Short communications

Protection of upland rice at Lake Alaotra (Madagascar) from black beetle damage (*Heteronychus plebejus*) (Coleoptera: Dynastidae) by seed dressing

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In Madagascar, growing demand for rice and resulting increased pressure on inundated lands has favoured the cultivation of upland rain-fed rice on hill slopes. Due to the instability of the ecosystem, this type of agriculture cannot meet the objectives of both sustainability and high yields if conventional tillage is used, particularly due to high erosion risk. Direct seeding, mulch-based cropping (DMC) systems have opened new prospects for upland rice. Just a few years after these methods were introduced and evaluated in the Central Highlands of Vakinankaratra, attacks by larvae (white grubs) and adult (black beetles) (*Heteronychus* spp.; Coleoptera: Dynastidae) on upland rice were demonstrably reduced, but the same beneficial effects were not observed at lower elevations (Ratnadass *et al.* 2006). Unless seeds are treated with an insecticide these particular pests remain one of the main obstacles to broader success with upland rice production and to the adoption of DMC systems, particularly in the medium-altitude Lake Alaotra region.

In Vakinankaratra (Randriamanantsoa & Ratnadass 2005) showed that seed treatment with Gaucho® T45 WS (35% imidacloprid, 10% thiram) at 5 g/kg seed resulted in a significant reduction of black beetle damage and a significant increase in yield under both mulch-covered and bare-ploughed soil management systems. However, the Alaotra Mangoro region has a different spectrum of black beetles: *H. plebejus* is the dominant species (Rajaonarison & Rakotoarisoa 1994), whereas *H. arator rugifrons* dominates in the highlands (Ratnadass *et al.* 2008; Randriamanantsoa *et al.* 2010).

Additionally, the high cost of the seed-dressing technique and the limited availability of the chemical put this practice out of reach of small farmers. This problem could be partially solved if the technique was subsidized or if generic active ingredients were made available, but the risk of selecting pesticide-resistant strains would remain. In addition, imidacloprid is suspected to have adverse environmental effects particularly for bees (see for instance Decourtye *et al.* 2005). It is thus critical to find more sustainable, cost-effective and eco-friendly alternatives to imidacloprid. From 2005–2008, tests were therefore conducted in this region to compare imidacloprid with two new neonicotinoids, clothianidin and thiamethoxam, and a biological seed-dressing treatment using a mixture of natural plant-derived products, to maximize effectiveness in terms of insect pest reduction economic benefit, and minimize environmental impact.

Field trials were conducted at Ambatofotsy (48°27′22.8″E 17°41′1.1″S, 765 m a.s.l.), located at the FOFIFA (National Centre of Research Applied to Rural Development) Mid-East Regional Research Centre (CRRME), Ambohitsilaozana, 15 km north of Ambatondrazaka, Alaotra Mangoro region, Madagascar.

One trial was conducted in each of the 2005–2006 and 2006–2007 seasons, in ploughed plots that had been fallow for several years, and a third trial was conducted in 2007–2008 in the 2005–2006 plot after

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rotation with cowpea in 2006–2007. The rice cultivar used was B22, an upland rice variety bred in Brazil, planted in hills at a rate of 7–10 seeds per hill, or about 70 kg/ha. Plots consisted of 15 6-m rows with a 0.30-m inter-row and 0.20-m intra-row spacing.

The imidacloprid and clothianidin formulations tested were Gaucho® T45WS (insecticide/fungicide: 350 g/kg imidacloprid + 100 g/kg thiram) and Poncho® 600 FS (insecticide: 600 g/l clothianidin) respectively, while the thiamethoxam formulation tested was Cruiser® 350 FS (insecticide: 350 g/l thiamethoxam). Locally purchased Calthir® (fungicide, 800 g/kg thiram) was tested both as a fungicide-only control and combined with clothianidin, while the fungicide tested in combination with thiamethoxam was Maxim® XL 035FS (25 g/l fludioxonil + 10 g/l mefenoxam (Metalaxyl-M)). Fungicides were used in all synthetic treatments because the reference product (Gaucho® T45WS) is itself a mixture of insecticide and fungicide; Maxim® XL 035FS was the fungicide recommended by the supplier of Cruiser® 350 FS. International suppliers were Syngenta-Switzerland for Cruiser® and Maxim® and Bayer CropScience, South Africa, for Gaucho® and Poncho®.

The organic treatment was a mixture of Umisan SS3®, a natural elicitor of plant defences against fungal infection, Umisan TY10®, a neem-tree seed extract, and Liquid Humus Elvisem®, a fertilizer derived from fermented organic materials. All three natural products were obtained from Elvisem-Europe, Chiavari, Italy.

In the 2005–2006 season, the trial (sown on 11 January 2006 due to late onset of rains) comprised six treatments with four replicates in a randomized complete block design: 1) untreated control; 2) thiram at 0.5 g a.i./kg of seeds; 3) imidacloprid + thiram at rates of, respectively, 1.75 and 0.5 g a.i./kg seed; 4) clothianidin + thiram at respectively 4.0 g and 0.5 g a.i./kg seed; 5) thiamethoxam + fludioxonil + mefenoxam at respectively 1.4, 0.1 and 0.04 g a.i./kg seed; 6) natural elicitor at 5 g/kg seed.

The seed required for the four replications of each treatment (50 g) was thoroughly mixed in a plastic bag with the exact amount of powder or liquid compounds of the dressing, measured with a precision scale and disposable pipettes, respectively.

In 2006–2007, the trial was repeated, replacing the natural elicitor treatment by dressing the seeds with a combination of this elicitor and commercial neem-tree seed extract (at 5 g/kg and 5 ml/kg seed respectively) plus a drop (approximately 1 ml) of liquid humus, and the addition of a seventh treatment, namely a second dosage of imidacloprid + thiram (0.875 and 0.25 g a.i./kg), corresponding to 2.5 g of commercial product per kg, which is the rate actually used by farmers. Based on the result obtained in 2006–2007, treatments in 2007–2008 were reduced to thiram at 0.5 g a.i./kg, imidacloprid + thiram at 0.875 and 0.25 g a.i./kg, clothianidin + thiram at 4.0 and 0.5 g a.i./kg, and three dosages of neem-tree seed extract (5 ml, 10 ml and 15 ml/kg) in combination with natural elicitor (5 g/kg) and liquid humus (one drop = about 1 ml).

In the 2005–2006 trial, all dead *Heteronychus* adults were manually collected in the trial plots, separated and counted according to species (Randriamanantsoa *et al.* 2010) after damage rating of the rice crop 20 days after sowing (DAS). For all three years, damage at the tillering stage, 20 DAS, of the central 96-hill square of each plot was rated on a 1–5 scale of tiller damage as follows: 1 = 0–20 %; 2 = 21–40 %; 3 = 41–60 %; 4 = 61–80%; 5 = 81–100 % damage (complete absence of tillers in a hill being marked 5). This was compared to earlier damage rating methods that considered separately percentage hills with no tiller, and other type of damage (Randriamanantsoa & Ratnadass 2005).

Grain yield (paddy weight) was recorded at harvest of the central 96-hill square of each plot (representing 5.76 m^2) in 2006–2007 and 2007–2008. Data were analysed with XLSTAT (Addinsoft 2009), using the non-parametric test module (Friedman test) for damage ratings, and the ANOVA module for grain yields. Correlations between damage ratings and grain yields were calculated using the Spearman's r in the correlation test module, with individual plot values.

In 2005–2006, the six treatments were applied within each of the four replicate blocks, but there was confusion as to which individual treatments had been allocated to particular plots, hence no comparison of treatments could be made. On the other hand, the trial served as a preliminary test to determine pest incidence and to improve the damage rating scale. We collected and identified 155 dead black beetles evenly distributed throughout all four blocks: *H. plebejus*: 68 ± 1.7 %; *H. arator*

Table 1. Effect of different seed dressings on black-beetle damage to paddy rice (cv B22) and paddy yield (Ambohitsilaozana, Madagascar, 2006–2008).

Treatment (a.i. and dose/kg of seeds)	Damage rating[a]		Paddy yield[b]	
	2006–07	2007–08	2006–07	2007–08
Untreated control	2.50 a	2.71 a	2652 c	3277 ab
Thiram @ 0.5 g	2.78 a	2.86 a	2027 c	2427 b
Imidacloprid @ 1.75 g + thiram @ 0.5 g	1.15 ab	–	4796 ab	–
Imidacloprid @ 0.875 g + thiram @ 0.25 g	1.24 ab	1.38 a	5013 a	4987 a
Clothianidin @ 4.0 g + thiram @ 0.5g	1.16 b	1.38 a	4722 ab	4805 a
Thiamethoxam) @ 1.4 g + fludioxonil @ 0.1 + mefenoxam @0.04 g	1.65 ab	–	4839 ab	–
Neem-tree seed extract @ 5 ml + natural elicitor @ 5 g + 1 drop liquid humus	2.00 ab	2.74 a	3668 b	2843 ab
Neem-tree seed extract @ 10 ml + natural elicitor @ 5 g + 1 drop liquid humus	–	2.73 a	–	3306 ab
Neem-tree seed extract @ 15 ml + natural elicitor @ 5 g + 1 drop liquid humus	–	2.64 a	–	2675 ab

[a]Rice seedling damage rating on a 1–5 rating scale from least to most severe. [b]Paddy yield in kg/ha. – Not applicable.

Means followed by the same letter in a column are not significantly different (Nemenyi test following Friedman test for damage rating, Newman-Keuls test following F-test for paddy yield) at $P = 0.05$.

rugifrons: 28 ± 1.0 %; *H. bituberculatus*: 4 ± 0.5 % (proportion means ± S.D., n = 4). These results agreed with earlier and concurrent observations on the same site (Rajaonarison & Rakotoarisoa 1994; Razafindrakoto *et al.* 2010) highlighting the dominant status of *H. plebejus*, but differed from observations in the Central Highlands where *H. arator rugifrons* is the dominant species (Ratnadass *et al.* 2008; Randriamanantsoa *et al.* 2010).

Furthermore, in 2005–2006, the ratings on the four blocks (theoretically all equivalent overall, since they all included all six treatments, as reflected by uniform recovery of dead adult *Heteronychus*) were remarkably homogenous using the second ('consolidated') rating scale, with CV of means of 5.5 %, compared with 18.2 % when using the earlier rating scale (which did not rate 'hills with no tillers' as 5), and 29.5 % for percentage of hills with no tillers.

In addition, in terms of improving the black-beetle damage rating method, in 2006–2007 and 2007–2008 the percentage of hills with no tillers in the fungicide only (thiram) treatment was not lower than in the control plot (data not shown), indicating that missing tillers were mainly due to insect rather than fungal damage. This confirmed the relevance of using a 1–5 rating scale by merging missing tiller symptom with >80 % damage class, rather than considering the percentage of hills with no tillers and 'milder' damage rating separately, as was previously the case (Randriamanantsoa & Ratnadass 2005). In both cropping seasons, damage rating, whether with the earlier or the new scale, was found to be significantly correlated with grain yield (26 d.f.). However, while in 2007–2008, Spearman's correlation coefficient had the same value (r = –0.64) for both scales, in 2006–2007 it was much higher with the new scale (r = –0.84) than the earlier one (r = –0.45).

In terms of seed-dressing efficacy, in 2006–2007, seedlings from seeds treated with clothianidin were significantly less damaged than those from both untreated control seeds and seeds treated with thiram alone. Seedlings from seeds treated with imidacloprid and thiamethoxam and those treated organically were not significantly more or less damaged than those from seeds treated with clothianidin on the one hand, and both controls on the other (Table 1). The four insecticide treatments all produced similar yields which were better than the organic treatment, although this was statistically significant only for imidacloprid at 0.875 g/kg (Table 1).

In 2007–2008, the same trend was observed in terms of damage rating; however, although the Friedman test was significant, no significant difference between treatments was detected with the Nemenyi multiple comparison method (Table 1), while the only significant differences in terms of yield were between the two neonicotinoid treatments and the treatment with fungicide (thiram) alone. The untreated control and all three biological treatments produced intermediate results (Table 1).

There are very few references on the use of neonicotinoid insecticides for the protection of upland rice. In South Africa, imidacloprid was shown to be more effective as a maize protectant as a seed dressing than acetamiprid and thiamethoxam against *H. arator* (Drinkwater 2003). However, *H. arator* is not the dominant species in the Lake Alaotra region, and the *H. arator* subspecies (*H. arator rugifrons*), which is endemic to Madagascar, differs from the *H. arator* species found elsewhere in the world (Randriamanantsoa *et al.* 2010).

In addition to confirming the efficacy of imidacloprid as a seed dressing at a rate of 2.5 g of commercial product per kg of seeds for the protection of upland rice from damage by *H. plebejus,* this study improved the black-beetle damage rating method, as evidenced particularly by the much stronger correlation (almost two-fold) one year out of two between grain yield and damage measured with the new scale as compared to the earlier one. The economic burden for farmers of using commercial imidacloprid-based seed-dressing products is being partly solved by the recent release of generic imidacloprid in Madagascar. However, despite the fact that seed dressing is generally considered to be less environmentally harmful than spraying pesticides, concerns remain about adverse effects of imidacloprid on the environment, and the risk of development of neonicotinoid resistance in black beetles (Elbert *et al.* 2008).

Among the alternatives to imidacloprid studied, application of clothianidin at 4 g a.i./kg seed appeared to be the most promising. However, as clothianidin is a compound of the same family as imidacloprid, the risk of cross-resistance should not be ruled out (Nauen *et al.* 2003). Therefore, novel rice seed treatments (anthranilic diamide insecticides, *e.g.* chlorantraniliprole) might be worth investigating (Cordova *et al.* 2006).

As thiamethoxam is an easy-to-cleave neonicotinoid precursor of clothianidin (Nauen *et al.* 2003), it has no advantage over clothianidin from the point of view of resistance management and appears to be less effective.

Furthermore, the toxicity to bees of clothianidin and thiamethoxam has recently been shown to be similar to that of imidacloprid (Iwasa *et al.* 2004). Although direct observations in Madagascar have shown that bees may feed on rice pollen, rice as a self-pollinated crop does not require a pollinating insect. Consequently, in the regions of Madagascar where bee-keeping is a significant income-generating activity, a 'push-pull' combination (Cook *et al.* 2007) of bee-repelling ('push') plants intercropped with neonicotinoid-treated upland rice, and border crops of highly bee-attractive ('pull') plants, such as buckwheat, could ensure a harmonious combination of rice-cropping and bee-keeping in these regions.

Due to the other benefits of DMC systems *vs* conventional upland rice cultivation (Ratnadass *et al.* 2006), and in the light of reduced pest damage observed under DMC systems in the Highlands (Randriamanantsoa & Ratnadass 2005), such studies should also be conducted at lower altitude under DMC systems, involving not only dead mulch, but also live cover intercrops or relay crops. Combining DMC and seed dressing could reduce the accumulation of pesticide residues in the soil and run-off water, as it was shown that DMC increases soil biological activity and decreases erosion (Ratnadass *et al.* 2006; Scopel *et al.* 2005). Results suggest that in some DMC systems, seed dressing, which is mandatory to control damage during the first years following the breaking with conventional management, is no longer necessary after several years of such DMC management (Ratnadass *et al.* 2008).

In addition to investigating such ways of reducing pesticide use, alternatives to synthetic pesticides should be sought. In line with promising results obtained earlier using ground seeds of *Melia azedarach* for rice seed dressing (Razafindrakoto 1997), the slight effect of combining biological products observed in 2006/07, is encouraging. Other formulations of neem extracts (higher concentrations, or dust formulation instead of liquid) should also be tested as foliar sprays rather than only as seed dressing. For instance, in Cameroon, foliar applications of natural neem extract were also found to be almost as effective as neonicotinoid products for the protection of sorghum cropped on receding soil moisture (*Muskwari*) from damage caused by the stem borer (*Sesamia cretica*) (Aboubakary *et al.* 2008).

Finally, the effect of entomopathogenic fungi (*Metarhizium anisopliae*) should be evaluated in combination with the DMC system for their potentially synergistic effects (Razafindrakoto *et al.* 2010).

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