

# Genetic diversity and plant propagation in the rare rainforest tree, *Ryparosa kurrangii*.

Bruce L. Webber & Ian E. Woodrow



*Cape Tribulation, Daintree lowlands rainforest, Queensland, Australia.*

**A report to the Australian Flora Foundation**

**June 2005**



**School of Botany  
The University of Melbourne  
Victoria, 3010 Australia**



---

**Table of contents:**


---

<b>Preface</b> .....	<b>2</b>
<b>Abstract</b> .....	<b>2</b>
<b>1 Introduction</b> .....	<b>3</b>
<b>2 Research methodology</b> .....	<b>4</b>
2.1 Vegetative propagation techniques .....	4
2.1.1 Aerial rooting .....	4
2.1.2 Field-sampled cuttings .....	4
2.2 Cyanogenic quantification.....	5
2.2.1 Sample harvesting and processing .....	5
2.2.2 Cyanide analysis .....	5
2.3 Frugivore-seed interaction trials.....	5
2.3.1 Fruit sampling .....	5
2.3.2 Seed treatment.....	5
2.3.3 Seed germination.....	6
2.4 Statistical analyses .....	7
<b>3 Results</b> .....	<b>7</b>
3.1 Vegetative propagation techniques .....	7
3.2 Cyanogenic quantification.....	8
3.3 Seed-frugivore interaction trials.....	8
<b>4 Discussion</b> .....	<b>10</b>
4.1 Vegetative propagation success and cyanogenic variation .....	10
4.2 Seed-frugivore interactions .....	11
4.3 Conclusions .....	12
<b>5 References</b> .....	<b>13</b>

## Preface

---

This research formed a small part of the doctoral studies of Bruce Webber, undertaken in the [Plant Physiology Research Group](#) in the [School of Botany](#) at [The University of Melbourne](#). Bruce was supervised by Dr Ian Woodrow and collaborated with a number of organisations including [The Royal Melbourne Zoological Gardens](#) and [Queensland Parks and Wildlife Service](#). All studies were conducted under Scientific Purposes permits issued by the Queensland Department of Environment and Heritage and with the full permission of private land owners. Bruce would like to acknowledge Peter Courtney, Paul Stewart-Higgs and Matt West (Royal Melbourne Zoological Gardens) who provided technical assistance with conducting the cassowary feeding trial and David Westcott (CSIRO TFRC Atherton) who provided invaluable advice on the life history of cassowaries and the implications of their interactions with rainforest fruits.

This work is described in greater detail in Bruce's doctoral thesis:

Webber B.L. (2005) Plant-animal interactions and plant defence in the rainforest tree, *Ryparosa*. PhD thesis, The University of Melbourne.

Parts of the above research funded by the AFF were published in 2004 and 2007:

Webber, B.L., Miller, R.E. and Woodrow, I.E. (2007) Constitutive polymorphic cyanogenesis in the Australian rainforest tree, *Ryparosa kurrangii* (Achariaceae). *Phytochemistry* **68**: 2068-2074.

Webber, B.L. and Woodrow, I.E. (2004) Cassowary frugivory, seed defleshing and fruit fly infestation influence the transition from seed to seedling in the rare Australian rainforest tree, *Ryparosa* sp. nov. 1 (Achariaceae). *Functional Plant Biology* **31**: 505-516.

Portions of this work have featured in *New Scientist* (2004, **2451**: 16), *Australian Life Scientist* (2004, **11**: 28) and *Australasian Science* (2005, **May**: 8).

For further information, please email Bruce ([b.webber@bigfoot.com](mailto:b.webber@bigfoot.com)) or Ian ([iewood@unimelb.edu.au](mailto:iewood@unimelb.edu.au)).

## Abstract

---

The rare Australian rainforest tree *Ryparosa kurrangii* (Achariaceae) has a very restricted distribution and is only known from a small strip of coastal lowland tropical rainforest in far north Queensland, Australia. Attempts at vegetative propagation of the species were unsuccessful, despite significant callusing and shoot production on field-sampled greenstick cuttings. This precluded an investigation of the relationship between cutting strike rates and plant tissue cyanogenic capacity of the taxon, which was found to be highly variable. At a population level, foliar cyanide concentration varied considerably between 0.54 – 4.77 mg CN g<sup>-1</sup> dw. A seed treatment trial found that cassowary gut passage significantly improved germination from 4% to 92%, and we were not able to replicate this result with simulated treatments. While high levels of fruit fly larval infestation accounted for reduced seed viability, this predation was apparently reduced by cassowary gut passage.

*Keywords*: Plant propagation, greenstick, germination, cyanide, cyanogenesis, secondary metabolite, frugivore, ratite, *Casuarium casuarium johnsonii*

## 1 Introduction

Amongst the Australian tropical flora that have their origins in the Indo-Malayan region is a rainforest tree taxon in the genus *Ryparosa* (Achariaceae; Flacourtiaceae *pro parte*). The Australian distribution of *Ryparosa*, as it is currently known, is restricted to tropical lowland rainforest of the Daintree region in far-north Queensland. Discovered in the mid 1960's, the specimens collected were assigned to *Ryparosa javanica* (BLUME) KURZ EX. KOORD. & VALETON based on the descriptions of Sleumer (1958; B.P.M. Hyland, pers. comm.). However, a recent revision of *Ryparosa javanica sensu lato* has described the Daintree populations as a single species, *Ryparosa kurrangii* B.L.WEBBER, which is endemic to Australia (Webber and Woodrow 2006; Figure 1). Significantly, *R. kurrangii* (i.e. *R. javanica pro parte*) is classified by the Queensland Nature Conservation Act as a rare species (Queensland Government 1992) and there is evidence that an upgraded conservation code may now be more appropriate (Webber 2005; Webber and Woodrow 2006).

Recent studies have identified *R. kurrangii* as a model plant in which to investigate plant-herbivore interactions and plant defensive chemicals (Webber 1999). The species is known to liberate volatile hydrogen cyanide (HCN) upon tissue disruption in a process known as cyanogenesis. Because cyanide is a respiratory toxin, its presence in plants has been shown to act as a trait conferring resistance against generalist herbivores (e.g. Gleadow and Woodrow 2002; Hruska 1988). Importantly, leaves from some plants contain levels of cyanide that are amongst the highest ever reported while individuals within populations seem to vary greatly in their (genetically based) capacity for cyanogenesis (Webber 1999).



**Figure 1:** *Ryparosa kurrangii* has large alternating leaves supported on long petioles.

To maximise both the research potential of this species and successful population restoration in cleared areas, a protocol needs to be established for the large-scale propagation of seedlings. This will allow controlled glasshouse-based studies to better understand the dynamics of rainforest cyanogenesis as well as responsible reforestation projects that encompass the full range of both genetic and cyanogenic diversity. Published studies on the germination characteristics of the Achariaceae are limited and there are none that deal with the germination processes of *Ryparosa*. Unfortunately, all previous attempts to establish vegetative cuttings of *R. kurrangii* have had very low success rates (B.L. Webber, unpub. results; N. I. J. Tucker, pers. comm.).

Initial experiments with seed germination (manual flesh removal and partial burial) had very low germination rates and the rare status of the tree precluded any large scale seed harvesting. Interestingly, the large apricot-like fruits of *R. kurrangii* fulfil all the morphological characteristics listed for avian frugivory in general (van der Pijl 1982) and cassowary frugivory in particular (Willson *et al.* 1989; Figure 2). In the Daintree lowland rainforests, cassowaries are one of only a few frugivores that can disperse large rainforest fruits, and are the only long distance dispersal vector for large seeds (Jones and Crome 1990; Noble 1991).



**Figure 2:** *Ryparosa kurrangii* fruit are large drupes borne on racemes on the lower trunk.

Given this background, the aim of the research was to capture the unique genetic diversity of the rare Australian rainforest tree, *R. kurrangii* and develop an efficient way of establishing glasshouse populations. More specifically, the objectives were to: (1) investigate the viability of vegetative propagation protocols to establish glasshouse *R. kurrangii* populations, (2) document variation in cyanide concentration at a population level and evaluate the effect of such variation on cutting establishment, and (3) quantify the effect of fruit treatment by frugivores on seed germination.

## 2 Research methodology

### 2.1 Vegetative propagation techniques

Due to the poor history of *R. kurrangii* propagation trials and the limits on vegetative sampling imposed by sampling permits, a limited suite of standard propagation methods were chosen to maximise the chance of success.

#### 2.1.1 Aerial rooting

Standard aerial rooting techniques were applied to a range of ‘finger-thick’ branches on mature trees in the field. To increase the chance of getting younger material, branches that were targeted had recent leaf production and were emerging from tubercles on the main trunk. In brief, moist sphagnum moss was wrapped around a freshly skinned stem and sealed tightly against the branch with heavy-duty black plastic and fibre-tape. Treatments were applied to 5 separate trees on 3 branches per tree. Trees were then examined 6 and 12 months later for callusing and/or root growth in the treated section of each branch.

#### 2.1.2 Field-sampled cuttings

Material for cuttings was taken from a range of mature trees (> 5 m height) across a number of populations in the Daintree lowlands. Material ranging from 2 cm diameter woody stem tissue through to pencil-thin green-stick cuttings was harvested to allow for final cutting pieces approximately 15 cm long with between 4 - 8 leaf nodes. These were transported back to Melbourne refrigerated in individual canisters containing wet tissue paper and planted out into a 50:50 sand:pine-bark potting mix within 24 hours of sampling. Before being planted, each cutting was treated with either 8000 ppm NAA (in alcohol), 5000 ppm IBA (in talcum powder) or a combination of both. Exposed leaves were treated in two ways: either totally removed or trimmed (leaving 4 - 5 cm of the basal portion of the leaf blade remaining). This created six possible treatment groups (three hormone, two leaf) for the cutting pieces. Cuttings were kept under 50% shade cloth on a misting bed in a tropical glasshouse and were examined every month for a period of six months for callusing, root growth and shoot growth.

## **2.2 Cyanogenic quantification**

### **2.2.1 Sample harvesting and processing**

Samples for population cyanide determination were collected from a permanent study population on a tributary of Myall Creek in the Mt Sorrow Valley (c. 16° 07' S, 145° 28' E; 50 m asl). Leaf material was sampled from a similar height (0.5 - 2.0 m) and pole pruning shears were used to sample material from greater heights where this was not possible. For each tree, five mature leaves (recently toughened) were sampled within an area of approximately 0.5 m<sup>2</sup>, usually from the same branch. Trees from which cutting material was sampled (Section 2.1.2) also had leaf material collected for cyanide determination. Using a leaf punch (15 mm diameter), two leaf discs were removed from either side of the midrib in the middle of each leaf, avoiding main veins. All vegetative samples were snap-frozen in liquid nitrogen, freeze dried, ground to a fine homogeneous powder and stored on desiccant at -20°C.

### **2.2.2 Cyanide analysis**

Cyanogen concentration was quantified by measuring the amount of hydrogen cyanide (HCN) released from ground tissue after hydrolysis (Brinker and Seigler 1989; Lambert *et al.* 1975). Trapped HCN was assayed using a miniaturised version of the method of Brinker and Seigler (1989). Standard curves, based on adding known concentrations of NaCN into the NaOH well, were used to calibrate the assay. The amount of cyanide detected with this method is directly proportional to the total concentration of cyanogens in the tissue and will be referred to as the amount of 'cyanide' in this work (mg g<sup>-1</sup> cyanide per dry weight plant tissue, CN<sub>M</sub>).

## **2.3 Frugivore-seed interaction trials**

### **2.3.1 Fruit sampling**

Total fruits collected were kept to a minimum for conservation management purposes. To conduct the germination trial, 125 ripe fruits were collected from approximately 20 trees across five populations in November 2002. They were kept cool for two days to avoid spoiling before the germination trial treatments were applied. Fruits were recognised as having two ecological units, hereafter referred to as 'flesh' (epicarp and mesocarp) and 'seed' ('endocarp', endosperm and embryo). Endocarp is used loosely to refer to the hard nutty layer protecting the seed, even though it is likely that this tissue is derived from the inner and outer integuments as observed in *Hydnocarpus* spp. (van Heel 1979).

All fruits were pooled and then stratified according to fruit fly larval infestation (presence or absence), based on sting marks (oviposition sites) on the epicarp surface. Infested fruits accounted for 55% of total fruits collected and ovipositing fruit flies were identified as *Adrama selecta* (Tephritidae; lodged with Australian National Insect Collection: ANIC 29-007416). Damage to dissected fruits not included in this study indicated infestation in flesh and seed tissue and it is likely that seed tissue damage happened before the endocarp had toughened.

### **2.3.2 Seed treatment**

Fruits were randomly assigned to each of six treatment groups, with an equal proportion of fruit fly larvae infested and uninfested (55% and 45% respectively) in each group. These groups included one control, one treatment that simulated scatter-hoarding animal interactions, two cassowary feeding trial treatments

and two treatments based on theories most popular in the literature for bird-improvement of seed germination (removal of pericarp germination inhibitors and endocarp scarification). The three treatments that involved removing the flesh beforehand were set up first and leftover fruits (after acquiring 20 seeds per treatment) were randomly allocated to the remaining three treatments while maintaining infestation proportions. The six treatments were as follows. The control group ('Control',  $n_{\text{Fruit}} = 15$ ,  $n_{\text{Seed}} = 24$ ) simulated no animal disperser interaction and seeds were left inside an intact fruit. Interactions with scatter-hoarding animals that chew flesh but do not swallow seeds ('Flesh Rem',  $n_{\text{Seed}} = 20$ ) was simulated by roughly removing the majority of the fruit flesh, leaving some remnants on the outside of the endocarp. Simulation of handling by animals such as cassowaries that swallow fruits and remove flesh during gut passage were carried out by carefully defleshing and then washing seeds several times with distilled water to remove every trace of pericarp from the endocarp. These seeds were then either scarified with sandpaper (P120, 3M Australia) over the entire endocarp surface ('Scarified',  $n_{\text{Seed}} = 20$ ) or soaked in tap water for 24 hours ('Soaked',  $n_{\text{Seed}} = 20$ ).

The remaining two groups of fruits were pooled ( $n_{\text{Fruit}} = 42$ ) and combined into the daily diet of two cassowaries (*C. c. johnsonii*; adult male #930897, adult female #780038) over two days at The Royal Melbourne Zoological Gardens (Parkville, Victoria, Australia). These were consumed by the cassowaries with a range of other fleshy fruits and subsequently passed almost entirely devoid of pericarp. Average gut retention time was approximately three to four hours and did not noticeably differ between birds. As many seeds as possible were retrieved from scats within the cassowary enclosure. Variation in the number of seeds per fruit precluded an analysis of the percentage of seeds that successfully survived gut passage (c.f. digestion); however, other studies have found that hard-coated seeds are consistently passed in cassowary scats (Crome and Moore 1990; Willson *et al.* 1989). Seed collection took place within 24 hours of egestion and seeds were randomly divided into two treatment groups based on different planting strategies outlined below ('Cass (Std)',  $n_{\text{Seed}} = 24$ ; 'Cass (Scat)',  $n_{\text{Seed}} = 28$ ). Given the period of time between cassowary egestion and seed collection, it is possible that some seeds may have passed through the gut more than once, as coprophagy is well known amongst ratites.

### 2.3.3 Seed germination

Seeds from each treatment were planted at an even spacing (4 cm) in germination trays and lightly pressed (to a depth of 0.5 cm) into the surface of a standard potting mix (pine-bark:sand:crushed quartz, 89:5.5:5.5 with 1 kg m<sup>-3</sup> gypsum; pH 5.8). The one exception was the 'Cass (Scat)' treatment in which seeds were embedded on top of the potting mix in a scat matrix (4 cm deep) comprised of cassowary faecal material collected at Melbourne zoo at the same time as the feeding trial. Trays were randomly rotated every two weeks under 60% shade cloth in a tropical glasshouse. Trays were kept moist throughout the trial and every two weeks subjected to a more vigorous application of water. Invasive monitoring of seed germination after planting was restricted to avoid damaging radicles and destructive harvesting of germinated seedlings at the conclusion of the trial was kept to a minimum. Seeds were monitored for germination success (the emergence of a radicle from the endocarp) over a period of eight months. All ungerminated seeds were dissected and examined for tissue condition and evidence of fruit fly larval infestation.

## 2.4 Statistical analyses

Statistical analyses were carried out using the software packages Minitab<sup>®</sup> (V13.1, Minitab Inc., Pennsylvania, USA) and SPSS (V11.5, SPSS Inc., Chicago, USA). Analyses of frequencies were carried out using tests of independence (also known as tests of association; Sokal and Rohlf 1995). The G-test statistic with the use of the conservative Williams correction was used for orthogonal comparisons and multiple non-orthogonal contrasts in testing for differences between seed treatment groups. Likelihood ratio tests were conducted using the Kolmogorov-Smirnov test to check for deviation from expected larval infestation rates in treatment seeds when expected counts ( $n_{\text{expected}}$ ) were greater than five. Low cell counts in some categories prevented statistical investigation of post-germination fate data; however, this limitation does not reduce the biological significance of the results and hence these results have been presented.

## 3 Results

### 3.1 Vegetative propagation techniques

Unfortunately, all efforts to vegetatively propagate *Ryparosa kurrangii* were unsuccessful in this study. Field-based aerial rooting experiments had no sign of callusing at the wound site or root formation in the surrounding peat matrix. For the field-sampled cuttings raised in the glasshouse, woody cuttings with leaf tissue dropped their half-leaves after approximately 4 weeks, while greenstick cuttings with half-leaves generally maintained their leaf tissue for the majority of the study. Greenstick cuttings with half-leaves were more likely to produce new shoots than those cuttings with all leaf material removed. At the conclusion of the trial, some cuttings had produced up to three shoots, with each shoot bearing up to 5 new leaves. Shoot production appeared independent of the amount of below-ground callus tissue.

Differing hormone treatment had no noticeable effect on the production of callus tissue or new shoots. The most obvious trend in cutting treatment was for tissue callusing between greenstick and woody cuttings (Table 1). While the majority of greenstick cuttings produced significant callusing, woody cuttings had very little callus tissue at the end of the study. Despite the fact that the majority of greenstick cuttings exhibited callusing, only one greenstick cutting ended up producing root tissue. At the end of the six-month trial, this seedling was gradually toughened with increasing time out of the misting bed. Within one year the only cutting that had produced root tissue had died.

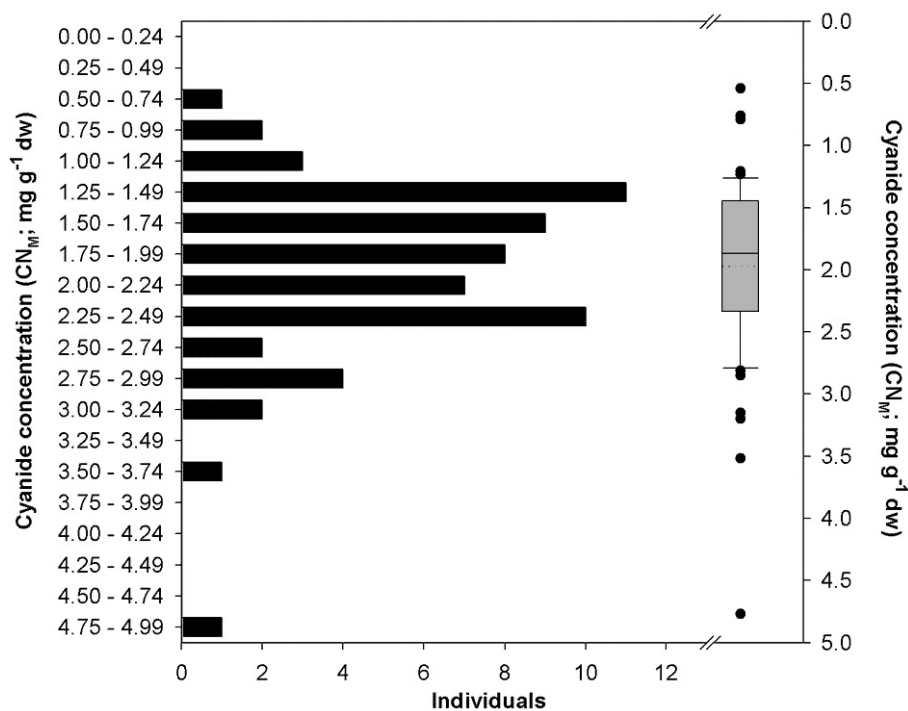
**Table 1:** Cutting morphology of field-sampled material of *Ryparosa kurrangii* raised in a misting bed in a tropical glasshouse. Greenstick and woody cuttings were sampled and leaves were trimmed (half-leaves) or entirely removed (no leaves) before treatment with hormones (NAA or IBA) and planting. Hormone treatment had no noticeable effect on cutting morphology.

	Greenstick cuttings	Woody cuttings
<b>Half-leaves</b>	Good shoot production. Good callus production. Root production rare (n = 1).	Moderate shoot production. Poor callus production. No root production.
<b>No leaves</b>	Moderate shoot production. Good callus production. No root production.	Poor shoot production. Poor callus production. No root production.



### 3.2 Cyanogenic quantification

The cyanogenic potential of mature leaves in *R. kurrangii* was quantified for a subset of individuals in the Myall Creek population ( $n = 61$ ). All trees tested in these populations were found to be cyanogenic (i.e. there were no acyanogenic morphs). Quantitative cyanogenic polymorphism was considerable with cyanide concentrations between 0.54 to 4.77 mg CN g<sup>-1</sup> dw leaf tissue (mean  $\pm$  1SE = 1.97  $\pm$  0.09; Figure 3).

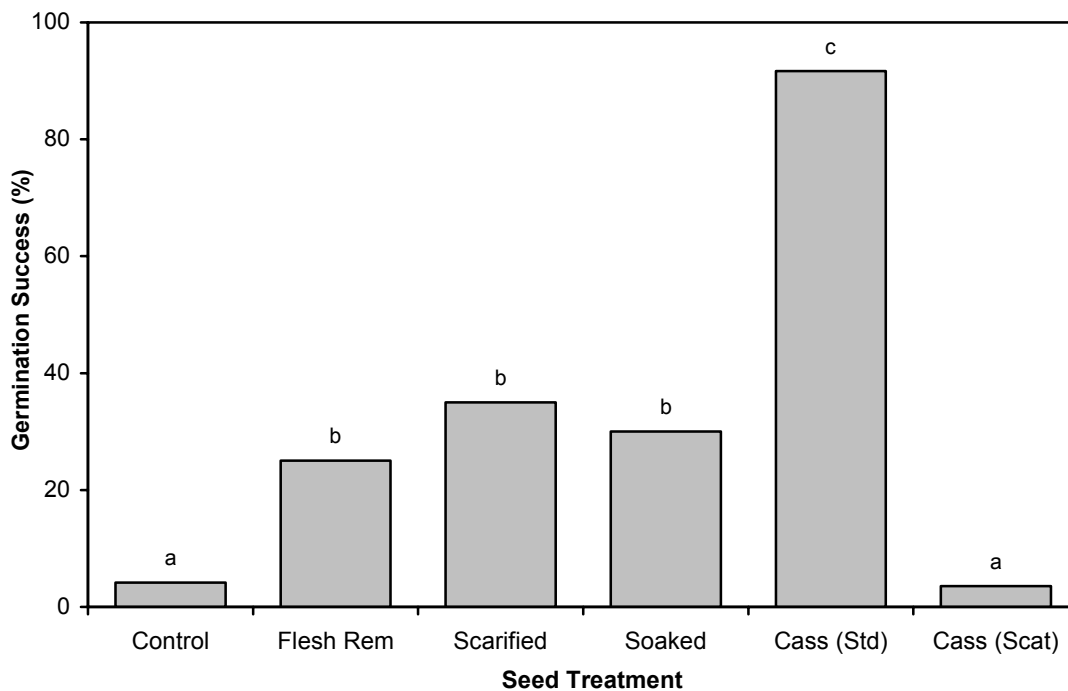


**Figure 3:** Variation in cyanogenic potential (CN<sub>M</sub>; mg g<sup>-1</sup> dw) for mature leaves of *Ryparosa kurrangii* individuals ( $n = 61$ ) located in the Daintree lowlands region in far north Queensland, Australia. The histogram shows individual CN<sub>M</sub> values while the boxplot indicates median (solid) and mean (dotted) CN<sub>M</sub> values.

The lack of success with vegetative propagation (Section 3.1) meant that no comparisons were able to be drawn between the cyanogenic potential of *R. kurrangii* individuals from which cutting material was sourced, and the strike rate of the cuttings produced.

### 3.3 Seed-frugivore interaction trials

Germination success (the emergence of a radicle from the endocarp) was recorded for each of the six seed treatments over a period of eight months and was significantly different between treatments ( $P < 0.01$ ; Figure 4). The 'Control' group had very poor germination success (4%). This was significantly lower than the flesh-chewed frugivory simulation ('Flesh Rem', 25%) and the cassowary gut passage simulation treatments ('Scarified', 35%; 'Soaked', 30%;  $P < 0.05$ ), which were in turn, not significantly different to each other ( $P > 0.05$ ). The highest germination success (92%) was recorded in standard-planted cassowary treatment seeds ('Cass (Std)'), significantly higher than any other treatment. In contrast, scat-planted cassowary treatment seeds had a low germination success (4%), which was not significantly different from that of the control treatment (Figure 4;  $P > 0.05$ ).

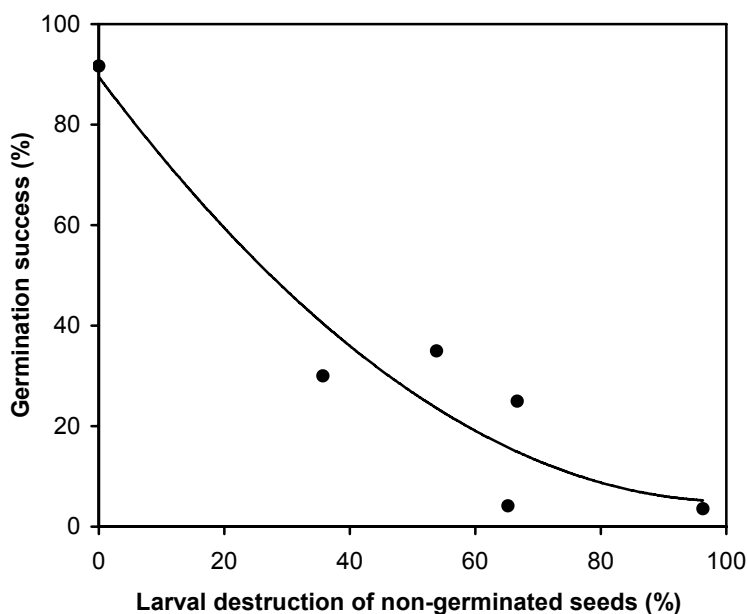


**Figure 4:** The effect of seed treatment on germination success in *Ryparosa kurrangii*. Germination (the emergence of a radicle from the endocarp) was significantly different between seed treatments ( $P < 0.01$ ) at the completion of the eight month trial. Seeds were either left intact inside the fruit ('Control',  $n = 24$ ), had the majority of the fruit flesh removed ('Flesh Rem';  $n = 20$ ), were thoroughly defleshed and then scarified ('Scarified';  $n = 20$ ) or soaked in water ('Soaked';  $n = 20$ ), or were consumed by cassowaries and planted normally ('Cass (Std)',  $n = 24$ ) or in a cassowary scat matrix ('Cass (Scat)',  $n = 28$ ). Treatments with different letters above the column are significantly different ( $P < 0.05$ ).

Those seeds that didn't germinate during the eight months were examined to assess viability and larval damage. No ungerminated seeds appeared viable at the completion of the trial. Those that were not infested by larvae had decomposing endosperm and shrunken embryos, while those that were infested had significant larval damage to all seed parts. The proportion of ungerminated seeds that were infested with larvae was then compared to the overall pre-trial infestation rate (55%) to see if infestation altered the propensity to germinate (95% confidence interval). This analysis assumes that infested seeds remained infested and uninfested seeds did not become infested during the trial. For 'Control', 'Flesh Rem', 'Scarified' and 'Soaked', the proportion of ungerminated seeds infested with larvae were 65, 67, 54 and 36% respectively (Table 2). Because these values were not significantly different from the pre trial infestation rate of 55%, it appears that for all the treatments that did not involve cassowary gut passage, infestation did not affect seed viability. For the 'Cass (Scat)' treatment, however, the assumption regarding infestation (above) did not hold because there were more infested seeds at the end of the trial than the beginning (Table 2). For the 'Cass (Std)' treatment, all but two seeds germinated indicating that both infested and uninfested seeds germinated, although no statistical analyses were possible on this treatment. However, when looking across treatments and taking into account all larval infestation, germination success was negatively correlated with the proportion of seed deaths attributable to larval destruction (Figure 5;  $y = -0.0083x^2 - 1.6695x + 89.5334$ ;  $r^2 = 0.91$ ;  $P = 0.03$ ).

**Table 2:** The fate of all ungerminated *Ryparosa kurrangii* seedlings after seed treatment and an eight month germination trial. Treatments are described in the legend to Fig. 2. At the conclusion of the trial, seeds were dissected and examined for fruit fly larval infestation. Before the trial, 55% of fruit showed signs of larval infestation and these fruit were randomly assigned in equal proportion to each of the six treatment groups. Differences between post-trial infestation rates in ungerminated seeds and the initial infestation rate were calculated using the Kolmogorov-Smirnov test ( $D_{\max}$ ) and are indicated with a \* ( $P < 0.05$ ; not possible for 'Cass (Std)' as  $n_{\text{expected}} < 5$ ).

Treatment	Infested		Uninfested		$D_{\max}$
	n	%	n	%	
Control	15	65	8	35	0.10
Flesh Rem	10	67	5	33	0.12
Scarified	7	54	6	46	0.01
Soaked	5	36	9	64	0.19
Cass (Std)	0	0	2	100	n/a
Cass (Scat)	26	96	1	4	0.41*



**Figure 5:** Overall germination success across seed treatments (as defined by the emergence of a radicle from the endocarp) in *Ryparosa kurrangii* seedlings as a function of larval destruction of ungerminated seeds. The polynomial regression for the relationship is significant ( $r^2 = 0.91$ ,  $P = 0.03$ ) and has the equation  $y = -0.0083x^2 - 1.6695x + 89.5334$ ;  $r^2 = 0.91$ ;  $P = 0.03$ .

## 4 Discussion

### 4.1 Vegetative propagation success and cyanogenic variation

When the cyanogenic potential of mature *Ryparosa kurrangii* leaves from individual trees in the field was quantified, considerable quantitative polymorphism was detected at a population level. Foliar cyanide concentration for the Myall Creek population averaged  $1.97 \text{ mg CN g}^{-1} \text{ dw plant tissue}$ , with concentrations generally above  $1 \text{ mg g}^{-1}$  and as high as  $4.77 \text{ mg g}^{-1}$  (Figure 3). Regrettably, the lack of success with establishing cuttings of uniform genetic background precluded any further investigation of the link between cyanogenesis (as an indicator of genetic diversity; Webber 1999) and propagation success rates. However, these results have clearly shown that cyanogenic polymorphism is an important

consideration in selecting genetic stock for revegetation work, as well as for conservation management planning of *R. kurrangii* populations in the Daintree region.

#### **4.2 Seed-frugivore interactions**

Consistent with the evidence that *R. kurrangii* fruit are ideally suited to interactions with large frugivores, it was found that removal of flesh, particularly by cassowary gut passage, significantly improved seed germination (Figure 4). Moreover, a lack of fruit treatment ('Control') resulted in very low seed germination success (4%), which is consistent with findings for a range of seeds from fruits with vertebrate dispersal syndromes (e.g. Howe 1990; Lamothe *et al.* 1990; Yagihashi *et al.* 1998). A number of reasons have been suggested for this effect, including seed germination inhibitors in the flesh (reviewed in (Bell 1999), endocarp abrasion (Barnea *et al.* 1991; Temple 1977; Traveset *et al.* 2001), vertebrate predation (e.g. Hulme 2002; Janzen 1970; Terborgh *et al.* 1993) and invertebrate predation (e.g. Dalling *et al.* 1997; Drew 1987). With regard to the latter, fruit fly larvae in 55% of the fruit before treatment, but this level was not sufficient to account for the low germination success. It seems that germination inhibitors in the flesh can also be ruled out as a factor, given that there was no significant difference between germination when flesh residue remained on the seed ('Flesh Rem') compared to when the flesh was removed ('Scarified' or 'Soaked'). The cassowary gut must, therefore, act in some additional way to improve the germination of *R. kurrangii* seeds. There is a shortage of information on digestive enzymes for ratites, and no available information for cassowaries (Angel *et al.* 1996); however, it is known that the pH of the emu gastro-intestinal tract varies from 2.5 - 3.2 in the proventriculus to 6.7 - 7.2 in the intestines (Angel *et al.* 1996; Davies 1978). It is possible that exposure to acidic conditions during gut passage may not only act favourably upon the endocarp to improve germination of seeds that are not infested with fruit fly larvae, but also impact on seed predation caused by these larvae (Drew 1987).

There has been only one other study involving cassowaries and the germination rate and success of rainforest seeds. This involved the dwarf cassowary (*Casuaris bennetti picticollis*) from Papua New Guinea, and it was found that germination was improved by cassowary gut passage, although this varied with plant species (Lamothe *et al.* 1990). In contrast to findings on *R. kurrangii*, manual flesh removal was able to simulate cassowary gut passage. For example, the germination rate of *Flacourita zippelii* seeds was over 90% for both cassowary treated and manually defleshed seeds, while that of *Prunus* sp. seeds was approximately 70% for both treatments (Lamothe *et al.* 1990).

There are three noteworthy attributes of cassowaries that are relevant to their potential role in the population dynamics of *R. kurrangii* and other species. First, the cassowary is the only co-occurring frugivore with sufficient gape width to internally process *R. kurrangii* seeds (Crome and Moore 1990; Noble 1991). Second, the gastro-intestinal (GI) tract of cassowaries is much shorter than in other ratites such as the emu and ostrich (Noble 1991) and does not have a crop, the food storage and prominent processing organ in other birds (Angel 1996; Fowler 1996). Together, these traits explain the relatively short gut retention time (GRT) for ratites, and cassowaries in particular. A short GRT means that the large majority of seeds ingested by cassowaries retain their viability and are voided whole with only the pericarps removed (Bentrupperbäumer 1992; Lott *et al.* 1995; Pannell 1997; Stocker and Irvine 1983). Finally, this apparently gentle digestive mechanism, also reported in other specialised frugivores (e.g. Murphy *et al.* 1993), may protect cassowaries from secondary metabolites commonly concentrated in fruit seeds, such as the cyanogens found in *R. kurrangii* (Stocker and Irvine 1983). Indeed, cassowaries do not

seem to be particularly affected by the consumption of fruits from cyanogenic plants. In the three known surveys on cassowary feeding preferences, seeds of several cyanogenic tree species have been identified in cassowary scats (Crome 1976; Lamothe *et al.* 1990; Stocker and Irvine 1983).

Given that removal of *R. kurrangii* fruit flesh also enhanced germination success, it is possible that ‘externally processing’ vertebrates play a significant role in plant dispersal. For the relatively large *R. kurrangii* fruit, these vertebrates are limited to musky rat-kangaroos and the white-tailed uromys, which are known to remove the majority of fruit flesh from and disperse seeds in caches of one or more at a distance from the parent tree (Dennis 2002; Theimer 2001). Fruit bats, however, are probably less important because they tend to prefer canopy fruits and rarely feed in the rainforest understorey (Kalko and Condon 1998; van der Pijl 1982).

Fruit fly also play an important role in determining the success of *R. kurrangii* seed germination. Interestingly, while fruit fly larval infestation in *R. kurrangii* fruits was a significant determinant of overall germination success across seed treatments (Figure 5), there was no detectable influence of larval infestation on the propensity of seeds to germinate within treatments (Table 2). In fact, post-infestation germination indicates that some initially infested *R. kurrangii* seeds remained viable after seed treatment. Having a relatively large seed size has been shown to maintain seed viability by providing a buffer against lethal seed predation (Dalling and Harms 1999; Harms and Dalling 1997; Mack 1998). It may be that a large seed size and vertebrate frugivore predation of fruit infesting invertebrates allows *R. kurrangii* seeds to maintain viability despite initial larval damage.

By spatially separating seeds and conducting the germination of treatment seeds in a glasshouse, the opportunity for post-treatment infestation in the majority of treatment groups was negligible. However there was an exception to this rule; in the ‘Cass (Scat)’ treatment, significant post-treatment infestation was clearly evident (Table 2). This predation may be explained by the scat matrix in which the seeds were planted. In captivity, healthy cassowaries exhibit a permanent state of diarrhoea due to the unusually high water and flesh content of exotic fruits in their diet (D. Black, pers. comm.). The combination of this attractive scat matrix used in the seed treatment trial and the fact that the scat material sat in a zoo enclosure for 24 h before collection meant that the chances of post-egestion larval infestation may be higher than expected in the field.

### **4.3 Conclusions**

To ensure the long-term survival of rare plant species, it is imperative to have no negative impact on natural populations during their study and documentation of their life history. Establishing glasshouse populations is often important in developing a detailed knowledge of rare plant species, and an efficient mechanism for generating multiple plants to study is the critical first step in this process. In this research on *R. kurrangii*, we were unable to find an efficient technique for vegetative propagation of the species using field-collected material. However, we were able to establish that a key process in improving *R. kurrangii* seed germination success is cassowary gut passage, and the degree of this success could not be replicated by simulated seed treatments. This finding is important for two reasons. Firstly, it appears that the rare *R. kurrangii* may have a close relationship with the increasingly endangered cassowary for long-term population sustainability. Secondly, it will allow for the establishment of a viable glasshouse population from sustainable (i.e. very limited) seed collection in the field. The resulting seedlings could

then be used for more intensive studies on vegetative propagation techniques, or for an investigation of tissue culture methods for establishing *R. kurrangii* plants of uniform genetic origin. The latter proposal would finally allow for the testing of the important link between plant genetic variation and cyanogenic polymorphism in this rare rainforest species.

## 5 References

---

- Angel CR (1996) A review of ratite nutrition. *Animal Feed Science Technology* **60**, 241-246.
- Angel CR, Scheideler SE, Sell JL (1996) Ratite nutrition. In 'Ratite Management, Medicine and Surgery'. (Eds TN Tully, Jr. and SM Shane) pp. 11-30. (Krieger Publishing: Malabar)
- Barnea A, Yom-Tov Y, Friedman J (1991) Does ingestion by birds affect seed germination? *Functional Ecology* **5**, 394-402.
- Bell DT (1999) The process of germination in Australian species. *Australian Journal of Botany* **47**, 475-517.
- Bentrupperbäumer J (1992) Conservation of a rainforest giant. *Wingspan* **8**, 1-2.
- Brinker AM, Seigler DS (1989) Methods for the detection and quantitative determination of cyanide in plant materials. *Phytochemical Bulletin* **21**, 24-31.
- Crome FHJ (1976) Some observation on the biology of the cassowary in northern Queensland. *Emu* **76**, 8-14.
- Crome FHJ, Moore LA (1990) Cassowaries in North-eastern Queensland: report of a survey and a review and assessment of their status and conservation and management needs. *Australian Wildlife Research* **17**, 369-385.
- Dalling JW, Harms KE (1999) Damage tolerance and cotyledonary resource use in the tropical tree *Gustavia superba*. *Oikos* **85**, 257-264.
- Dalling JW, Harms KE, Aizprúa R (1997) Seed damage tolerance and seedling resprouting ability of *Prioria copaifera* in Panamá. *Journal of Tropical Ecology* **13**, 481-490.
- Davies SJF (1978) The food of emus. *Australian Journal of Ecology* **3**, 411-422.
- Dennis AJ (2002) The diet of the musky rat-kangaroo, *Hypsiprymnodon moschatus*, a rainforest specialist. *Wildlife Research* **29**, 209-219.
- Drew RAI (1987) Reduction in fruit fly (Tephritidae: Dacinae) populations in their endemic rainforest habitat by frugivorous vertebrates. *Australian Journal of Zoology* **35**, 283-288.
- Fowler ME (1996) Clinical anatomy of ratites. In 'Ratite Management, Medicine and Surgery'. (Eds TN Tully, Jr. and SM Shane) pp. 1-10. (Krieger Publishing: Malabar)
- Gleadow RM, Woodrow IE (2002) Constraints on effectiveness of cyanogenic glycosides in herbivore defense. *Journal of Chemical Ecology* **28**, 1297-1309.
- Harms KE, Dalling JW (1997) Damage and herbivory tolerance through resprouting as an advantage of large seed size in tropical trees and lianas. *Journal of Tropical Ecology* **13**, 617-621.
- Howe HF (1990) Seed dispersal by birds and mammals: implications for seedling demography. In 'Reproductive Ecology of Tropical Forest Plants'. (Eds K Bawa and M Hadley) pp. 191-218. (Parthenon: London)
- Hruska AJ (1988) Cyanogenic glucosides as defence compounds. A review of the evidence. *Journal of Chemical Ecology* **14**, 2213-2217.
- Hulme PE (2002) Seed-eaters: seed dispersal, destruction and demography. In 'Seed Dispersal and Frugivory: Ecology, Evolution and Conservation'. (Eds DJ Levey, WR Silva and M Galetti) pp. 257-273. (CABI Publishing: Wallingford)
- Janzen DH (1970) Herbivores and the number of tree species in tropical forests. *The American Naturalist* **104**, 501-528.
- Jones RE, Crome FHJ (1990) The biological web - plant/animal interactions in the rainforest. In 'Australian Tropical Rainforests: Science - Value - Meaning'. (Eds LJ Webb and J Kikkawa) pp. 74-87. (CSIRO Publications: Melbourne)

- Kalko EKV, Condon MA (1998) Echolocation, olfaction and fruit display: how bats find fruit of flagelliferous cucurbits. *Functional Ecology* **12**, 364-372.
- Lambert JL, Ramasamy J, Paukstelis JV (1975) Stable reagents for the colourimetric determination of cyanide by modified König reactions. *Analytical Chemistry* **47**, 916-918.
- Lamothe L, Arentz F, Karimbaram R (1990) Germination of cassowary egested and manually defleshed fruit. *Paua New Guinea Journal of Agriculture, Forestry and Fisheries* **35**, 37-42.
- Lott RH, Harrington GN, Irvine AK, McIntyre S (1995) Density-dependent seed predation and plant dispersion of the tropical palm *Normanbya normanbyi*. *Biotropica* **27**, 87-95.
- Mack AL (1998) An advantage of large seed size: tolerating rather than succumbing to seed predators. *Biotropica* **30**, 604-608.
- Murphy SR, Reid N, Yan Z, Venables WN (1993) Differential passage time of mistletoe fruits through the gut of honeyeaters and flowerpeckers: effects on seedling establishment. *Oecologia* **93**, 171-176.
- Noble JC (1991) On ratites and their interactions with plants. *Revista Chilena de Historia Natural* **64**, 85-118.
- Pannell CM (1997) A new, cassowary-dispersed, species of *Aglaia* (*Meliaceae*, section *Amoora*) from Papua New Guinea. *Kew Bulletin* **52**, 715-717.
- Queensland Government (1992) 'Nature Conservation Act.' (Government Printer: Queensland)
- Sleumer HO (1958) Flacourtiaceae. In 'Flora Malesiana, Series 1 (Spermatophyta)'. (Ed. CGGJ Van Steenis) pp. 1-106. (Noordhoff-Kolff N V: Djakarta)
- Sokal RR, Rohlf FJ (1995) 'Biometry: The principles and practice of statistics in biological research.' (W H Freeman and Company: New York)
- Stocker GC, Irvine AK (1983) Seed dispersal by cassowaries (*Casuarius casuarius*) in North Queensland's Rainforests. *Biotropica* **15**, 170-176.
- Temple SA (1977) Plant-animal mutualism: coevolution with Dodo leads to near extinction of plant. *Science* **197**, 885-886.
- Terborgh J, Losos E, Riley MP, Bolaños Riley M (1993) Predation by vertebrates and invertebrates on the seeds of five canopy tree species of an Amazonian forest. *Vegetatio* **107/108**, 375-386.
- Theimer TC (2001) Seed scatterhoarding by white-tailed rats: consequences for seedling recruitment by an Australian rain forest tree. *Journal of Tropical Ecology* **17**, 177-189.
- Traveset A, Riera N, Mas RE (2001) Passage through bird guts causes interspecific differences in seed germination characteristics. *Functional Ecology* **15**, 669-675.
- van der Pijl L (1982) 'Principles of dispersal in higher plants.' (Springer-Verlag: Berlin)
- van Heel WA (1979) Flowers and fruits in Flacourtiaceae. IV. *Hydnocarpus* spp., *Kiggelaria africana* L., *Casearia* spp., *Berberidopsis corallina* Hook. f. *Blumea* **25**, 513-529.
- Webber BL (1999) Cyanogenesis in the tropical rainforest tree, *Ryparosa javanica*. Honours Thesis, The University of Melbourne.
- Webber, BL (2005) Plant-animal interactions and plant defence in the rainforest tree, *Ryparosa*. PhD Thesis, The University of Melbourne, Australia.
- Webber, BL, and Woodrow, IE (2006) Morphological analysis and a resolution of the *Ryparosa javanica* species complex (Achariaceae) from Malesian and Australian tropical rainforests. *Australian Systematic Botany* **19**: 541-569.
- Willson MF, Irvine AK, Walsh NG (1989) Vertebrate dispersal syndromes in some Australian and New Zealand plant communities, with geographic comparisons. *Biotropica* **21**, 133-147.
- Yagihashi T, Hayashida M, Miyamoto T (1998) Effects of bird egestion on seed germination of *Sorbus commixta*. *Oecologia* **114**, 209-212.