Seed production and maturation of *Limnocharis flava* (L.) Buchenau in the field and glasshouse

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Summary *Limnocharis flava* is the target of an eradication program but little is known about the reproductive biology of this aquatic plant. Field and glasshouse studies showed that seedlings exhibited relatively high survival (64%) and that fruits containing over 1000 seeds could be produced on young plants in a minimum of 46 days, at any time of the year. Mature fruits, follicles and seeds were also found to be buoyant. These findings were incorporated into an eradication program and influenced the frequency of infestation monitoring.

Keywords *Limnocharis flava,* eradication, reproduction, aquatic.

INTRODUCTION

Limnocharis flava (L.) Buchenau. (limnocharis) (Limnocharitaceae) is an anchored, perennial aquatic herb native to tropical areas of Central and South America. It is a serious weed of irrigated rice paddies and irrigation channels in Indonesia and Malaysia and has invaded wetland areas in Sri Lanka, India and many South East Asian countries (Waterhouse 2003). Limnocharis was first discovered in Australia in 2001 and was included in a tropical weed eradication program which commenced that year; the location, size and management of Australian limnocharis infestations are described by Brooks and Galway (2006).

Under an eradication program, all infestations need regular monitoring and control to prevent fresh seed input, as additional seed increases the time needed to exhaust the seed bank (Panetta 2004). Limnocharis produces flowers on short pedicels at the end of a stalk (peduncle) growing from the base of the plant. Flowers mature to form a buoyant compound dry papery fruiting aggregate (fruit) with wedge-shaped follicles. Each fruit can contain 1000 seeds (Kotalawala 1976). Vegetative plantlets (bulbils) are also produced at the apex of flower stalks (Nayar and Sworupanandan 1978), but these tend to establish close to parent plants (Song *et al.* 2000). Controlling plants prior to flowering also prevents plantlets forming.

The time to reach maturity drives the frequency with which infestations are revisited. Little information has been published on the rate of limnocharis reproduction (J. Weber unpubl. report), apart from field and glasshouse observations in southern China (Song *et al.* 2000). Research conducted in conjunction with an eradication program can provide local data on the minimum age to maturity (Vitelli *et al*. 2006), thereby lowering the chance of 'reproductive escapes'. In this study the reproductive development of limnocharis was observed in field and glasshouse situations to determine the reproductive biology at a local level.

MATERIALS AND METHODS

Field Field studies were conducted on part of a limnocharis infestation at Feluga (145°56'40"E, 17°52'53"S, where dense clumps and scattered limnocharis plants were discovered over 0.25 ha in July 2003. This site is a permanent spring fed stream in a tropical lowland environment receiving an average annual rainfall of 4000 mm and mean annual maximum and minimum temperatures of 27.4 and 18.2°C respectively. Beyond the research area limnocharis was controlled by the eradication program staff.

Between 22 December 2003 and 20 December 2005, 90 newly emerged, similarly sized seedlings (smallest identifiable, four leaf stage) in the research zone were tagged. While we aimed to identify and tag ten new seedlings every second month, variable field emergence limited the tagged plants to the following dates and plant numbers. **2003:** 22/12 (7 plants). **2004:** 24/2 (1), 3/6 (9), 21/7 (14), 22/9 (10), 1/12 (10). **2005**: 25/1 (9), 20/5 (4), 27/9 (6), 20/10 (11), 20/12 (9). Tagged seedlings were observed monthly for signs of maturity and were controlled if a flowering stalk started to form.

Glasshouse Observations on seed production were conducted in a quarantine glasshouse facility in Charters Towers (146°16'04"E, 20°05'43"S). Field collected limnocharis seedlings (from Black River,

146°37'43"E, 19°12'33"S) were grown in individual pots that were kept waterlogged in troughs of fresh aerated water, under a 30°C, 12 h day and 25°C, 12 h night regime. In December 2003 and January 2004, the number of fruit buds (immature fruit) on the first two flowering stalks of 21 plants was counted. Time between flowering and seed set was recorded for 10 of these stalks. Fruits from an additional 125 cultivated mature stalks were collected from the same plants on 25 April and 14 May 2004. The length and width of 50 mature fruits were measured and the number of follicles and seeds from each of the 50 fruits were recorded. A random sub-sample of fresh fruits, follicles and seeds were also weighed.

RESULTS

Field The minimum time for a newly emerged seedling to produce a flowering stalk was 23 days. Two other plants produced flower stalks 35 days after tagging (Figure 1). The average time to flowering was 113.8 days (±SD 63.6, median 90 days), with 61% of the plants flowering in less than 100 days (Figure 1). These times may be slight underestimates as the exact age of field plants when tagged was not known. The date of tagging was not correlated with the period taken to flower (data not shown). Flowering of the remaining 39% of plants occurred between 111 and 280 days after tagging. Thirty-four tagged plants died before reaching maturity; including three plants which died more than 305 days after tagging without producing a flower stalk. 62% of plants survived to maturity.

Glasshouse The average time from flowering to seed set was 28.9 days (SD ±6.77, n = 10), with a range of 23 to 45 days (median 26.5 days). The 23 day minimum value was recorded on one stalk, with two stalks maturing in 24 days.

The first flower stalks contained fewer buds than those produced by plants five months later (Table 1). Fruits produced under glasshouse conditions contained an average of 15 follicles and 1038 seeds (Table 2). First flowering and older stalks could produce between 7000 and 9200 seeds per stalk respectively. The 11–16 mm diameter mature fruits weighed an average of 0.185 g (n = 11); the average follicle weight was 0.0115 g (n = 8). The total weight of 200 seeds was 0.0417 g. Mature fruits, follicles and seed readily floated in containers of fresh water under glasshouse conditions where germination was observed (but not assessed). As the number of stalks per plant was not recorded, seed production per plant could not be determined from this data, though this is a priority for future trials.

Table 1. Average number of fruit buds on the first two limnocharis flower stalks (peduncles) produced by young plants and a sample from plants five months later in the glasshouse.

	First stalk produced	Second stalk produced	Older plant stalks
Number of observations	19	15	125
Average of fruit buds ±SD	7 ± 2.3	5.9 ± 2.4	8.9 ± 2.4
Range of fruit bud counts	3–10	1–10	2–13

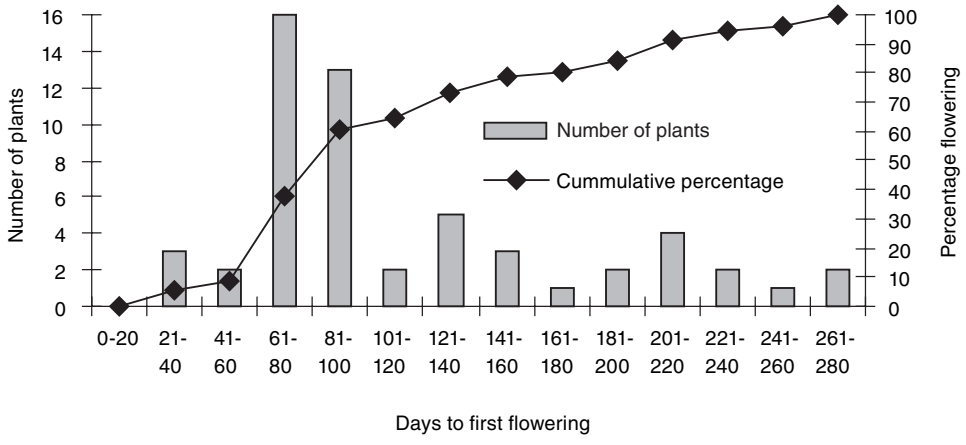

Figure 1. Time taken for newly emerged limnocharis plants to produce a flowering stalk in the field. (n = 56).

Table 2. Characteristics of 50 mature limnocharis fruits produced in the glasshouse.

	Fruit width (mm)	Fruit length (mm)	Seeds per fruit	Follicles per fruit	Seeds per follicle (n = 759)
Maximum	15.5	19	1547	18	115
Minimum	11.5	12.5	524	12	6
Average	13.32	15.32	1038.5	15.18	68.42
±SD	0.72	1.18	235.4	1.04	19.46

DISCUSSION

Survey frequencies for eradication targets should account for local maturity rates and the risk of missed plants seeding before the next survey (Vitelli *et al*. 2006), with consideration given to site access, seasonal emergence patterns, plant visibility, search areas and resources. With the eradication program managing limnocharis at 19 locations across tropical north Queensland by July 2007, the results of local reproductive studies were important to the success of the eradication efforts.

In north Queensland, a small proportion of limnocharis seedlings can take only 23 days to produce a flowering stalk under field conditions. Under glasshouse conditions, mature fruits can form on flowering stalks in a minimum of 23 days, giving a minimum of 46 days for limnocharis seed production. Limnocharis could therefore produce thousands of seeds per flowering stalk in less than two months, with plantlets also likely to form on the flowering stalks. These reproductive propagules could readily be dispersed by water. In November 2005 this information was incorporated into the limnocharis eradication program (K. Galway pers. comm. 2006), resulting in an increase in the frequency of visits to all naturalised limnocharis populations (n = 8). Between 3/4/04 and 31/12/04 the Feluga site was visited four times at an average interval of 79 days, while in 2005, 11 visits were conducted on average 38 days apart. Since 2006 visit intervals have averaged 27 days (T. Sydes pers. comm. 2007), thereby removing the risk that any plants missed in one survey could mature before the next survey.

Environmental and or genetic differences between geographically distinct introduced populations can result in differences in the reproductive behaviour of weeds. Reliance on the observations of Song *et al*. (2000), who found that transplanted Chinese limnocharis plants produce seed in 3–11 months, with 560 seeds per fruit in glasshouse conditions could have misinformed the local limnocharis eradication program. Incorporating research into eradication programs can refine and enhance survey and control activities and increase confidence that weed species can be eradicated.

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