American Journal of Plant Sciences, 2014, 5, 535-540
Published Online March 2014 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2014.55068

Seed Germination and Production of *Erythrina mulungu* and *Erythrina velutina* Plantlets

Ana Maria Soares Pereira[1*], Verena Trevizani Alves Souza[1], Juliana da Silva Coppede[1], Suzelei de Castro França[1], Bianca Waléria Bertoni[1], Ana Valéria Vieira de Souza[2]

[1]Department of Biotechnology, University of Ribeirão Preto, Ribeirão Preto, Brazil
[2]Embrapa Semiárido, Centro de Pesquisa Agropecuaria do Tropico Semi-Arido, Petrolina, Brazil
Email: [*]apereira@unaerp.br

Received 28 November 2013; revised 17 January 2014; accepted 23 February 2014

Abstract

Species of the genus *Erythrina* are frequently used as ornamental plants and in projects to restore degraded areas. Also, extracts from its shoots and stems are popularly used as a herbal medicine with validated activity on the central nervous system. The objective of this study was to evaluate *E. mulungu* and *E. velutina* seed germination and seedling development potential aiming commercial scale production of those species. Seeds stored for one or twelve months at 10˚C ± 2˚C were sown in sand, soil or Plantmax® substrates and evaluated for germination and seedling development. Subsequently, seedlings sprouted in Plantmax® were transferred to polyethylene bags and kept in greenhouse, under direct sunlight for plant development (plant height, stem diameter and root length pivoting) evaluations. Four-month-old plantlets were transplanted to the field and after a period of one year the collar diameter and shoot height (of each plant were measured. Seed germination rates of both species grown in soil and in Plantmax® were significantly high (over 80%). Storing seeds for 1 month did not inhibit germination. However, seeds stored for twelve months had germination reduced by more than a quarter. The development of plants in greenhouse and in the field was satisfactory, indicating the viability of producing *E. mulungu* and *E. velutina* on a commercial scale, in order to meet the expanding market demand for herbal medicines.

Keywords

Medicinal Plants; Herbal Medicines; Cerrado; Caatinga; *Rhizobium*

[*]Corresponding author.

How to cite this paper: Pereira, A.M.S., *et al.* (2014) Seed Germination and Production of *Erythrina mulungu* and *Erythrina velutina* Plantlets. *American Journal of Plant Sciences*, **5**, 535-540. http://dx.doi.org/10.4236/ajps.2014.55068

1. Introduction

The plant genus *Erythrina* [Leguminosae—Papilionoideae (Fabaceae)] comprises approximately 115 species distributed all around the Neotropics, across South Africa, and all over the Himalayas and southern United States [1].

In Brazil, the most customarily found species are *Erythrina velutina* Willd. and *Erythrina mulungu* Mart. Ex Benth., which are well known for their multipurpose use. Those species are used as ornamental plants. Because of its deep pivoting root system and rapid growth, it is also used in the restoration of degraded areas and in riparian vegetation recovery. *Erythrina* is considered a pioneer species in ecological succession [2]-[5]. The sedative action of the *Erythrina* tea that is popularly consumed in Brazil has been reported in ethnobotanical studies [6] [7]. Moreover, numerous pharmacological studies carried out with animals validated the anxiolytic, anticonvulsant and antinociceptive effects of the hydroalcoholic extract of *E. velutina* and *E. mulungu* stem bark and flowers [8]-[17]

However, even being a species relevant to the development of scientific researches, especially considering its diversity of use and validated therapeutic efficacy, only a few studies on *Erythrina* seed germination characteristics and production of seedlings have been carried out and those works are restricted to seed physical dormancy caused by its water-impermeable seed coat [18]-[21].

In view of the importance of studies aiming the large scale production of *Erythrina* species for further establishment of sustainable management and production of plantlets with commercial purposes, in this work the germination potential of *E. mulungu* and *E. velutina* seeds and plant development in greenhouse and in field conditions were evaluated in order to achieve this plant mass production and enable its use in flora recovery programs and also to provide crude material for the development of phytopharmaceutical formulations.

2. Material and Methods

This work was carried out in the Plant Biotechnology Department at the Universidade de Ribeirão Preto – UNAERP, Ribeirão Preto, SP. *E. velutina* seeds were collected in the cities of Boa Nova (S14˚23'58,5" W42˚21'14,3" 562 m), Lagoas (S13˚14'30,1" W40˚01'02.6" 607 m) and Santo Estevão (S12˚28'36,1" W39˚18'17,5" 158 m) in the state of Bahia. *E.* mulungu seeds were collected in the cities of Ribeirão Preto (S21˚06'24,9" W47˚45'41,1" 503 m) and Rifaina (S20˚06'06,8" W47˚26'54,5" 616 m) in the state of São Paulo and in Sacramento (S19˚51'58,0" W47˚25'49,1" 801 m) in the state of Minas Gerais. All collected seeds were gathered in a single seed pool before starting the experiments.

To evaluate seed viability, *E. velutina* and *E. mulungu* seeds (1200 un) were packed separately in kraft paper bags (200 seeds of each species per bag) and stored for 1 and 12 months in a dry cold chamber (30% relative humidity) and controlled temperature (10˚C ± 2˚C).

Before sowing, seeds were immersed for 20 h in benomyl solution (1% w/v) under continuous agitation (100 rpm), dipped for 1 hour in calcium hypochlorite solution (0.5% w/v) and washed with autoclaved distilled water. After disinfestation seeds were mechanically scarified (at the opposite end of the embryo) using electric scarifier (Dremel-3957CT-multpro 10,000 RPM). Following scarification, seeds were soaked for 24 h in autoclaved distilled water under agitation (100 rpm).

To evaluate seed germination 3 different experiments were carried out in polystyrene trays containing soil, sand, or Plantmax® substrate. The trays were kept for 30 days in phytotronics germination chamber (MA 1403/UR Marconi®) under 28˚C ± 2˚C temperature and 60% relative humidity. For each experiment *E. velutina* and *E. mulungu* seeds stored for 1 and 12 months (4 treatments with 50 seeds per repetition) were sowed (at 1 cm profundity) in sand, soil or Plantmax®. The experiment was conducted in a completely randomized design and germination speed and seedling emergence rates were evaluated daily during 1 month, using as a parameter of germination the hypocotyl length ± 2 cm.

Germination was evaluated calculating percentage of emerged seedlings and the speed of emergence index was determined according to the formula proposed by Maguire [22], IVE = G1/N1 G2/N2 + + ... + Gn / Nn where: G1, G2, Gn = number of seedlings germinated in the first, second, until the last count and N1, N2, Nn = number of days from the first, second until the last count.

Plants grown in Plantmax® substrate for 30 days were transplanted to polyethylene bags (15 × 25 cm) containing soil and cattle manure (3:1) as substrate, kept in greenhouse under direct sunlight and irrigated once a day during 4 months. The collar diameter, shoot height and pivoting root system elongation of each plant were

measured 2 and 4 months after the transplant of seedlings. Experiments were carried out in a randomized block design with 100 plantlets of each species divided into 4 groups of twenty five plants.After cultivation in polybags containing soil and cattle manure substrate for a period of 4 months the plantlets were transplanted to field in pits of 40 × 40 × 40 cm, fertilized with 2 kg·m^2 of cattle manure observing the spacing of 3 × 3 m in the medicinal plant cultivation area of the University of Ribeirão Preto, located in the city of Jardinópolis (SP). During the period one year the plantlets were watered twice a week and hand weeding was performed every two months. After one year of field cultivation the development of *E. velutina* and *E. mulungu* plants was evaluated measuring collar diameter and shoot height of each plant.

Statistical Analysis

The experimental design adopted was the randomized block design in a 3 × 2 factorial structure with 3 replications and 20 plants per plot. Obtained data were compared by analysis of variance (ANOVA) using the software SISVAR V.4.3 and the means were compared by the Scott-Knott test at a level of significance of 5%.

3. Results and Discussion

Mechanical scarification was an efficient method for breaking tegument dormancy of *E. velutina* and *E. mulungu* seeds, since it improved seed imbibition and germination between the fourth and fifteenth day. Obtained results corroborate those reported by Silva *et al.* [18] on the validation of mechanical scarification as an efficient method for breaking dormancy of *Erythrina* seeds. According to Rolston [23] and Carvalho & Nakagawa [24], the seed dormancy of leguminous plants is related to impermeability of seed coat to water due to the hard seed coat structure which restrict the entry of moisture into the seeds and so mechanical scarification facilitates germination. Lazarotto *et al.* [25] reported that seeds of *Erythrina crista-galli* presented dormancy and low percentage of germination on the tenth week after anthesis.

Examining *E. mulungu* and *E. velutina* seed germination it was evidenced that according to the classification of cotyledons characteristics proposed by Miquel [26] those species are phanero-epigeal-reserve type (PER)

Evaluating germination of *E. velutina* seeds stored for different periods of time and sown in different substrates it was observed that germination rates of seeds stored for one month ranged between 85.8 and 91.6%, and there was no statistical difference between the treatments (**Table 1**). However, seeds stored for twelve months showed significant reduction on germination ratios regardless the substrate used. Even though, a reduction in the germination speed index (GSI) was observed for seeds stored for twelve months in relation to seeds stored for 1 month, in either one of the three types of substrate tested, the difference was not significant (**Table 2**). The GSI (4.39) obtained for seeds sown in sand was similar to the results (GSI 4 and GSI 5.1) reported by Cardoso *et al.* [27] and Matthew *et al.* [28], respectively, when investigating the influence of sowing position and depth on germination of *E. velutina* seeds sown in seed boxes containing sand substrate and kept in a greenhouse.

Bento *et al.* [29] investigating the physiological quality of *E. velutina* seeds observed homogeneity among germination tests, germination speed index and accelerated aging. Both, data obtained by those authors and data recorded in this present work may be used as reference when focusing on large-scale production of *E. velutina* plantlets for commercial purposes.

Table 1. Mean germination rates (%) of *E. velutina* and *E. mulungu* seeds stored for 1 or 12 months and sown in three different substrates.

Substrate	Seed Germination Rates			
	E. velutina Time of storage (month)		*E. mulungu* Time of storage (month)	
	1	12	1	12
Plantmax®	85.83aA	55.50aB	84.99aA	34.00aB
Soil	91.66aA	62.00aB	54.16bA	49.05aB
Sand	89.16aA	56.50aB	63.33bA	40.50aB

Means followed by the same lowercase letters in the vertical and uppercase letters in the horizontal do not statistically differ from each other by Scott-Knott ($p < 0.05$).

Table 2. Average data of germination speed index of *E. velutina* and *E. mulungu* seeds stored for 1 or 12 months and sown in three different substrates.

Substrate	Germination Speed Index—GSI			
	E. velutina Time storage (month)		*E. mulungu* Time storage (month)	
	1	12	1	12
Plantmax®	3.14aA	3. 19aA	3.09aA	1.73aA
Soil	4.15aA	3.94aA	2.24aA	2.02aA
Sand	4.39aA	2.93aA	2.96aA	2.09aA

Means followed by the same lowercase letters in the vertical and uppercase letters in the horizontal do not statistically differ from each other by Scott-Knott ($p < 0.05$).

Regarding *E. mulungu*, higher germination percentage (84.99%) was achieved with seeds sown in Plantmax® substrate if compared with germination rates verified for seeds sown in soil and in sand substrates (54.16 and 63.33%), respectively. Independently of the type of substrate tested the GSIs did not statistically differ between them. As for the species E. velutina, storage for twelve months resulted in the reduction of seed germination for all tested substrates (**Tables 1** and **2**).

Medeiros & Zanon [30] reported that germination of *Machaerium stipitatum* (Leguminosae-Papilionoideae) seeds stored at 10˚C was reduced by more than 40%, comparable to what happened with *E. velutina* and *E. mulungu* seeds in this study.

Values recorded for collar diameter, shoot height and root elongation of plantlets grown in polybags in the greenhouse are shown in **Table 3**. During the evaluations it was possible to observe total development of the plants, which grew rapidly and presented statistical differences between species. Comparing *E. mulungu* and *E. velutina* plantlets after 2 and 4 months of cultivation it was observed that plant height of four-month-old plants was enhanced three times. However, no significant differences were observed for root elongation in plants cultured.

Considering that the slow growth of aerial parts of tree species has been constantly reported, obtained results can be extremely interesting, especially if deeming large-scale production when the main goal is to produce higher number of quality plants. RAMOS *et al.* [31] reported that *Amburana cearensis*, an important species for the herbal industry after 4 months of cultivation under intense sunlight presented showed enhanced shoot height (15). Moreover, among the variables to determine plant development during initial phase of growth, one important characteristic is shoot elongation. According to Carneiro [32] shoot height is particularly correlated to the development of plantlets transplanted to field.

Values obtained for collar diameter root elongation also demonstrated the effective development of the seedlings (**Table 3**). Plantlets cultured under direct sunlight, did not present stem etiolation and a reasonable growth of the main root was observed. For both species after 4-month cultivation, the average values recorded for those two variables almost doubled, compared to 2-month-old plantlets (**Table 3**).

According to Carneiro [32], species which present superior stem diameter development assure better-quality plantlets, more resistant to adaptation to field conditions. Collar diameter ratio is very important when evaluating the plant potential to survive and grow after transplanting to field.

E. velutina shoot height in the first four months of cultivation was superior to *E. mulungu*, but 12 months after transplanting to field the difference for that variable was not significant, though *E. velutina* collar diameter values were significantly higher compared to *E. mulungu* (**Table 3**).

After 2 months of culture, the occurrence of *Rhizobium* nodules on the radicular system of 98% of the plants of the two species was observed. Those nodules measured between 0.1 and 0.8 cm and were located in the hypocotyl region. The symbiosis between Rhizobium and species of the genus *E. crista-galli*, *E. variegata* (syn. *E. indica*) and *E.costaricensis* has been extensively investigated [33]-[35] but this is the first report of Rhizobium nodules on *E. mulungu* and *E. velutina* roots.

The symbiosis between Rhizobium and plant species is very interesting because it propitiates biological nitrogen fixation and that may have been one of the factors that enhanced *E. velutina* and *E. mulungu* growth. However additional specific studies are necessary to corroborate those findings.

Table 3. Average data of development of 2, 4 and 12-month-old *E. velutina* and *E. mulungu* plants.

Species	Plant height (cm)	Collar diameter (cm)	Root elongation (cm)
		2 meses	
Erytrina mulungu	9.9b	0.32b	18.3b
Erytrina velutina	16.0a	0.54a	27.1a
		4 meses	
Erytrina mulungu	31.5b	0.59b	42.9a
Erytrina velutina	46.6a	1.47a	53.8a
		12 meses	
Erytrina mulungu	126.6a	2.5b	Dno
Erytrina velutina	155.6a	5.6a	Dno

Dno = Data not obtained. Means followed by the same letters in the vertical do not statistically differ from each other by Scott-Knott ($p < 0.05$).

4. Conclusion

There is no complexity in producing *E. velutina* and *E. mulungu* plantlets. Moreover, besides the interest on the chemical, pharmacological and clinical properties regarding those species, large-scale production are viable and simple to be implemented in order to meet the market demands for herbal medicines.

Acknowledgements

This research was supported by the State of São Paulo Foundation (FAPESP) under project number 2012/12762-0.

References

[1] Kass, D.L. (1994) *Erythrina* Species: Pantropical Multipurpose Tree Legumes. In: Gutteridge, R.C. and Shelton, H.M. Eds., Forage Tree Legumes in Tropical Agriculture, CAB International, Wallingford, 84-96.

[2] Maixner, A.E. and Ferreira, L.A.B. (1978) Contribution to the Study of Native Forest and Fruit Essences in the State of Rio Grande do Sul: *Erythrina crista-galli* L. (Corticeira do Banhado). *Wheat and Soybean*, **28**, 3-27.

[3] Lorenzi, H. (1998) Brazilian Trees: Guide for Identification and Cultivation of Brazilian Native Plants. 2nd Edition, Plantarum, Nova Odessa, 205 Pages.

[4] Backes, P. and Irgang, B. (2002) Southern Trees: Identification Guide & Ecological Interest: The Main Species Native to South Brazil. Instituto Souza Cruz, Rio de Janeiro, 326 Pages.

[5] Hardt, E., *et al*. (2006) Riparian Forest Restoration in Sand Minings of the Corumbataí River Basin: Efficacy in the Recovery of Biodiversity. *Scientia Forestalis*, **70**, 107-123. http://www.ipef.br/publicacoes/scientia/nr70/cap11.pdf

[6] Albuquerque, U.P. and Andrade, C.H.L. (2002) Use of Savanna Plant Resources: The Case of the Wild Pernambuco (Northeast of Brazil). *Interciência*, **27**, 336-334.
http://www.etnobotanicaaplicada.com.br/gerenciador/uploadfiles/23b7feb96d134ee60549d26c4ebb5679.pdf
http://dx.doi.org/10.1590/S1516-05722010000100006

[7] Teixeira, S.A. and Melo, J.I.M. (2006) Medicinal Plants Used in the City of Jupi, Pernambuco, Brazil. *IHERINGIA, Series Botanica*, **61**, 5-11.
http://www.fzb.rs.gov.br/publicacoes/iheringia-botanica/Ih61-p5-11.pdf

[8] Onusic, G.M., *et al*. (2010) Effect of Acute Treatment with a Water-Alcohol Extract of *Erythrina mulungu* on Anxiety-Related Responses in Rats. *Brazilian Journal Medical Biology Research*, **35**, 473-477.
http://www.scielo.br/pdf/bjmbr/v35n4/4445.pdf
http://dx.doi.org/10.1590/S0100-879X2002000400011

[9] Onusic, G.M., *et al*. (2010) Effects of Chronic Treatment with a Water-Alcohol Extract from *Erythrina mulungu* on Anxiety-Related Responses in Rats. *Biological Pharmaceutical Bulletin*, **26**, 1538-1542.
http://www.jstage.jst.go.jp/article/bpb/26/11/1538/_pdf
http://dx.doi.org/10.1590/S0100-879X2002000400011

[10] Vasconcelos, S.M., *et al*. (2003) Antinociceptive Activities of the Hydroalcoholic Extracts from *Erythrina velutina* and *Erythrina mulungu* in Mice. *Biological Pharmaceutical Bulletin*, **26**, 946-949.
http://www.jstage.jst.go.jp/article/bpb/26/7/946/_pdf
http://dx.doi.org/10.1016/j.jep.2006.09.023

[11] Marchioro, M., *et al*. (2005) Anti-Nociceptive Activity of the Aqueous Extract of *Erythrina velutina* Leaves. Fitoterapia, **76**, 637-642. http://www.sciencedirect.com/science/article/pii/S0367326X05001772 http://dx.doi.org/10.1016/j.fitote.2005.07.002

[12] Dantas, M.C., *et al*. (2004) Central Nervous System Effects of the Crude Extract of *Erythrina velutina* on Rodents. *Journal Ethnopharmacology*, **94**, 129-133. http://dx.doi.org/10.1016/j.jep.2004.05.007

[13] Vasconcelos, S.M., *et al*. (2011) Central Activity of Hydroalcoholic Extracts from *Erythrina velutina* and *Erythrina mulungu* in Mice. *Journal Pharmacy and Pharmacology*, **56**, 389-393.

[14] Vasconcelos, S.M., *et al*. (2007) Anticonvulsant Activity of Hydroalcoholic Extracts from *Erythrina velutina* and *Erythrina mulungu*. *Journal Ethnopharmacology*, **110**, 271-274.

[15] Ribeiro, M.D., *et al*. (2011) Effect of *Erythrina velutina* and *Erythrina mulungu* in Rats Submitted to Animal Models of Anxiety and Depression. *Brazilian Journal Medical Biololy Research*, **39**, 263-270. http://www.scielo.br/pdf/bjmbr/v39n2/v39n2a13.pdf

[16] Flausino Jr., O.A., *et al*. (2007) Anxiolytic Effects of Erythrinian Alkaloids from *Erythrina mulungu. Journal Natural Products*, **70**, 48-53. http://dx.doi.org/10.1021/np060254j

[17] Flausino Jr., O.A., *et al*. (2007) Effects of Erythrinian Alkaloids Isolated from *Erythrina mulungu* (Papilionaceae) in Mice Submitted to Animal Models of Anxiety. *Biological Pharmacceutical Bulletin*, **30**, 375-378.

[18] Silva, K.B., *et al*. (2007) Breaking Dormancy of *Erythrina velutina* Willd Seeds. *Brazilian Journal of Biosciences*, **5**, 180-182. http://www6.ufrgs.br/seerbio/ojs/index.php/rbb/article/viewFile/264/180

[19] Silva, A.J.C., *et al*. (2010) Breaking Dormancy of *Erythrina crista-galli* Seeds. *Bulletin of Forestry Research*, **53**, 65-78. http://www.cnpf.embrapa.br/publica/boletim/boletarqv/boletim53/BPF_53_p65-78.pdf

[20] Teketay, D. (1994) Germination Ecology of Two Endemic Multipurpose Species of *Erythrina* from Ethiopia. *Forest Ecology and Management*, **65**, 81-87. http://dx.doi.org/10.1016/0378-1127(94)90160-0

[21] Carvalho, N.M., *et al*. (1980) Germination of Seeds of Native Forest Species (*Erythrina speciosa* Andr.). *Revista Brasileira de Sementes*, **2**, 81-88.

[22] Maguire, J.D. (1962) Speed of Germination-Aid in Selection and Evaluation for Seedling Emergence and Vigor. *Crop Science*, **2**, 176-177. http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x

[23] Rolston, M.P. (1978) Water Impermeable Seed Dormancy. *The Botanical Review*, **40**, 365-396. http://dx.doi.org/10.1007/BF02957854

[24] Carvalho, N.M. and Nakagawa, J. (2000) Seeds: Science, Technology and Production. 4th Edition, FUNEP, 5 Jaboticabal, 88 Pages.

[25] Lazarotto, M., *et al*. (2011) Physiological Maturation of *Erythrina crista-galli* L. *Forestry Science*, **21**, 9-16. http://cascavel.ufsm.br/revistas/ojs-2.2.2/index.php/cienciaflorestal/article/viewFile/2742/1668

[26] Miquel, S. (1987) Morphologie Fonctionnele de Plantules D'espèces Forestières du Gabon. *Bulletin du Muséum National d'Histoire Naturelle*, **9**, 101-121.

[27] Cardoso, E.A., *et al*. (2010) *Erythrina velutina* Seedling Emergence in Different Positions and Depths of Sowing. *Rural Science* [*Online*], **38**, 2618-2621. http://www.scielo.br/pdf/cr/v38n9/a34v38n9.pdf http://dx.doi.org/10.1590/S0103-84782008000900034

[28] Matheus, M.T., *et al*. (2010) Overcoming Dormancy in Seeds of two *Erythrina* Species. *Revista Caatinga*, **23**, 48-53. http://periodicos.ufersa.edu.br/revistas/index.php/sistema/article/view/1387/4736

[29] Bento, S.R.S.O., *et al*. (2010) Efficiency of Current Tests to Evaluate Physiological Quality of Mulungu (*Erythrina velutina* WILLD) Seeds. *Brazilian Journal of Seeds*, **32**, 111-117. http://www.scielo.br/pdf/rbs/v32n4/12.pdf http://dx.doi.org/10.1590/S1413-70542009000500023

[30] Medeiros, A.C.S. and Zanon, A. (2000) Storage of Sapuva (*Machaerium stipitatum*) Seeds. *Boletim de Pesquisa Florestal*, No. 40, 57-66. http://www.cnpf.embrapa.br/publica/boletim/boletarqv/boletim40/medeiros.pdf

[31] Ramos, K.M.O., Felfili, J.M., Fagg, C.W., *et al*. (2004) Initial Development and Biomass Production of *Amburana cearensis* (Allemão) AC Smith, under Different Shading Conditions. *Acta Botanical Brasilian*, **18**, 95-101.

[32] Carneiro, J.G.A. (1995) Production and Quality Control of Forest Plantlets. UFPR/FUPEF, Curitiba, 451 Pages.

[33] Bhowmick, P.K. and Basu, P.S. (1987) IAA Production by *Rhizobium* sp. from a Leguminous Tree, *Erythrina indica*. *Folia Microbiológica*, **32**, 142-148. http://dx.doi.org/10.1007/BF02883243

[34] Krishnan, H.B. and Pueppke, S.G. (2010) Cultivar-Specificity Genes of the Nitrogen-Fixing Soybean Symbiont, *Rhizobium fredii* USDA257, Also Regulate Nodulation of *Erythrina* spp. *American Journal of Botany*, **81**, 38-45. http://www.jstor.org/pss/2445560

[35] Frioni, L., *et al*. (2001) Differentiation of Rhizobia Isolated from Native Legume Trees in Uruguay. *Applied Soil Ecology*, **16**, 275-282. http://dx.doi.org/10.1016/S0929-1393(00)00124-4