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Reproductive ecology and conservation prospects of a threatened medicinal plant *Curculigo orchioides* Gaertn. in Nepal

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Abstract: Lack of biological information is a major constraint to the development of the medicinal plant sector in Nepal. We monitored phenology, population structure, and regeneration strategies of *Curculigo orchioides,* a threatened medicinal herb of tropical to subtropical Asia, for 1 year at five sites in the inner Terai, Central Nepal. Only 20 - 26 % of mature individuals were in the reproductive phase during the phenologically most active months (June - July), and about 55 % of flowering individuals developed fruits. Soil moisture, stored reserves, and biotic pressure appeared to govern phenological patterns. Fruiting frequency was high under conditions of a partially open canopy and a thin litter layer. Seeds showed physiological dormancy and germinated 10 - 12 months after dispersal in natural habitats. Clonal propagation from leaves was induced by mild mechanical damage, high soil moisture, and humidity. Low regenerative potential through sexual reproduction and high vulnerability to habitat disturbance appear to be the major constraints to maintaining natural populations of *C. orchioides.*

Resumen: La carencia de información biológica es una limitación fuerte para el desarrollo del sector de plantas medicinales en Nepal. Nosotros monitoreamos la fenología, la estructura poblacional y las estrategias de regeneración de *Curculigo orchioides*, una hierba medicinal amenazada de Asia tropical y subtropical, durante un año en cinco sitios en el Terai interior, centro de Nepal. Sólo 20-26 % de los individuos maduros estuvieron en fase reproductiva durante los meses más activos fenológicamente (junio-julio), y alrededor de 55 % de los individuos que florecieron produjeron frutos. La humedad del suelo, las reservas acumuladas y la presión biótica parecieron controlar los patrones fenológicos. La frecuencia de fructificación fue alta en condiciones de dosel abierto parcialmente y una capa delgada de mantillo. Las semillas mostraron latencia fisiológica y germinaron 10-12 meses después de la dispersión en los hábitats naturales. La propagación clonal a partir de hojas fue inducida por un daño mecánico ligero, y por una humedad y un contenido de agua altos en el suelo. El potencial regenerativo bajo por medio de reproducción sexual y la alta vulnerabilidad a las perturbaciones del hábitat parecen ser las limitantes principales para el mantenimiento de las poblaciones naturales de *C. orchioides*.

Resumo: A falta de informação biológica é um dos principais entraves para o desenvolvimento do sector das plantas medicinaisno Nepal. Assim, durante um ano e em cinco sítios no interior de Terai, no centro do Nepal, monitorizamos a fenologia, a estrutura populacional, e as estratégias de revitalização da *Curculigo orchioides*, uma erva medicinal ameaçada da Ásia tropical e subtropical. Apenas 20-26 % dos indivíduos adultos estavam em fase reprodutiva durante os mesesfenologicamente mais activos (Junho- Julho), e cerca de 55 %

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dos indivíduos em floração desenvolveram frutos. A humidade do solo, as reservas armazenadas, e a pressão biótica pareceram governar os padrões fenológicos. A frequência dafrutificação foi alta em condições de coberto parcialmente aberto e de uma camada fina de folhada. As sementes apresentaram dormência fisiológica e germinaram 10-12 meses após a sua dispersão em habitats naturais. A propagação clonal das folhas foi induzidaatravés dano mecânico leve, alta humidade do solo e humidade. O baixo potencial de regeneração por propagação sexuada e uma elevada vulnerabilidade à perturbação do habitat parecem ser os principais obstáculos para a manutenção de populações naturais de *C. orchioides*.

Key words: Clonal propagation, phenology, population decline, reproductive success, seed set.

Introduction

In the context of increasing global interest in medicinal plants as potential sources of new bioactive molecules (Cordell 2000; Dahanukar *et al.* 2000; Ji *et al.* 2009), conservation of medicinal plants as well as of the knowledge of their uses is vital for the future of human health care. Though the volume of literature on Nepalese medicinal plants has been increasing rapidly, most (> 50 %) of this is related to inventory, and only a small proportion (< 10 %) has focused on biology (Ghimire 2008). Biological information such as reproductive ecology, life history strategies, distribution, habitat preference, and plants' responses to environmental perturbations are essential for conservation and sustainable use of ethnomedicinal plants, especially when they are collected in large volumes from wild populations. However, this information has been lacking for most of the ethnomedicinal plants native to Nepal.

Curculigo orchioides Gaertn. (Hypoxidaceae) is a medicinal herb of tropical to subtropical regions in Asia, and occurs naturally on grassy slopes and forest understories. Due to its wide range of uses in ethnomedicine, both in the Ayurvedic and Chinese systems of traditional health care, a large number of phytochemical and pharmacological investigations have been undertaken (Shrestha *et al.* 2008). However, biological characters of this plant have not been investigated in detail. Wild population of *C. orchioides* are reported to be declining rapidly, and the plant has been assigned to various threat categories, from vulnerable (Bhattarai *et al.* 2001) and threatened (Manandhar 2002) to endangered (Jasrai & Wala 2001; Prajapati *et al.* 2003; Suri *et al.* 1999).

Population decline of this plant has been attributed to anthropogenic factors (over exploitation, over grazing, forest floor denudation) and to the plant's own biology, including poor seed set and low germination (Gupta & Chadha 1995; Jasrai & Wala 2001). However, empirical data to support or reject these assumptions has been lacking (Shrestha *et al.* 2008). We report some aspects of the reproductive ecology of *C. orchioides.* We also report temporal and spatial patterns in phenology, regenerative strategies and population abundance and propose explanations for patterns observed. The major questions we asked were: (1) What is the pattern of phenological activity of *C. orchioides*? (2) What are the major environmental factors that have shaped phenology and determine regeneration and population size? and, (3) Can reproductive biology help us understand the causes of population decline of this species?

Materials and methods

Study area

The study area (27° 37-42´ N, 84° 13-26´ E) is a dun valley (Chitwan valley), lying in the tropical region of Central Nepal. Average temperature ranges from 15 °C in January to 28.4 °C in May (Fig. 1). The area receives monsoon rain from June to September, which accounts for about 70 % of the total annual rainfall (2427 mm). The rest of the year is dry with a few showers of winter rain. The area has deciduous to semi-deciduous tropical forests with sal (*Shorea robusta* Gaertn.) forest as the dominant type, and riverine *Acacia-Dalbergia* forest as a minor component (Stainton 1972).

Five sampling sites, which were relatively less disturbed and had large populations of *Curculigo orchioides,* were chosen subjectively. Each site lies within a different community-managed forest, na-

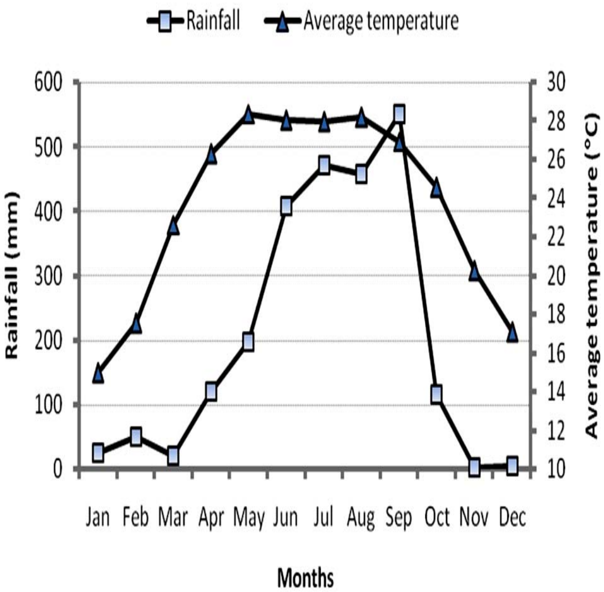

Fig. 1. Average monthly rainfall and temperature of the study area from 2003-2007 recorded at Bharatpur weather station. This station lies about 15 km east of the sampling site in Nawalparasi, and about 5 km west of the sampling site at Barandabhar. (Source: Department of Hydrology and Meteorology, Government of Nepal).

mely Dhuseri, Chautari, Sundari, Sansari and Barandabhar (Table 1). Barandabhar is on the lowland plain of Chitwan district, near Beeshazar lake, and is about 20 km east of the other sites. The Barandabhar forest is a part of the buffer zone area to the north-east of Chitwan National Park and is frequently visited by wild animals from the park (e.g., rhino, deer, and wild boar). The soil of this forest is alluvial and moist, but not flooded, due to the shallow water table and the adjoining lake. The other four forests lie in the foothills of Mahabharat range within a distance of 5 km of each other, in Nawalparasi district, where the soil is relatively dry due to the raised topography. Wild boar and peacocks are common wildlife in these forests.

Study species

Curculigo orchioides Gaertn. (family: Hypoxidaceae, vernacular name: Kalo musali, Fig. 2) is a perennial herb, with a vertical black rootstock at the base of which is a bundle of white fleshy roots. The rootstock is the perennating organ while the leaves and other above ground parts senesce annually. Leaves are narrow and crowded at the top of the rootstock, which generally lies near the soil surface. Inflorescence is a spike and appears almost at ground level. Flowers are yellow; the first 1-2 flowers are bisexual and the remainders are male. Fruits are fleshy and hidden in the leaf bases. Seeds are shiny black with fine striations on the surface and a hook-like projection at the proximal end.

Fruits contain 1-15 seeds (average: 7 seeds/fruit, n = 263) with a seed size of 5 mg seed^{-1} (average of 3 measurements with 100 seeds in each). Average seed output per flowering plant per year is estimated to be 12.

Fig. 2. *Curculigo orchioides*. (A) mature individual with flowers and fruit, (B) flowering individual tagged for monitoring phenology, (C) rootstock with cluster of fleshy roots at the base, (D) fruits, (E) seeds, and (F) seedling.

Rootstock of this plant has been used in traditional medicine as an aphrodisiac, a tonic to restore vigor, and against asthma, bronchitis, jaundice, dysentery, diarrhoea, leucorrhoea and male sterility (Shrestha *et al.* 2008). The rootstock

Table 1. Characteristics of the sampling sites.

Sampling sites	Elevation (m asl)	Latitude/ Longitude	Slope (°)	Soil characters	Litter cover (%)	Livestock grazing	Tree canopy cover (%)	Shrub canopy cover (%)
Dhuseri	450	27° 41.6′ N 84° 13.7′ E	0-10	Brown to reddish brown; coarse to fine	55	Banned	65	45
Chautari	660	27° 42.5′ N 84° 14.2′ E	10-20	Brown; abundant pebbles on the surface	50	Goat grazing	35	30
Sundari	450	27° 42.2′ N 84° 16.0′ E	10-20	Reddish brown; fine	64	Banned	65	55
Sansari	425	27° 42.3′ N 84° 16.7′ E	20-30	Brown; coarse to fine	27	Goat grazing	30	65
Barandabhar	410	27° 37.4′ N 84° 26.8′ E	0	Dark brown; sandy loam	10	Banned†	60	50

† Although wild animals like rhino, deer, wild boar, etc. come to this forest in significant number from adjoining Chitwan National Park.

contains glycosilated phenolics, triterpenic saponins and aliphatic ketones, and some of these secondary metabolites show immunostimulatory (Bafna & Mishra 2006; Lakshmi *et al.* 2003), antioxidative (Tang *et al.* 2004; Wu *et al.* 2005), estrogenic (Vijayanarayanan *et al.* 2007) and anti-osteoporotic activities (Cao *et al.* 2008).

Phenology observations

At each sampling site, four plots (10 m × 10 m) were subjectively chosen and marked permanently. The plots were relatively undisturbed and had high density of *Curculigo orchioides*. Altogether, 200 quadrats lying within 20 plots were sampled. In each plot, 10 quadrats (1 m × 1 m) were located randomly. The number of individuals in each phenophase - seedling, vegetative (mature but without flower and fruit), flowering and fruiting - were recorded in each quadrat to determine population-level phenology. Observations were repeated every month, starting in June 2007. Tree canopy and ground vegetation (shrub layer) cover were estimated visually.

The 20 best looking flowering individuals in each plot were tagged (Fig. 2B). Altogether, 400 individuals lying within 20 plots were tagged for monitoring phenological changes. Plants were tagged in June 2007 and the phenological stage of each plant was recorded; monthly observation at the marked plots was continued until October 2007 when the plants began to senesce. In September, due to harsh weather conditions (continuous heavy rainfall) we could not make observations. At each monthly observation the phenophases of tagged individuals were recorded as vegetative, flowering or fruiting.

Environmental variables

In October, litter cover (%) on the ground was estimated, and a 200 g soil sample was collected in each quadrat from 10 - 15 cm depth to determine organic matter and total nitrogen. There were altogether 200 soil samples, with 10 samples from each plot.

Soil samples were air dried in the shade for a week and passed through a fine sieve (mesh size 0.5 mm). Organic matter (Walkley-Black method) and total nitrogen (mirco-Kjeldahl methods) were determined in each soil sample following the methods described in Gupta (2000). Mean values of soil organic matter and total nitrogen were calculated for each plot.

Germination experiment

In preliminary experiments, seeds of *Curculigo orchioides* did not germinate when placed between moist blotting paper for 3 months, indicating dormancy. When these seeds were tested using 0.1 % solution of 2,3,5 triphenyl-tetrazolium chloride, they were found viable.

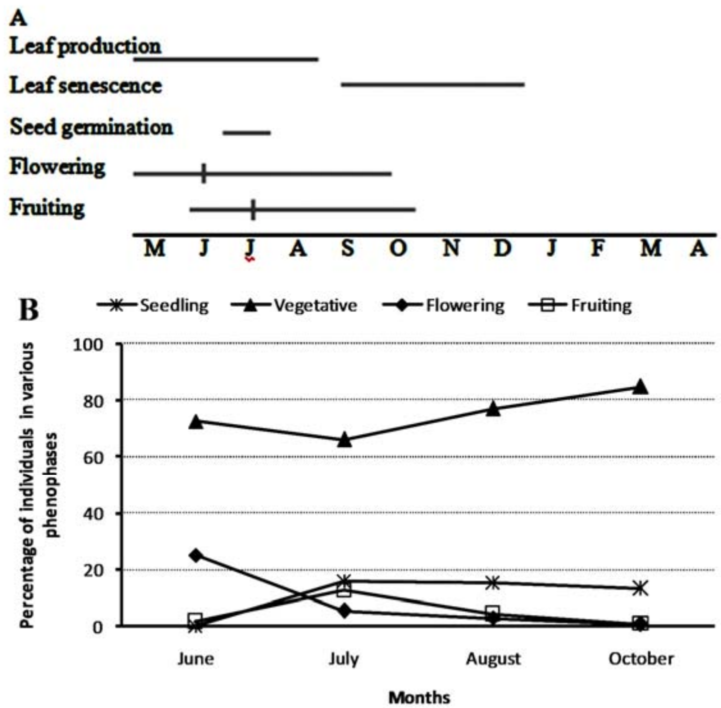

Fig. 3. Phenological patterns of *Curculigo orchioides*. (A) phenological events in one year starting from May; vertical lines in flowering and fruiting indicate peak frequency of the respective phase. (B) Change in percentage of individuals in various phenophases during growing season across the entire sampling sites.

Then seeds were tested for permeability of seed coat to water using the method followed by Hidayati *et al.* (2001) with some modifications. Three replicates of 20 seeds each (total seeds = 60) were weighed to the nearest 1 mg after soaking for about 2 minutes and subsequent surface-drying by blotting paper. This initial measurement was considered as the dry mass (M_0). The seeds were then placed between three layers of blotting paper placed in Petri dishes and saturated with distilled water. After 1 hour, the seeds were surface-dried by blotting paper, and reweighed to get wet mass (M_t). The process was repeated every hour for the first 10 hours and every 24 hours for the next week. For each measurement time, the percentage increase in mass (ΔM) was calculated as: $\Delta M = (M_t - M_0) / M_0 \times 100$.

For germination, seeds were soaked in various concentrations of gibberellic acid (GA_3) solution (100, 250 and 500 ppm) for 24 h. Half of the seeds soaked in each concentration of GA_3 were placed in Petri dishes (five seeds in each of two Petri dishes) lined with a double layer of moist blotting paper. Another half of the seeds were placed in Murashige-Skoog (MS) medium. Seeds placed in MS medium were surface sterilized by 70 % ethanol (1 min) and then 1 % sodium hypochlorite (5 min) before inoculation. Seeds soaked in distilled water for 24 h were considered as control. Seeds were left to germinate and were observed for 6 weeks.

Data analysis

From the quadrat-level data, percentage of individuals in various phenophases was calculated for each sampling month for each sampling site as well as from the combined data. Tagged individuals data were also used to calculate percentage of individuals in vegetative, flowering and fruiting stages for each sampling month and site.

Relationship between seedling density of *Curculigo orchioides* and litter cover on the ground was analyzed by linear regression. Since most of the phenological activities (e.g., germination, flowering, and fruiting) occurred in July, percentage of individuals in each phenophase was calculated for each plot from this month's data of quadrat sampling and tagging. The phenological data from July was used to understand the variation of per-

Table 2. Percentage of tagged individuals of *Curculigo orchioides* producing various numbers of bouts of inflorescences across the sampling sites.

Bouts of inflorescences	Sampling sites				
	Dhuseri	Chautari	Sundari	Sansari	Barandabhar
1-bout	27	63	53	59	54
2-bouts	33	36	35	36	42
3-bouts	32	1	12	5	4
4-bouts	8	0	0	0	0

centage of individuals in various phenophases (e.g. flowering, fruiting) with environmental variables such as litter cover, soil OM, total N and canopy using regression analysis. Statistical Package for Social Sciences (SPSS, version 11.5, 2002) was used for statistical analysis.

Results

Phenological patterns

The emergence of new leaves from the apical bud of underground rootstocks was initiated in May (Fig. 3A) when temperature was the highest and soil was moistened by a few showers of rain (Fig. 1). Plants occurring at moist but sunny sites produced inflorescence together with new leaves. June-July was phenologically the most active period when most flowering, fruiting and seed germination occurred. During that phenologically active period, 27 % of individuals in June and 18 % in July were in reproductive stage (flowering and fruiting) while 73 % and 66 %, in June and July, respectively, were in the vegetative stage (Fig. 3B); in July, the remaining 16 % of the population was made up of seedlings. When only mature individuals were used in analysis, excluding seedlings, 74 % in June and 80 % in July were in the vegetative stage.

There was a single flowering peak in June at the beginning of the monsoon (Fig. 3). However, flowering was not synchronized and it continued until October at some sites. During a growing season some individuals produced up to four bouts of inflorescences (Table 2). Average number of inflorescence/plant was 1.63 (n = 579). If plants produced > 1 inflorescence/plant during a growing season, they did this in two ways. They either produced inflorescences in sequence, such that a plant would have > 1 inflorescence at a time but at different developmental stages (Fig. 2A). Alternatively, they produced inflorescences at intervals of a few weeks to a month, such that at any given time a plant would have only one inflorescence. There was asynchrony in flowering within an inflorescence. The first flower produced was always bisexual and it opened 3-5 days before subsequent flowers did; a few individuals produced > 1 bisexual flowers per inflorescence. Therefore, the average number of bisexual flowers/inflorescence was 1.13 (n = 31). The bisexual flowers mostly withered before anthesis of male flowers.

Table 3. Percentage of tagged individuals of *Curculigo orchioides* in various phenophases. Individuals in flowering stage were tagged in June. Values inside the parenthesis represent total number of individuals.

Phenophases	Months of observations			
	June	July	August	October
Vegetative	0	24	61	92
		(87)	(190)	(272)
Flowering	100	21	13	4
	(410)	(76)	(40)	(12)
Fruiting	0	55	26	4
		(204)	(80)	(12)
Total	100	100	100	100
	(410)	(367)	(310)	(296)

Fruiting occurred from June to October across the sites, with a single fruiting peak during July (Fig. 3). At the lowland moist site, Barandabhar, fruiting occurred earlier than at other sites. About 55 % of flowering individuals in June bore fruits in July (Table 3). However, the percentage of individuals fruiting varied across the sites; it was 89 % at Barandabhar while at other sites it varied between 40 and 55 %.

Seed maturation took nearly a month. Immature seeds were white and fleshy while the mature ones were dark black and hard (Fig. 2E). Mature seeds emerged from the fruit (berry) after gelatinization of the fleshy wall. Seeds were dis-

Table 4. Results of linear regression analysis between some reproductive (response) and environmental (predictor) variables. Degrees of freedom (d.f.) for all analyses is 18.

Responses	Predictors	R-square	F-value	Significance
Flowering in June (%)	Tree canopy	0.480	16.61	0.001
	Tree × shrub canopy	0.366	10.41	0.005
	Soil N	0.248	5.93	0.025
Flowering in July (%)	Soil OM	0.207	4.7	0.044
Vegetative in July (%)	Tree canopy	0.198	4.43	0.05

persed by ants as well as by surface runoff during the rainy season. Small ants used the seed content as food. Seeds remained dormant until June of the following year and germinated when the rainy season began in July (Fig. 3). Litter accumulation on the ground had a negative effect on the seedling population. Seedling density declined with increasing litter cover (linear regression, $P < 0.001$, $R^2 = 0.50$).

Environmental factors and phenological patterns at population level

Flowering was influenced by canopy cover and soil characters (Table 4, Fig. 4). The percentage of flowering individuals increased with increasing

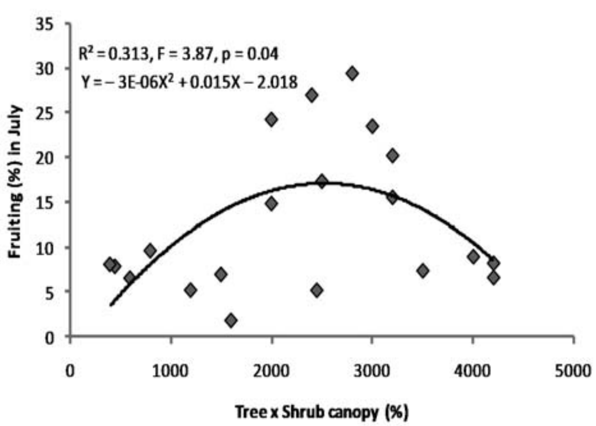

Fig. 4. Impact of tree × shrub canopy cover on fruiting frequency. The fitted line is based on quadratic regression model.

tree canopy, tree × shrub canopy and soil N content. Flowering increased with organic matter though the relation was only marginal. Fruiting increased with increasing tree canopy, but the relation changed when shrub cover was used as a component of canopy cover. There was a unimodal relationship between fruiting and tree × shrub canopy with highest fruiting at an intermediate level of canopy cover (Fig. 4). Similar pattern of unimodal variation, though not significant, was observed between tree × shrub canopy and the frequency of flowering individuals that bore fruits in July ($P = 0.114$). Percentage of individuals in vegetative stage increased with increasing tree canopy. The canopy and soil variables (OM and N) had no effect on frequency of juvenile individuals, and flowering individuals that bore fruits.

Seed germination

In natural habitats seeds of *Curculigo orchioides* germinated 10 - 12 months after dispersal. Freshly collected seeds did not germinate when placed between moist filter papers for more than 90 days, indicating dormancy, although these seeds remained viable (embryos were stained pink by tetrazolium solution). When placed between moist filter papers, there was an increase in seed mass by 30.5 % over air dry mass. Seeds treated with GA_3 also did not germinate either on moist filter paper or with an MS medium. In short, we could not identify the environmental condition to break seed dormancy of *C. orchioides* from the experiments that we performed.

Vegetative propagation

Underground rootstock was mainly a means of winter perennation. When upper part of the rootstock was damaged by human collection or animal foraging, there was often development of more than one shoot (Fig. 5A). These adventitious shoots detached from each other and became independent individuals.

Clonal propagation from leaves (Fig. 5B-C) was observed during the second half of the growing season (September-October). When midribs of mature leaves were mildly damaged and the injured part came in direct contact with moist surface soil a few whitish swellings were formed along the midrib, which resembled tiny calluses. Calluses were also observed at the proximal end of completely detached leaves. Wild animals, especially wild boar, were the main biotic factors of mechanical damage to the leaves or whole plant.

Fig. 5. Clonal propagation in *Curculigo orchioides*. A) Branching in rootstock after removal of uppermost part, B-C) Clonal propagation from leaves.

During September-October, wild boar selectively foraged on the rootstock of this plant. These callus-like structures developed into globular bulbils, which produced leaves and roots. A complete plantlet was formed while still attached to the leaf midrib. We observed this phenomenon at the three sampling sites (Dhuseri, Sundari and Barandhabhar) that were relatively moister than the other sites. Among them, frequency of leaves with such plantlets was higher at Barandhabhar, the wettest site.

Discussion

Phenological patterns

Phenological patterns are the result of a compromise between a variety of selective pressures, such as seasonal climatic changes, resource availability, and the presence of pollinators, predators, and seed dispersers (Fenner 1998). In *Curculigo orchioides*, flowering began with the pre-monsoon showers, reaching a peak during early monsoon (Fig. 3). This pattern is common in seasonally dry tropical forests, such as of the present study area, where flowering is induced by rainfall and is often concentrated in the transition from the late dry season to the early wet season (Murali & Sukumar 1994; Rathcke & Lacey 1985; van Schaik *et al.* 1993). Resource availability influences the timing of phenological events (Rathcke & Lacey 1985). Flowering during pre- to early monsoon in *C. orchioides*, together with leaf flushing, could be possible due to presence of adequate assimilate in the perennial rootstock. Moisture, temperature and the presence of adequate stored reserves could have imposed bottom-up selective pressure on the timing of flowering (Elzinga *et al.* 2007). An extended flowering in *C. orchioides* from May to October could be attributed to the production of inflorescences in one to four bouts at intervals of a few weeks to a month (Table 2).

Curculigo orchioides had short (nearly one month) flower to fruit duration which could possibly be due to utilization of reserved food for fruit development (Murali & Sukumar 1994). Fruiting during the early wet season is a common tendency of plants in seasonal dry tropical forest, which minimize seedling mortality in the next dry season (van Schaik *et al.* 1993). However, early fruiting in *C. orchioides* could have no effect on seedling mortality in the same year. Seed dormancy postponed germination until early in the next wet season (July), which ensured establishment of seedlings before the next dry season began. Seed dormancy in this plant appears to be an adaptive mechanism to time germination so as to avoid unfavourable weather conditions for subsequent establishment (Finch-Savage & Leubner-Metzger 2006). During the late growing season the plant was foraged extensively by wild animals for the rootstock, which is nutrient rich (with a calorific value of 1630 KJ/100 g dry mass; Suresh 2008) and an aphrodisiac (Ramawat *et al.* 1998). Therefore, early flowering and fruiting ensured successful completion of seed production before the plant was damaged by wild animals. Any phenotype that would flower and produce fruits late in the growing season could most likely be damaged before the fruits matured.

Seeds had no active mechanism of dispersal and were dispersed by ants and surface runoff during the rainy season. It is not clear whether there are chemical attractants in seeds to attract ants, which has been considered an adaptive modification in ant dispersed seeds (Bennett &

Krebs 1987; Howe & Smallwood 1982). But we observed that ants foraged seeds of *C. orchioides* for their nutritious cotyledons. Unlike the post-monsoon fruiting peak of most understorey herbs of moist tropical forests (Bhat & Murali 2001), the mid-monsoon fruiting peak of *C. orchioides,* together with its unwettable seed coat could potentially help seeds to float on the surface of runoff water in the rainy season (Howe & Smallwood 1982). It appears that fruiting phenology in *C. orchioides* could have been shaped by its seed dispersal mode and the potential damage by animals during the late growing season.

Curculigo orchioides showed spatial variation in timing of phenological events, with earlier unset of flowering and fruiting at the moister site. This pattern of difference between moist and dry sites is a common feature in tropical forests at the community as well as the species level (Newstrom *et al.* 1994). At the community level, flowering peaked during the late dry season at moist sites but during the early wet season at dry sites in dry tropical forests (Murali & Sukumar 1994). Individual species of tropical regions also followed the same pattern (Newstrom *et al.* 1994).

Reproductive success

About one-fourth of the mature individuals of *Curculigo orchioides* entered the reproductive phase, and only about half of the flowering individuals developed fruits (Fig. 3B, Table 3). This indicates a relatively low regenerative potential of *C. orchioides* through sexual reproduction. Reproductive success of this plant appeared to be determined by canopy and litter cover (Table 4, Fig. 4). A similar pattern in reproductive success has been reported for the herbaceous monocots in Amazonian tropical forests (Costa *et al.* 2002). Effect of tree canopy on regeneration is modified by litter accumulation (Dupuy & Chazdon 2008). Among the sampling sites, highest percentage (89 %) of tagged (in June) flowering individuals bore fruits in July at Barandabhar, where litter accumulation was the lowest (Table 1). Litter accumulation had significantly negative impact on the seedlings density of *C. orchioides* (P = 0.001), which is a common effect of litter accumulation on herbaceous species (Berendse 1999). Negative effect of litter accumulation has been also reported for seedlings of small-seeded trees (Dupuy & Chazdon 2008). The combined result of these two effects was the decline in total density of this plant with increasing tree canopy (unpublished data, BB Shrestha & PK Jha). Reproductive success was high in plots with partially open canopy and accumulation of a thin layer of litter.

Freshly collected seeds showed dormancy. An increase in seed mass by 30.5 % over dry mass when placed between moist filter paper is typical for permeable seed coat (Carol C. Baskin, University of Kentucky, USA, per. com. Nov. 20, 2009). This excluded the possibility of physical dormancy. Since the seeds did not germinate when treated with GA_3, it appears that seeds of *C. orchioides* have deep physiological dormancy (Baskin & Baskin 1998; Carol C. Baskin, per. com. Nov 20, 2009).

Clonal propagation in *Curculigo orchioides* from leaves was the combined effect of mechanical damage, probably, high humidity and availability of adequate moisture in soil. High humidity slows down the drying rate of detached or damaged leaves, and provides sufficient time for the development of plantlets. The indiscriminate damage to adult plants by animals might have induced regeneration capacity from leaf midrib. To our knowledge, this is the first report of natural regeneration of *C. orchioides* from leaves. This observation is supported by the earlier reports that *in vitro* direct regeneration of *C. orchioides* plantlet was the best from mid rib region of leaf (Prajapati *et al.* 2003; Suri *et al.* 1999). Identification of the specific environmental conditions essential for natural regeneration of *C. orchioides* from leaves can help to improve the techniques of *in vitro* micro-propagation at a large scale.

There was temporal separation between sexual reproduction and clonal propagation. Seasonality in clonal propagation in a tropical understorey herb (*Aechmea magdalenae*) was attributed to variation in abiotic factors such as light and moisture (Villegas 2001). However, in *C. orchioides*, seasonality in clonal propagation also appeared to be due to variation in activity of wild animals that damaged the plant.

Implications for conservation

The two regenerative strategies in *Curculigo orchioides*, sexual and vegetative, appear to be separated spatially as well as temporally. The relative proportion of sexually and vegetatively produced offspring in the population is determined by the complex interplay between genetic and environmental factors (Ceplitis 2001). The role of genetic variation in selecting the mode of reproduction in *C. orchioides* is not known at present, but some of the environmental factors such as high humidity and soil moisture appeared to favor bulbil formation from damaged leaves. There appeared to be a trade-off in regenerative strategies between moist/shady and dry/open habitats. Sexual reproductive success was low at sites with high canopy cover (high shade and thereby high moisture) where frequency of clonal propagation from leaves was high. Clonal propagation from leaves was virtually absent in the dry sites with low canopy cover, where sexual reproductive success was high. This could be the reason for the species having a wide range of distribution in the landscape. However, given the high anthropogenic disturbance and habitat degradation, this plant appears to be vulnerable due to its limited dispersal range of seeds and propagules. Plants with ant or gravity dispersed seeds are the most vulnerable to habitat degradation and fail to re-colonize during restoration (McLachlan & Bazely 2001). Recolonization of such species would be possible only if they are actively reintroduced. Thus habitat degradation could be an important reason for declining natural population size of *C. orchioides* in India as suggested by Jasrai & Wala (2001).

The low reproductive success of this plant observed in the present study area corroborates earlier reports, that natural populations of *C. orchioides* lack adequate regeneration (Gupta & Chadha 1995). Low regenerative potential through sexual reproduction and high vulnerability to habitat disturbance appear to be the major constraints for maintaining natural population of *C. orchioides*. Although the plant has evolved additional strategies for regeneration through leaves and rootstock, they are not adequate to maintain natural populations in the context of increasing anthropogenic disturbances. Therefore, conservation management of this plant in the forest understorey should focus on maintaining partial open canopy and thin litter accumulation to maximize regenerative potential through sexual reproduction.

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