microbial assistance in the conversion of the substrates into the desired products (Arantes *et al.* 1999).

Currently, patchouli oil is one of the important natural essential oils because of its base and lasting aromatic character. The yellowish brown coloured viscous oil obtained from the leaves and stems have an intensely camphorous odour, a character that is utilized in the cosmetic industries to scent perfumes, flavour toothpastes and other health and self-care products. The global demand for patchouli oil has been noted to increase annually with Indonesia as the major producer of this oil (Robbins 1983; Tao 1983). The main buyers of the patchouli oil are the USA, Switzerland and France.

Preliminary studies on the chemical constituents in the PNG cultivar of patchouli (Wossa *et al.* 2004a) has shown the PNG oil to contain superior patchouli alcohol content at 70 percent as compared to the oil from Indonesia, Philippines, Malaysia, China and other South East Asian nations, which have patchouli alcohol compositions between 30 - 40 percent. In view of the economic potential in the cultivation and extraction of patchouli oil as an alternative agribusiness in PNG, we report herein the chemical constituents in the volatile oil extracts from patchouli from three localities and discuss these data in the light of the global market trends. This report is part of an ongoing study to document the chemical constituents in the essential oils obtained from the diversity of aromatic plants of PNG (Rali *et al.* 2003; Wossa *et al.* 2004a; 2004b; 2005).

MATERIALS AND METHODS

Samples of Patchouli were obtained from Rabaul, Port Moresby and the Mekeo area of the Central Province. The stem cuttings of the Rabaul cultivar was grown in a Port Moresby backyard garden to compare its oil yield and chemical composition with that of the samples from Rabaul. A native cultivar from the Mekeo area was also used in this study for comparative analysis of the chemical constituents.

The matured leaf samples of the patchouli were collected while fresh and the volatile oils obtained through exhaustive hydrodistillation. The distillates were extracted with diethyl ether and the ether removed under reduced pressure to afford yellowish brown-coloured pure oils. The oils were dried with anhydrous magnesium sulphate and stored at 4 degrees Celsius until further analysis. The analyses of the oils were done using a gas chromatograph coupled to a mass spectrometer (GC and GC/MS) and the individual components identified on the basis of their individual retention indices.

The analyses of the oil constituents were done as previously described (Wossa *et al.* 2005). The individual oil constituents were identified on the basis of their respective retention indices and confirmed by comparison with the mass spectral data of the authentic reference compounds or with the library of the published data (Adams 1995).

RESULTS AND DISCUSSION

The results of the GC and GC/MS analysis of the patchouli oil obtained from Rabaul, Port Moresby and Inawabui in Mekeo (Table 1) indicate that the Rabaul cultivar of patchouli has a higher patchouli alcohol content than the Mekeo cultivar with compositions at 71.8 and 43.7 percent compositions respectively. Such differences can be expected due to the various environmental and genetic factors that may be involved in the genesis of the different chemical constituents in the two cultivars of patchouli. Similarly, the Port Moresby cultivar was found to be superior in yield and composition of the patchouli alcohol than the Mekeo cultivar with compositions at 71.7 percent and 43.7 percent respectively.

On the other hand, the chemical constituents and composition of the Port Moresby cultivar were noted to be similar to that of the Rabaul cultivar. This result was as expected because the cuttings were from the Rabaul cultivar and were cultivated in the backyard garden in Port Moresby to see the possible effects of environmental factors on oil yield and chemical composition. The similar oil yield and chemical constituents and composition led us to infer that the environmental factors had minimal effect on the yield and composition of the patchouli oil. These results further suggest that the Rabaul cultivar has a higher patchouli alcohol content compared to that reported from other South East Asian cultivars. The comparison of the patchouli alcohol contents from the Mekeo cultivar with that of the other South East Asian cultivars can be categorized as being similar on the basis of the patchouli alcohol composition at around 40 percent, however they differ in the composition of the other constituents. This study, further shows that the three patchouli cultivars analyzed so far belong to the patchouliol-type with high patchouli alcohol contents and other non-oxygenated constituents.

On the basis of the chemical compositional data on patchouli oil presented, it can be realized that the patchouli alcohol content in the patchouli oil from Rabaul are higher compared to those reported from other patchouli oil producing countries in the region. This implies that patchouli oil from PNG can be readily accepted on the global patchouli oil market on the basis of the high patchouli alcohol contents. These results further suggest that patchouli can be encouraged and cultivated as a rural based agro industry in PNG. With the current global market for patchouli oil fluctuating between US$ 12 – 27 per kilogram, PNG could do well in introducing patchouli oil production as an alternative income earner in the country.

It is therefore recommended that further studies into the effects of the soil nutrients and chemistry, irrigation systems, age of crop at harvest, other important environmental as well as genetic factors be pursued to identify the optimum conditions for maximum yield of the patchouli alcohols with higher patchouli alcohol content from the patchouli cultivars

Table 1. **Volatile chemical constituents (% area) of *Pogostemon cablin* from three localities in PNG**

Chemical Constituents	Rabaul	Port Moresby	Mekeo
alpha-pinene	-	-	1.2
alpha-guaiene	7.5	4.6	7.8
seychellene	3.9	3.2	6.2
gamma-patchoulene	-	-	3.7
beta-patchoulene	-	-	1.8
alpha-patchoulene	1.7	1.5	1.4
delta-guaiene	9.9	6.5	9.5
pogostol	5.1	-	-
Patchouli alcohol	**71.8**	**71.7**	**43.7**
beta-caryophyllene	-	1.1	2.4
aciphyllene	-	1.2	-
beta-patchoulene	-	2.2	-
viridiflorol	-	1.4	-
Selina-3,7(11)-diene	-	-	5.1
Benzyl benzoate	-	-	1.7
$C_{15}H_{22}O$	-	6.6	0.7
$C_{15}H_{24}$	-	-	1.9
$C_{15}H_{26}O$	-	-	0.9

- = not detected.

of PNG. Such studies will pave the way for commercial production of patchouli oil in PNG as an alternative revenue earner.

CONCLUSION

This study has given new insight into the commercial potential for the patchouli oil production in PNG in terms of its oil yield and chemical constituents. The Rabaul cultivar of patchouli was found to contain higher patchouli alcohol content compared to the Mekeo cultivar while the patchouli alcohol content in the Mekeo cultivar were noted to be within the marketable range. It was also noted that the cultivation of the cuttings from the Rabaul cultivar in Port Moresby gave similar yield and composition, suggesting that the environmental factors had minimal effect on the oil yield and chemical composition.

In view of the commercial potential in the cultivation and extraction of the patchouli oil as an alternative revenue earner in the country, further studies into the soil nutrients and chemistry, the irrigations systems, age of plant at harvest, other environmental factors and genetic composition needed to be established to assist farmers in cultivating *Pogostemon cablin* for the extraction of its oil for the global patchouli oil markets.

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ha - hectare
mm - millimetre
cm - centimeter
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a.s.l. - above sea level
yr - year
wk - week
h - hour
min - minute
s - second
k - kina
n.a. - not applicable or not available
n.r. - not recorded
var - variance
s.d. - standard deviation
s.e.m.- standard error of difference
d.f. - degrees of freedom

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