Pod classification and its role in rubber vine (*Cryptostegia grandiflora*) germination and emergence

F.F. Bebawi and **J.R. McKenzie**, Tropical Weeds Research Centre, Queensland Department of Natural Resources, PO Box 187, Charters Towers, Queensland 4820, Australia.

Summary

A method for classifying seed pods of the woody weed rubber vine (*Cryptostegia grandiflora* (Roxb.) R.Br.) was developed as an aid for evaluating the effect of imposed control techniques on seed stored in pods. Rubber vine pods were qualitatively classified into four classes: juvenile, immature, mature and ripe. Pod characteristics and seed behaviour within the four designated classes were assessed to determine whether they displayed distinct characteristics. Pod features measured were fresh weight (FWT), dry weight (DWT), relative water content (RWC), dorsal length (DL), dorsal width (DW) and dorsi-ventral width (DVW). Both seed germination and seedling emergence were assessed for seeds from each of the pod classes. Four out of the six pod characteristics (FWT, RWC, DL, DVW) differed significantly from one another. Seeds from mature and ripe pods averaged 99% germination compared with 13.7 and 0.3% for immature and juvenile pods respectively. Similarly, seedling emergence of mature and ripe pods averaged 92%, with both juvenile and immature pods averaging less than 2%. Mean germination and seedling emergence rates increased with seed maturity stage.

Introduction

Rubber vine, native to Madagascar, was first introduced into Australia as an ornamental shrub around 1875 (Anderson 1993). In the early 1940s, it was cultivated as a potential source of rubber (Howard 1994). Since then, it has successfully invaded the rangelands of tropical and subtropical Queensland, particularly in areas where the annual rainfall is between 400 and 1400 mm (McFadyen and Harvey 1990). It is a poisonous weed (McGavin 1969) and displays a bimorphic growth habit (Grice 1996). Along watercourses, it climbs over dense tall trees forming what is commonly described as 'rubber vine towers'. In the absence of adjacent tall trees to support its climbing habit, rubber vine assumes a shrubby growth form which is commonly described as 'free stands'.

Chippendale (1991) estimated that rubber vine cost primary industry in Queensland around eight million dollars per annum. However, this figure did not include the cost of chemicals used for control and is therefore a conservative estimate.

There are a range of existing control techniques for rubber vine, with refinement of these and development of new techniques being continually undertaken. One area that has received little attention to date has been the impact of imposed control techniques on seed located within pods on the plant at the time of application. For example, numerous studies (Harvey 1981, Harvey 1982, Harvey 1987a,b,c, Harvey 1989, McFadyen and Harvey 1990, Vitelli 1990, Vitelli *et al.* 1994) have been undertaken on the efficacy of foliar herbicides on rubber vine, but recordings have generally been restricted to measuring the impact on the plant itself. Whether these foliar applications affected seed located within pods at the time of application was not identified. Likewise, the impact of fire on the viability of seeds stored in pods is not known and is of particular interest for wet season burns, when a large number of pods may be present on the plant at the time of burning. In the case of rubber vine, answering these questions becomes complicated as flowers and fruit, although predominantly produced between the months of December and May (Brown *et al.* 1996) in high rainfall areas, can be produced throughout the year if suitable environmental conditions occur (Curtis 1946, Grice 1996). Consequently, at any one time it is possible to find pods of varying maturity on individual rubber vine plants (Parsons and Cuthbertson 1992). How pods of varying maturity respond to treatments such as the use of chemicals and fire has not been investigated. Before these questions can be fully answered it is necessary to first develop a classification scheme which will cover all pods present on a plant at any one time. Such a classification would be useful for future ecological studies, allowing the responses of different pod classes to be tested against a range of control strategies.

Literature on systems describing fruit or pod development is very scarce. However, phenological indices have been used as a basis for physiological work. West and Wein (1971) developed a phenology index for *Atriplex nuttallii* and *Hilaria jamesii* where characters related to flowering, fruit development, and fruit dissemination were used. To date, descriptions of rubber vine pods in the literature have been relatively general and have referred more to the size and shape of the pods, than variations in pod characteristics with maturity. Parsons and Cuthbertson (1992) described rubber vine fruit as a large greenish pod (follicle), 10–12 cm long, 3–4 cm wide, produced in pairs horizontally opposed and diverging from the tip of a short but common stalk, sharply 3-angled and tapering into a long beak. Tomley (1995) reported the pods to be 10–15.4 cm long and 2.1–4 cm in diameter.

Rubber vine is a prolific seeder. Mature plants are capable of producing as many as 30 pods at any one time, with each pod containing between 270 and 350 seeds (Symontorone 1943, Grice 1996). Consequently, a dense one-hectare infestation of rubber vine may produce one billion seeds annually (Alford 1992). When combined with germination levels greater than 90% (Grice 1996), the reproductive potential of rubber vine is fully realized.

This paper presents a pod classification system for rubber vine based on qualitative features. Pod characteristics and seed behaviour of four designated classes are compared and the implications for development of control techniques discussed.

Materials and methods

Plant material

Approximately 1000 rubber vine pods were randomly collected during June 1996 from rubber vine populations located on cattle properties, roadsides, and creeks in the vicinity of Charters Towers (Lat. 20° 5'S, Long. 146° 16'E). It is interesting to note here that during our collection trips we came across rubber vine fruits (pods / follicles) consisting of three basally united pods (Figure 1). The occurrence of this phenomenon was very rare. Nevertheless, it contrasted with the conventional fruit form, which usually consisted of two basally-united pods and the rare single pod form reported by other workers (Tomley 1995, Grice 1996).

Qualitative features

Three distinct qualitative features (pod colour, pod woodiness and woody pods without or with fibrous woody patches) commonly observed within the bulk sample of pods were chosen to develop a three tiered classification system based on primary, secondary, and tertiary pod groups (Figure 2).

The primary pod group was dichotomously divided on the basis of colour: green or brown. The secondary group arose from pods which were green in colour. These were dichotomously subdivided on the basis of pod woodiness:

woody or non-woody. Those that were woody, i.e. very hard in texture and resisting bending when squeezed between fingers, were shifted to the tertiary pod group. At this stage the pods were still green in colour but woody in texture. Finally, the tertiary group was dichotomously sub-divided on the basis of the degree of pod woodiness: with external brown woody fibrous patches or without external brown woody fibrous patches.

Pod characteristics

To determine whether the four pod classes selected on a qualitative basis had distinct quantitative features, pod fresh weight (FWT), dry weight (DWT), relative water content (RWC), dorsal length (DL), dorsal width (DW), and dorsi-ventral width (DVW) measurements were taken on pods of each of the four pod classes.

All pod measurements reported in this study represent measurements of a double basally-united pod, hereafter referred to as double pod (Figure 3). DL, DW, and DVW parameters were separately taken for each pod of the double pod but later added to give pod DL or averaged to give pod DW and DVW. The DW and DVW measurements were taken at a distance of 3 cm from the fusion point of the double pod. Measurements were taken from individual pods constituting the double pod because the orientation of the pod dimensions was not exactly opposite but manifested a slight concavity on the pod dorsal side.

Double pods collected from the field were first sorted into their designated classes. For each pod class, 200 pods were then randomly selected and subjected to quantitative measurements. Pod FWT, DL, DW, and DVW were initially recorded. Pods were then placed in size 2 (12 cm × 25 cm) Trojan Satchel paper bags and air-dried for 60 days before being oven dried at 40°C to constant weight. The choice of this temperature was to prevent seed damage within the pod. The RWC was calculated gravimetrically using the formula:

$$\% RWC = (FWT - DWT) / FWT \times 100$$

For each feature measured, data were subjected to analysis of variance. Where F values were significant at the 5% level, means were compared using tests for least significant differences (LSD). Statistical evaluation of parameter stability was performed by regression analysis using the regression co-efficient 'b' in a Finlay and Wilkinson (1963) style analysis, regressing parameter totals on block totals of all parameters. In such an analysis a regression coefficient value of 1.0 indicates average stability whereas values >1.0 indicate low stability and values <1.0 indicate high stability.

Seed behaviour

Germination and seedling emergence studies were undertaken to determine what proportion of seeds within pods of the identified classes had the ability to germinate and emerge when buried in soil. Both germination and emergence was tested within six months from the time of collection.

Germination. For each of the four pod classes, six replicates of 50 undamaged seeds were obtained by sub-sampling from bulk samples. Seeds were surface sterilized against fungal growth for 5 min in a 1% solution of sodium hypochlorite before being placed in 9 cm petri-dishes filled with 10 mL of deionized water. Four petri-dishes, representing each of the four pod classes, were randomly stacked in each of six TA650 Smith and Nephew plastic containers (9 × 10.7 cm in diameter) with the lid sealed to reduce evaporation. The containers were placed in black plastic bags to ensure exclusion of light because rubber vine seeds have a definite preference for germination in the dark (Sen 1968). Next the bags were placed in a controlled environment glasshouse set at a day and night temperature of 30±1°C and 20±1°C, respectively.

Grice (1996) indicated that 90% of rubber vine seed would germinate within 10 days of moisture becoming available. In this study, germinated seeds (identified by radicle emergence) were counted and removed daily for 15 days to ensure that seed of all treatments had adequate time to germinate. Cumulative germination percentages were calculated on the basis of total seed numbers. Statistical analysis was performed on arcsin transformed data, which was later back-transformed. Mean germination rate was calculated mathematically by dividing cumulative germination by number of days required to reach maximum germination.

Seedling emergence. For each of the four pod classes, 10 replicates of 20 undamaged seeds were obtained by sub-sampling from bulk samples. Individual seed samples were sown into 285 mL plastic tumblers filled with coarse sand. Before sowing, the bases of the tumblers were perforated to provide drainage, with filter paper (42.5 mm diameter) then inserted inside each tumbler to prevent sand loss. Seeds were carefully placed on the sand surface and covered with additional sand to a depth of 1 cm. Following sowing, all tumblers were placed in a controlled environment glasshouse set at a day and night temperature of 30±1°C and 20±1°C respectively. All tumblers were irrigated daily to field capacity. This was achieved by adding water until it drained off at the perforated base of the tumbler.

In Queensland, freshly collected rubber vine seeds planted in soil or potting mix in a glasshouse held at 30°C maximum, 15°C minimum, exhibited germination (emergence) levels in excess of 95% within 6 to 10 days (Dale 1980). In the present study, emerged seedlings (identified when the cotyledons became visible) were counted and removed daily for 27 days to ensure that seedlings from all pod classes had adequate time to emerge. Cumulative emergence percentages were calculated on the basis of total seed numbers. Statistical analysis was performed on arcsin transformed data, which was later back-transformed. Mean seedling emergence rate was also calculated using the method described for seed germination.

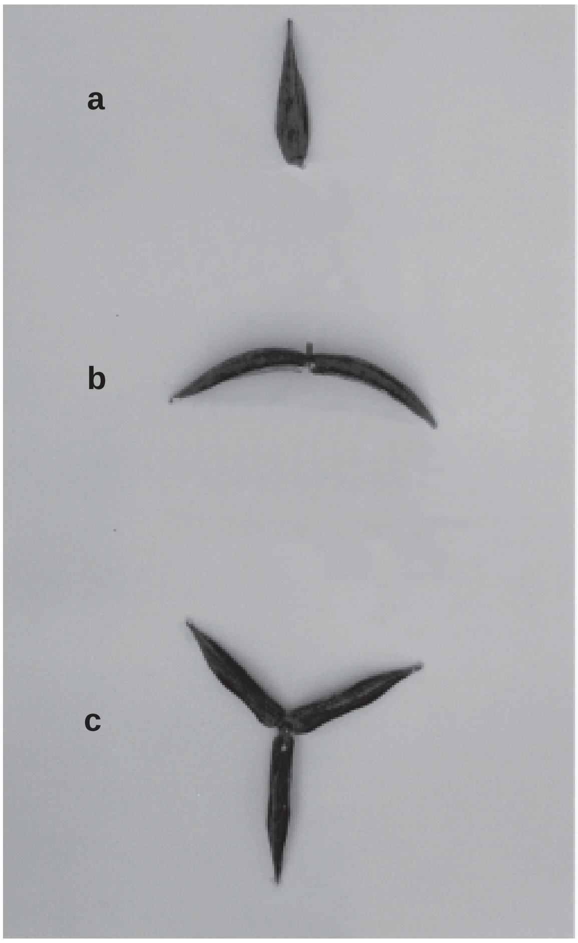

Figure 1. Three types of pods: (a) single pod, (b) double pod (two basally united pods), and (c) triple pod (three basally united pods) which may be found on rubber vine plants.

Results and discussion

This study has developed a process for classifying rubber vine pods into four classes based on their level of maturity. The process undertaken involved selection of distinct visual and textural features associated with each of the four classes. These qualitative features were used as a basis for pod characteristics and seed behaviour assessment of the designated pod classes.

Pod characteristics

Significant differences (P<0.05) occurred between pod classes for all quantitative features measured (Table 1). Furthermore, in four out of the six features (FWT, RWC, DL, DVW) all pod classes differed significantly from one another.

Pod FWT peaked in the immature pod class at 71.4 g and decreased thereafter, with mature and ripe pods weighing 6 and 68% less than immature pods respectively. In contrast, pod DWT was positively correlated ($r^2 = 0.92$) with pod class maturity. The DWTs of immature, mature, and ripe pods were 2.5, 3.4, and 3.5 times greater than those of the juvenile pod class. Pod RWC was inversely correlated ($r^2 = -0.55$) with pod class maturity. RWC decreased gradually between juvenile, immature and mature pod classes and then markedly between mature and ripe pods. By the time pods reached the ripe stage they had lost, on average, 94% of their maximum water content, which occurred in juvenile pods.

The relative water content of pods may play an important role in determining the survival of seeds during fires. Whilst the tolerance of seed to heat generally increases as the moisture content of the seed decreases (McKell *et al*. 1962), the outcome may be different for dry seed stored in pods with low RWC at the time of exposure. It is envisaged that ripe pods may be more easily ignited because of their very dry state, and consequently could be consumed by the fire as a part of the fuel load. Pods with higher RWC, on the other hand, may be less susceptible to ignition. The development of a pod classification scheme will now allow these questions to be answered. By exposing pods within the different classes to fires the impact on the pods themselves and the viability of seed within pods can be recorded. Differential responses between pod classes could then be directly related to the distinct features of the classes.

The physical size of pods generally increased between juvenile and mature classes, with a decline in size then occurring between mature and ripe pods (Table 1). Measurements of DL, DW, and DVW of mature pods were 29, 29, and 15% greater than juvenile pods respectively. Pod DL, DW, and DVW then declined by 6, 16, and 30% respectively between mature and ripe classes. The significant decline in pod DVW through shrinkage signals the onset of the ripening process.

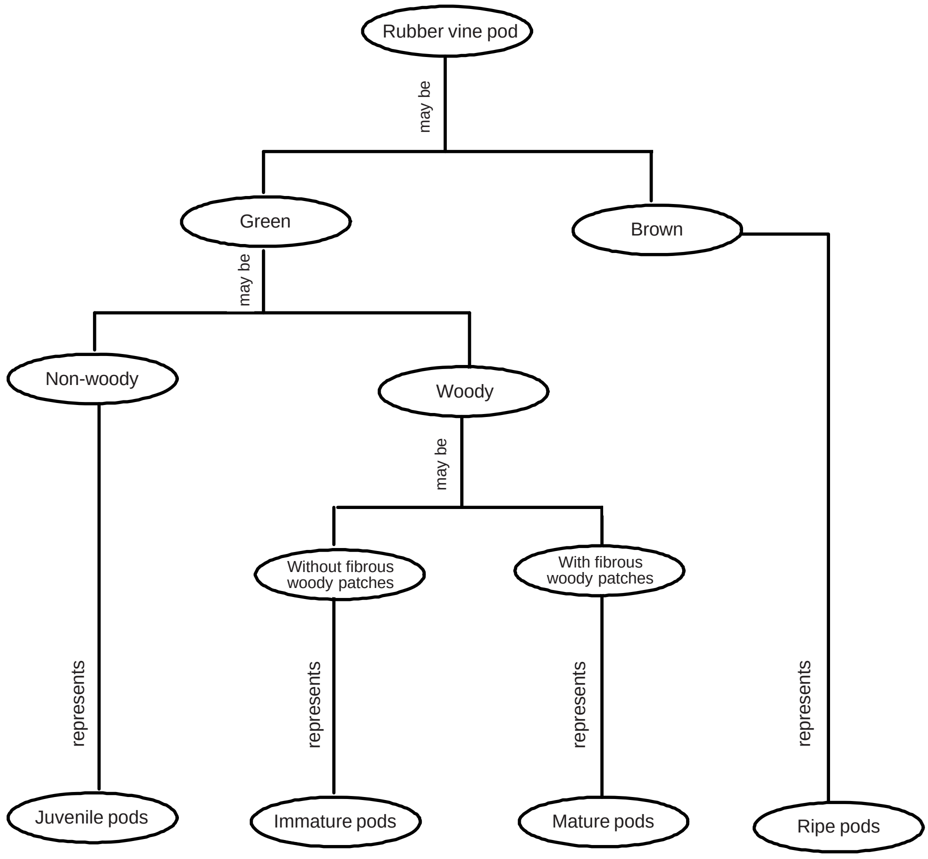

Figure 2. Classification scheme of rubber vine pods.

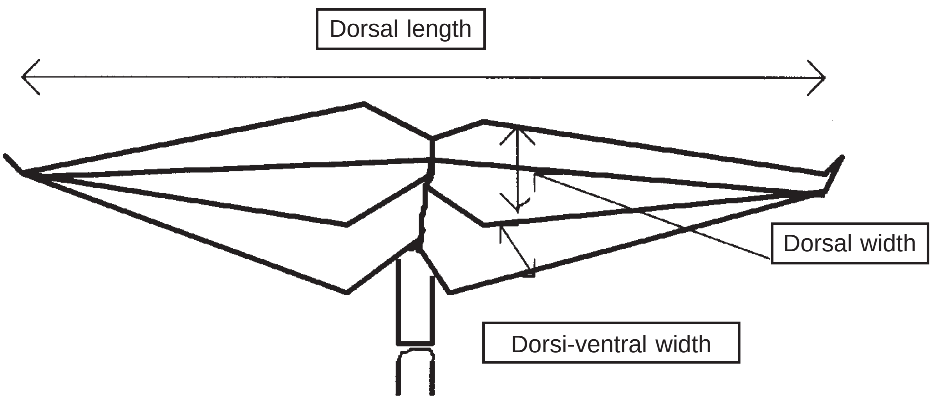

Figure 3. Dorsal length, dorsal width, and dorsi-ventral width aspects of a double pod of rubber vine.

Measurements of both pod DL (when halved to give the average length of the single pod) and pod DW concur with those reported by Parsons and Cuthbertson (1992) and Tomley (1995).

Regression lines obtained by regressing block totals of DL, DW, and DVW against DL, DW, and DVW totals indicate that pod DL had the highest slope, with a 'b' value of 0.81 compared to pod DVW, which had the lowest slope with a 'b' value of 0.07 (Figure 4). These values mean that pod DL was the least stable pod parameter and therefore the most affected by pod development stage, compared to pod DVW. The pod DW slope value of 0.10 indicates that pod DW was less sensitive to pod maturity processes compared to pod DL and pod DVW. Regression analysis also identified a strong positive correlation between pod FWT and pod DWT ($r^2 = 0.88$), pod DVW ($r^2 = 0.98$), and RWC ($r^2 = 0.95$) and a weak correlation between pod FWT and pod DL ($r^2 = 0.48$).

Seed behaviour

Germination. Highly significant differences (P<0.01) were detected in seed germination between pods of different maturity stages (Table 1). Seed germination and mean germination rate increased with seed maturity stage (Table 1, 2). While all

Table 1. (a) Mean fresh weight (FWT), dry weight (DWT), relative water content (RWC), dorsal length (DL), dorsal width (DW), and dorsi-ventral width (DVW) of a double pod of rubber vine as affected by pod maturity stage; (b) Germination and seedling emergence response of rubber vine seed obtained from juvenile, immature, mature, and ripe pods. Means followed by the same letter are not significantly different at P<0.05.

	(a) Pod characteristics						(b) Seed behaviour	
Pod maturity stage	FWT (g)	DWT (g)	RWC (%)	DL (cm)	DW (cm)	DVW (cm)	Cumulative germination (%)	Seedling emergence (%)
Juvenile	38.4 c	6.0 c	84.4 a	19.2 c	2.8 c	2.7 c	0.3 c	1.0 b
Immature	71.4 a	15.5 b	78.3 b	23.9 b	3.6 a	3.3 a	13.7 b	1.5 b
Mature	66.9 b	20.4 a	69.5 c	24.8 a	3.6 a	3.1 b	99.9 a	89.0 a
Ripe	22.7 d	20.8 a	8.8 d	23.4 b	3.0 b	2.1 d	99.9 a	94.7 a

pod classes produced some germinable seed, mature and ripe pods averaged 333 and 7 times greater germination than immature and juvenile pods respectively. Zimmerman and Weis (1982) working on fruits of *Xanthium strumarium* also found a strong positive correlation between fruit maturity and germination percentage.

Assuming that rubber vine pods contain an average of 279 seeds (Grice 1996), the number of germinable seeds in juvenile and immature pods would be in the range of one to 39. While one seed may appear negligible, it has the potential to germinate and grow into a plant capable of producing more than 8000 wind-dispersed seeds in a single reproductive episode (Grice 1996).

Seedling emergence. As for seed germination, highly significant differences (P<0.01) were detected in seedling emergence between pod classes (Table 1). In contrast to seed germination, no significant differences (P>0.05) in emergence occurred between juvenile and immature pod classes or between mature and ripe pod classes. However similar to seed germination, seedling emergence rate increased with seed maturity stage (Table 2). Seedling emergence was, on average, 73 times greater for seeds from mature and ripe pods than from juvenile and immature pods. Results thus indicate that the potential of rubber vine seed to germinate and emerge increases with pod maturity stage.

Conclusions

It is concluded from the present study that seed pods of rubber vine can be classified into four classes: juvenile, immature, mature and ripe pods, with each class displaying many distinct qualitative and agronomic features. Of particular importance is the high germinability and emergence potential of seeds within mature and ripe pods, which should be targeted when developing control techniques. Nevertheless, the staggered production of rubber vine pods all year round means that prevention of recruitment will be difficult, unless all reproductive plants are killed, as the seed bank is continually being replenished. Such a strategy provides a fail safe mechanism for the survival and expansion of rubber vine in hazard ridden environments.

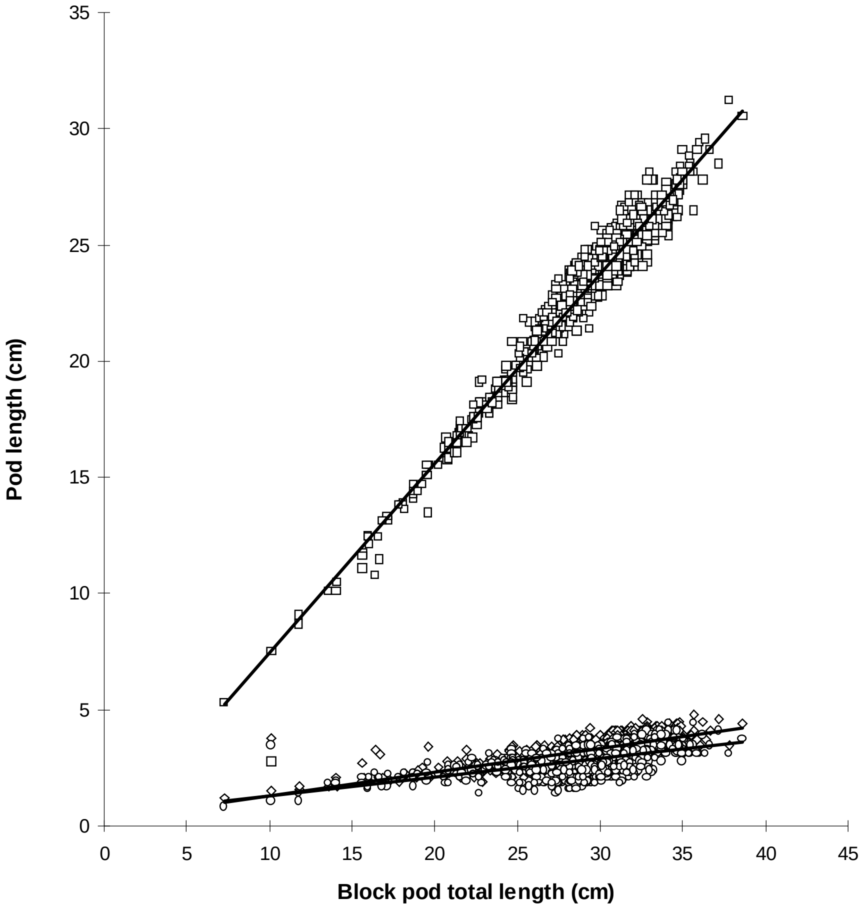

Figure 4. Regression lines of dorsal length (DL), dorsal width (DW), and dorsi-ventral width (DVW) of rubber vine pods. ◇ DW (y=0.1036x +0.4793; R2=0.5885). □ DL (y=0.8164x - 0.7334; R2=0.9565). ○ DVW (y=0.0799x + 0.4793; R2=0.3397).

Table 2. Mean germination and seedling emergence rate of ripe, mature, immature, and juvenile seeds of rubber vine expressed as per cent per day.

	Seed maturity stage			
Parameter	Ripe	Mature	Immature	Juvenile
Mean germination rate	14.2	9.10	1.50	0.04
Mean seedling emergence rate	4.30	3.36	0.16	0.09

Acknowledgments

The authors wish to thank Dr. T. Stanley, Dr. S.D. Campbell, Mr. J.S. Vitelli, and Mrs. B. Madigan for their encouragement and support. The authors also wish to extend their thanks to Dr. Dane Panetta for his constructive comments of the manuscript and the Von Wald family and the Lavery family for their kind permission to use their cattle properties for rubber vine research purposes.

References

Alford, T. (1992). Introduction. *In* 'Control and management of rubbervine', ed. Bob Shepherd, p. 2. (Queensland Department of Primary Industries, Brisbane).

Anderson, E. (1993). 'Plants of central Queensland'. (Queensland Department of Primary Industries, Brisbane).

Brown, J.R., Grice, A.C., Vitelli, J., Lindsay, A.M., Campbell, S.D., Jeffrey, P.L., Keir, M. and Barker, M. (1996). Managing tropical woodlands to control exotic woody weeds. Final Report, MRC Project. CS219, CSIRO Tropical Agriculture, Aitkenvale, Queensland.

Chippendale, J.F. (1991). The potential returns to research into rubber vine (*Cryptostegia grandiflora* R.Br.) in north Queensland. M.Agric.Sci. Thesis, University of Queensland.

Curtis, J.T. (1946). Some factors affecting fruit production by *Cryptostegia*. *American Journal of Botany* 33, 763-9.

Dale, I.J. (1980). Factors affecting the distribution of rubber vine (*Cryptostegia grandiflora* R.Br.) in north Queensland. M.Agric.Sci. Thesis, University of Queensland.

Finlay, K.W. and Wilkinson, G.N. (1963). The analysis of adaptation in a plant breeding programme. *Australian Journal of Applied Research* 14, 742-54.

Grice, A.C. (1996). Seed production, dispersal and germination in *Cryptostegia grandiflora* and *Ziziphus mauritiana*, two invasive shrubs in tropical woodlands of northern Australia. *Australian Journal of Ecology* 21, 324-31.

Harvey, G.J. (1981). Studies on rubber vine (*Cryptostegia grandiflora*): II Field trials using various herbicides. *Australian Weeds*, 1, 3-5.

Harvey, G.J. (1982). Studies on rubber vine (*Cryptostegia grandiflora*): IV The effects of herbicide formulations, carrier, and placement on control of rubber vine. *Australian Weeds* 1, 3-5.

Harvey, G.J. (1987a). A comparison of different salt formulations of MCPA, 2,4-D, 2,4,5-T, picloram and dicamba, and of salt additives to these herbicides on rubber vine. Proceedings 8th Australian Weeds Conference, Sydney, pp. 39-44.

Harvey, G.J. (1987b). Low-volume application of herbicides for control of rubber vine. Proceedings 8th Australian Weeds Conference, Sydney, pp. 45-8.

Harvey, G.J. (1987c). The toxicity of picloram, dicamba, 2,4-D and other growth regulating herbicides to rubber vine. Proceedings 8th Australian Weeds Conference, Sydney, pp. 49-54.

Harvey, G.J. (1989). Biological activity of 2,4-D esters on rubber vine (*Cryptostegia grandiflora*): Dependence on vapour pressure and molecular weight and isomerism of the alcohol substituent. *Australian Journal of Agricultural Research*, 40, 685-90.

Howard, R.A. (1994). The role of botanists during World War II in the Pacific Theatre. *Bot. Review* 60,197-257.

McFadyen, R.E. and Harvey, G.J. (1990). Distribution and control of rubber vine, *Cryptostegia grandiflora*, a major weed in Queensland. *Plant Protection Quarterly* 5, 152-5.

McGavin, M.D. (1969). Rubber vine (*Cryotostegia grandiflora*) toxicity for ruminants. *Queensland Journal of Agric. Anim*., 26, 9-19.

McKell, C.M., Wilson, A.M. and Kay, B.L. (1962). Effective burning of rangelands infested with Medusahead. *Weeds* 10, 125-31.

Parsons, W.T. and Cuthbertson, E.G. (1992). 'Noxious weeds of Australia', pp. 176-9. (Inkata Press, Melbourne).

Sen, D.N. (1968). Ecology of desert plants and observations on their seedlings. II. Germination behaviour of seeds in Asclepiadaceae. *Osterr. Bot. Zeit*. 115, 18-27.

Symontorone, R. (1943). Notes on the *Cryptostegia* plant. *Tropical Agriculture* 20, 195-7.

Tomley, A.J. (1995). The biology of Australian weeds 26. *Cryptostegia grandiflora* R.Br. *Plant Protection Quarterly*, 10, 122-30.

Vitelli, J.S. (1990). Effect of soil moisture on the efficacy of 2,4-D and metsulfuron-methyl on rubber vine – preliminary investigation. Proceedings 9th Australian Weeds Conference, Adelaide, pp. 407-10.

Vitelli, J.S., Mayer, R.J., and Jeffrey, P.L. (1994). Foliar application of 2,4-D/picloram, imazapyr, metsulfuron, triclopyr/picloram, and dicamba kills individual rubber vine (*Cryptostegia grandiflora*) plants. *Tropical Grasslands* 28, 120-6.

West, N.E. and Wein, R.W. (1971). A plant phenological index technique. *Bioscience* 21, 116-17.

Zimmerman, J.K. and Weis, M. (1982). Fruit size variation and its effects on germination and seedling growth in *Xanthium strumarium*. *Canadian Journal of Botany* 61, 2309-15.