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# BIOMASS AND CAFFEOYL PHENOLS PRODUCTION OF *ECHINACEA PURPUREA* GROWN IN TAIWAN

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SUMMARY

*Echinacea purpurea* has been introduced in to Taiwan and grown successfully. However, information regarding the effects of the growing climate on its active constituents (e.g. caffeoyl derivatives) and biomass production is very limited. In this study the biomass of field-grown *E. purpurea* plants harvested during three different crop seasons was compared. The content of caffeoyl phenols and the production of aerial plant parts were also assayed. The results indicated that both morphological and agronomic traits were affected by crop season, with spring-grown plants producing more stems and flowers but fewer leaves than autumn-grown plants. Autumn-grown plants produced more caffeoyl phenols, particularly cichoric acid and caftaric acid, in leaf and flower tissues than spring grown plants. Thus, transplanting *E. purpurea* seedlings in the autumn and harvesting the aerial parts at the beginning of winter first, and then harvesting the rhizome-regenerated plants again in the following summer are technically feasible. This farming system would give commercial cultivation of *E. purpurea* in Taiwan a great competitive advantage over other growing regions, provided that an environmentally suitable population is selected and established in Taiwan.

## INTRODUCTION

*Echinacea* are herbaceous perennials of the Asteraceae family native to North America, with wild populations ranging from the eastern and central United States to southern Canada. They are widely used for wild flower establishment, perennial gardening and as a cut flower (Wartidiningsih and Geneve, 1994). However, three species of *Echinacea* are used medicinally: *E. purpurea, E. pallida* and *E. angustifolia* (Mistríková and Vaverková, 2007) because of their antiviral, antibacterial and immunostimulatory benefit to human (Barrett, 2003; Merali *et al*., 2003; Miller, 2005; Murch *et al*., 2006). All three species of *Echinacea* have shown immune-modulating activity (Binns *et al*., 2002). This activity appears to result from the combined effects of caffeoyl phenols, alkylamides and polysaccharides, which are present in all three *Echinacea* species but in different amounts (Briskin, 2000; Speroni *et al*., 2002; Randolph *et al*., 2003). Wild *Echinacea* populations are reportedly threatened by over-harvesting and anthropogenic modifications of habitats, and therefore, *Echinacea* species used medicinally are now being cultivated to meet the huge market demand (Li, 1998). Cultivated *Echinacea* populations are mainly located in the USA and Canada; nevertheless, Europe, Russia

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and Australia also have well-established *Echinacea* cultivation (Kreft, 2005; Letchamo *et al.*, 2002; Seidler-Lozykowska and Dabrowska, 2003; Willis and Stuart, 1999).

*E. purpurea* has been studied extensively in Europe and North America. Many types of phytomedicine are commercially produced from the aerial portions of *E. purpurea* for the prevention and treatment of the common cold and other upper respiratory infections, and the stimulation of immunomodulation (Goel *et al.*, 2005; Lindenmuth and Lindenmuth, 2000; Mahady *et al.*, 2001; Vimalanthan *et al.*, 2005). It has been recently introduced in to Taiwan and appears to grow well (Lin, 2003). However, information regarding the effects of genetic diversity, growing climates and cultivation practices on active constituents (e.g. caffeoyl derivatives) and biomass production of *E. purpurea* is still very limited. It is known that the phytochemical traits of medicinal plants, depend on growing sites, climate conditions, cultural practices, vegetation phases and genetic modifications, and vary considerably within and between wild and cultivated populations (Millauskas *et al.*, 2004). The objectives of the present study were to examine the biomass production of an *E. purpurea* population, which was selected in a previous study (Lin, 2003), grown in different seasons. Several caffeoyl derivatives were also determined and compared between the *E. purpurea* populations harvested. The collected data may help us to select and breed a morphologically superior *E. purpurea* population with desirable active constituents that are adapted to the environmental conditions of Taiwan.

## MATERIALS AND METHODS

Seeds of *Echinacea purpurea* selected from a consecutive mass selection (the spring crop of 2003) were used. In June 2003, selected seeds were soaked in running water for 8 h, and then planted in 104-plugs filled with a mixture of peat moss and vermiculite (3:1) at a depth of 1.5 cm, and watered as necessary (Figure 1A). The indoor-raised seedlings with four to five leaves were transplanted to the experimental farm of the Department of Agronomy, National Chung Hsing University in July 2003. The seedlings were planted on raised two-row beds (1 m wide and 6 m long with 30 cm bed spacing) covered with silver-black polyethylene sheets for weed control (Figure 1B). The plant spacing was 30 × 30 cm. Pre-plant fertilizers were applied at the rates of 100 kg N $ha^{-1}$, 60 kg $P_2O_5$ $ha^{-1}$ and 100 kg $K_2O$ $ha^{-1}$.

For biomass determinations, plant samples, composed of two rows 3 m long, were taken at the full flower stage (Figure 1E). For 2003 autumn crops (Figure 1C), the plants were harvested in December 2003. The number of flowers produced per plant was counted and recorded, and the plants were then cut at 10 cm above ground level. The plots were irrigated after the completion of harvest. The plants regenerated from rhizomes in January 2004. The regenerated and developed plants (Figure 1D) were harvested again in June 2004 (2004 spring crop) using the same harvest procedures as for the previous crop. The rhizome re-generated plants resumed growth and development in July 2004 (Figure 1F) and harvested in December 2004 (2004 autumn crops). All the harvested plants were separated into leaves, stems and flowers for biomass determinations. All the sampled materials were dried in a forced hot air dryer

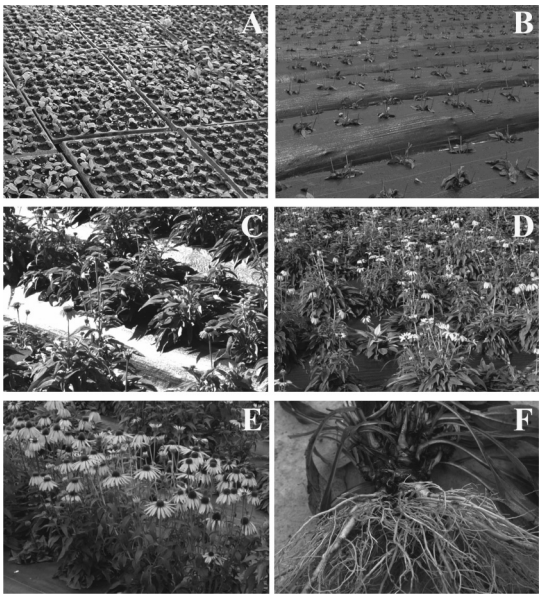


Figure 1. The growth and development of *E. purpurea* plants. (A) seedlings plugs in nursery. (B) transplanted seedlings. (C) transplanted *E. purpurea* plants started to flower. (D) rhizome-regenerated *E. purpurea* plants started to flower. (E) the *E. purpurea* plants at full bloom. (F) 18-months-old rhizomes and roots.

at 43 °C to a moisture content of 10% after drying for 4–7 d, and weighed for biomass determinations.

The total phenol content was estimated by a colorimetric assay based on procedures described by Taga *et al.* (1984). Fifty milligrams of dried ground tissue were extracted using 3 ml 60% (v/v) methanol containing 0.3% (v/v) HCl for 60 min, and then centrifuged at 18 000 *g* for 15 min. A 10 $\mu$l aliquot of tissue extract was dissolved in 200 $\mu$l of 2% (v/v) $Na_2CO_3$, and 10 $\mu$l of the Folin and Ciocalteu's phenol reagent (50%, v/v) were added. The mixture was left to stand at room temperature for 30 min. Absorbance measurements were taken at 725 nm using a spectrophotometer, and caffeic acid was used in the construction of the standard curve.

For caffeic acid derivatives determinations, the tissue extract used for total phenol determination (20 $\mu$l) was filtered through a 0.2 $\mu$m syringe filter (Minisart RC 15, Sartorius) and then analysed using a HPLC (Hitachi, Japan) consisting of a pump (L-7100), column oven (655A-52), UV-VIS detector (L-4200) (330 nm) and auto sampler (L-7200) (Hu and Kitts, 2000). The column used was Mightysil RP-18 GP 5 $\mu$m 150 × 4.6 mm (Kanto, Tokyo, Japan). Two eluents were used: acetonitrile/water 10:90 and acetonitrile/water 25:75. Various levels of caftaric acid, chlorogenic acid, cynarin, echinacoside and cichoric acid were used in the construction of standard curves.

The experimental design was a randomized complete block design with four replicates. All data were subjected to an analysis of variance and when a significant ($p < 0.05$) *F* ratio occurred for treatment effects, a least significant difference (LSD) was calculated.

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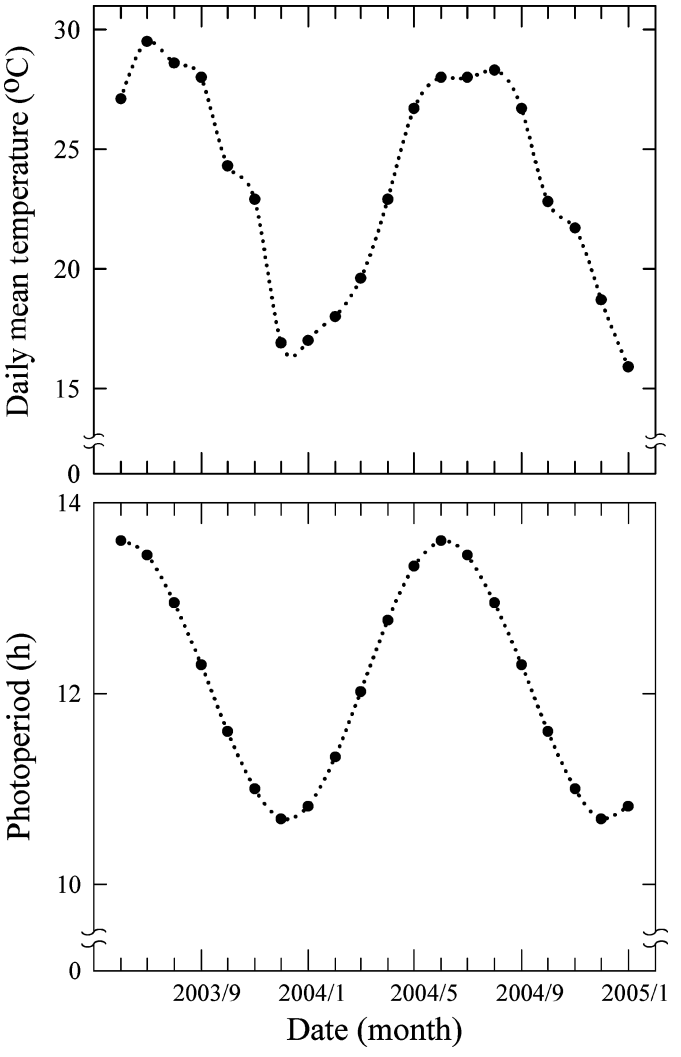


Figure 2. The changes in (A) daily mean temperature and (B) photoperiod during the growth and sampling of *E. purpurea* plants.

## RESULTS

Significant differences in daily mean temperature (Figure 2A) and photoperiod (Figure 2B) were found between spring and autumn crop seasons. Daily mean temperature peaked in July at about 29 °C and dropped to a minimum of 16 °C in December in 2003 (2003 autumn crop season). In the following year, daily mean temperature gradually increased from 17 °C in January to around 28 °C in July (2004 spring crop season), and then followed by another temperature decline (2004 autumn crop season). Similar patterns were also noted for daily photoperiods (Figure 2B).

Both seedling-transplanted (2003 autumn growing samples) (Figure 1C) and rhizome-regenerated (2004 spring and autumn growing samples) (Figure 1D)

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*E. purpurea* plants grew vigorously under natural conditions. As shown in Table 1, both morphological and agronomic traits in harvested plants were highly variable, as indicated by the relatively greater standard deviations of the samples examined. Crop season was found to affect the morphological traits of *E. purpurea* plants (Table 1). The spring-grown plants generally grew taller and produced more flowers than autumn-grown plants. As a result, spring-harvested *E. purpurea* produced more biomass in the aerial portion of the plants than autumn-harvested plants (Table 1). Moreover, the seedling-transplanted *E. purpurea* (2003 autumn season) grew better and produced more biomass than the *E. purpurea* regenerated from rhizomes (2004 autumn season) (Table 1).

The average content of total phenolics in harvested *E. purpurea* leaves and flowers is presented in Tables 2 and 3, respectively. As with the morphological and agronomic traits, the content of total phenolics in leaf and flower tissues also varied greatly (Tables 2 and 3). Both leaf and flower tissues showed that the content of total phenolics was higher from autumn-harvested plants than from spring-harvested plants.

Large variations were also found in the content of total caffeic acid derivatives in leaves and flowers (Tables 2 and 3). As shown in Table 2, the contents of caffeic acid derivatives in leaf tissues differed by harvest season. The leaves harvested in the autumn had more caffeic acid derivatives than leaves harvested in the spring. Among the five caffeic acid derivatives examined in the present study, leaves harvested in both autumn 2003 and 2004 had the highest cichoric acid content and followed by caftaric acid (Table 2). The cichoric acid and caftaric acid produced were at the same level for the leaf tissues harvested from the spring season crop, which were relatively lower than that of the leaf tissues harvested from the 2003 or 2004 autumn seasons (Table 2). In all cases, the contents of chlorogenic acid, cynarin and echinacoside were relatively low in comparison with those of cichoric acid or caftaric acid (Table 2).

The contents of total caffeic acid derivatives in flowers (Table 3) were consistently higher than those in leaves (Table 2). The contents of total caffeic acid derivatives in flowers also differed by harvest season (Table 3). The autumn-harvested flowers had more caffeic acid derivatives than spring-harvested flowers. In all three seasons, harvested flowers contained the highest cichoric acid content and followed by caftaric acid and chlorogenic acid. The contents of cynarin and echinacoside were relatively low compared to cichoric, caftaric and chlorogenic acids (Table 3).

## DISCUSSION

The objective in commercial *E. purpurea* production is to produce high biomass with a high bioactive compound content (i.e. caffeoyl phenols). The content of bioactive compounds varies between *E. purpurea* plant organs, with the content of caffeoyl phenols in leaves and flowers considerably higher than in underground parts (Stuart and Wills, 2000; Thygesen *et al.*, 2007). That is why the majority of *E. purpurea* preparations in the commercial market, ranging from direct pressed juices to freeze-dried ethanolic or hydrophilic extracts are made from whole or powered dried leaves and flowers (Barrett, 2003; Perival, 2000). Therefore, in the present study, only the aerial parts of the *E. purpurea* plant were sampled for biomass and caffeoyl phenols production.

Table 1. The morphological and agronomic traits of *Echinacea purpurea* grown under different seasons. The morphologic traits (plant height and produced flowers) were expressed on per plant basis. The agronomic traits were expressed on g dry weight per plant basis.

| | Plant height (cm) | | Flowers | | Aerial part (g) | | Flower (g) | | Stem (g) | | Leaves (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Growing season | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* |
| 2003 Autumn | 46.21 | 0.61 | 19.40 | 0.89 | 92.95 | 2.58 | 31.59 | 1.18 | 16.83 | 0.79 | 44.53 | 0.88 |
| 2004 Spring | 50.01 | 0.76 | 45.93 | 1.11 | 112.89 | 3.23 | 51.19 | 1.47 | 30.22 | 0.98 | 31.48 | 1.10 |
| 2004 Autumn | 46.63 | 1.00 | 19.52 | 1.44 | 61.52 | 4.26 | 25.61 | 1.94 | 11.43 | 1.30 | 24.48 | 1.44 |
| | ANOVA | | | | | | | | | | | |
| Growing season | $F_{(2,1075)} = 8.27$, $p = 0.0003$ | | $F_{(2,1077)} = 194.95$, $p < 0.0001$ | | $F_{(2,1077)} = 46.21$, $p < 0.0001$ | | $F_{(2,1077)} = 74.19$, $p < 0.0001$ | | $F_{(2,1077)} = 83.78$, $p < 0.0001$ | | $F_{(2,1078)} = 87.84$, $p < 0.0001$ | |
| 2003 Autumn v. 2004 Spring | $F_{(1,1075)} = 15.54$, $p < 0.0001$ | | $F_{(1,1077)} = 350.03$, $p < 0.0001$ | | $F_{(1,1077)} = 23.25$, $p < 0.0001$ | | $F_{(1,1077)} = 108.27$, $p < 0.0001$ | | $F_{(1,1077)} = 112.93$, $p < 0.0001$ | | $F_{(1,1078)} = 86.05$, $p < 0.0001$ | |
| 2003 Autumn v. 2004 Autumn | $F_{(1,1075)} = 0.11$, $p = 0.7447$ | | $F_{(1,1077)} = 0.01$, $p = 0.9306$ | | $F_{(1,1077)} = 39.78$, $p < 0.0001$ | | $F_{(1,1077)} = 6.87$, $p = 0.0089$ | | $F_{(1,1077)} = 12.60$, $p = 0.0004$ | | $F_{(1,1078)} = 143.15$, $p < 0.0001$ | |
| 2004 Spring v. 2004 Autumn | $F_{(1,1075)} = 7.56$, $p = 0.0061$ | | $F_{(1,1077)} = 207.29$, $p < 0.0001$ | | $F_{(1,1077)} = 92.32$, $p < 0.0001$ | | $F_{(1,1077)} = 110.16$, $p < 0.0001$ | | $F_{(1,1077)} = 132.99$, $p < 0.0001$ | | $F_{(1,1078)} = 15.65$, $p < 0.0001$ | |

Table 2. The contents (mg $g^{-1}$ dry weight) of total phenolics and caffeic acid derivatives in the leaves of *Echinacea purpurea* grown under different seasons.

| | Total phenolics | | Total caffeic acid derivatives | | Cichoric acid | | Caftaric acid | | Chlorogenic acid | | Cynarin | | Echinacoside | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Growing season | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* |
| 2003 Autumn | 83.92 | 0.80 | 62.45 | 0.79 | 40.32 | 0.63 | 20.63 | 0.24 | 0.20 | 0.01 | 0.51 | 0.00 | 0.79 | 0.02 |
| 2004 Spring | 45.13 | 1.43 | 13.41 | 1.40 | 6.30 | 1.12 | 6.68 | 0.43 | 0.11 | 0.02 | 0.19 | 0.00 | 0.13 | 0.04 |
| 2004 Autumn | 108.28 | 1.42 | 46.43 | 1.40 | 29.34 | 1.11 | 15.92 | 0.42 | 0.18 | 0.02 | 0.36 | 0.00 | 0.63 | 0.04 |
| | ANOVA | | | | | | | | | | | | | |
| Growing season | $F_{(2,980)} = 508.36$, $p < 0.0001$ | | $F_{(2,980)} = 459.77$, $p < 0.0001$ | | $F_{(2,980)} = 353.29$, $p < 0.0001$ | | $F_{(2,980)} = 413.19$, $p < 0.0001$ | | $F_{(2,858)} = 3.58$, $p = 0.0284$ | | $F_{(2,969)} = 55.30$, $p < 0.0001$ | | $F_{(2,891)} = 114.87$, $p < 0.0001$ | |
| 2003 Autumn v. 2004 Spring | $F_{(1,980)} = 563.80$, $p < 0.0001$ | | $F_{(1,980)} = 912.27$, $p < 0.0001$ | | $F_{(1,980)} = 701.03$, $p < 0.0001$ | | $F_{(1,980)} = 817.56$, $p < 0.0001$ | | $F_{(1,858)} = 3.87$, $p = 0.0495$ | | $F_{(1,969)} = 105.06$, $p < 0.0001$ | | $F_{(1,891)} = 229.66$, $p < 0.0001$ | |
| 2003 Autumn v. 2004 Autumn | $F_{(1,980)} = 224.19$, $p < 0.0001$ | | $F_{(1,980)} = 97.12$, $p < 0.0001$ | | $F_{(1,980)} = 74.48$, $p < 0.0001$ | | $F_{(1,980)} = 94.77$, $p < 0.0001$ | | $F_{(1,858)} = 1.71$, $p = 0.1919$ | | $F_{(1,969)} = 22.70$, $p < 0.0001$ | | $F_{(1,891)} = 14.05$, $p = 0.0002$ | |
| 2004 Spring v. 2004 Autumn | $F_{(1,980)} = 986.57$, $p < 0.0001$ | | $F_{(1,980)} = 274.41$, $p < 0.0001$ | | $F_{(1,980)} = 211.08$, $p < 0.0001$ | | $F_{(1,980)} = 235.74$, $p < 0.0001$ | | $F_{(1,858)} = 7.07$, $p = 0.0080$ | | $F_{(1,969)} = 20.18$, $p < 0.0001$ | | $F_{(1,891)} = 90.57$, $p < 0.0001$ | |

Table 3. The contents (mg $g^{-1}$ dry weight) of total phenolics and caffeic acid derivatives in the flowers of *Echinacea purpurea* grown under different seasons.

| | Total phenolics | | Total caffeic acid derivatives | | Cichoric acid | | Caftaric acid | | Chlorogenic acid | | Cynarin | | Echinacoside | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Growing season | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* | Mean | *s.e.* |
| 2003 Autumn | 191.32 | 2.27 | 126.16 | 1.80 | 94.93 | 1.49 | 23.91 | 0.35 | 5.92 | 0.26 | 0.30 | 0.00 | 1.10 | 0.03 |
| 2004 Spring | 186.43 | 2.42 | 115.97 | 1.92 | 85.89 | 1.58 | 23.69 | 0.37 | 4.91 | 0.27 | 0.44 | 0.00 | 1.04 | 0.03 |
| 2004 Autumn | 201.63 | 2.62 | 126.90 | 2.08 | 94.81 | 1.72 | 24.71 | 0.40 | 5.50 | 0.30 | 0.42 | 0.00 | 1.46 | 0.04 |
| | ANOVA | | | | | | | | | | | | | |
| Growing season | $F_{(2,492)} = 9.41$, $p < 0.0001$ | | $F_{(2,492)} = 10.09$, $p < 0.0001$ | | $F_{(2,492)} = 10.81$, $p < 0.0001$ | | $F_{(2,491)} = 1.88$, $p = 0.1542$ | | $F_{(2,492)} = 3.64$, $p = 0.0269$ | | $F_{(2,491)} = 22.26$, $p < 0.0001$ | | $F_{(2,491)} = 39.47$, $p < 0.0001$ | |
| 2003 Autumn v. 2004 Spring | $F_{(1,492)} = 2.22$, $p = 0.1368$ | | $F_{(1,492)} = 15.50$, $p < 0.0001$ | | $F_{(1,492)} = 17.41$, $p < 0.0001$ | | $F_{(1,491)} = 0.22$, $p = 0.6419$ | | $F_{(1,492)} = 7.26$, $p = 0.0073$ | | $F_{(1,491)} = 36.65$, $p < 0.0001$ | | $F_{(1,491)} = 1.71$, $p = 0.1912$ | |
| 2003 Autumn v. 2004 Autumn | $F_{(1,492)} = 8.85$, $p = 0.0031$ | | $F_{(1,492)} = 0.03$, $p = 0.8659$ | | $F_{(1,492)} = 0.00$, $p = 0.9703$ | | $F_{(1,491)} = 2.17$, $p = 0.1412$ | | $F_{(1,492)} = 1.18$, $p = 0.2786$ | | $F_{(1,491)} = 27.33$, $p < 0.0001$ | | $F_{(1,491)} = 52.72$, $p < 0.0001$ | |
| 2004 Spring v. 2004 Autumn | $F_{(1,492)} = 18.31$, $p < 0.0001$ | | $F_{(1,492)} = 14.64$, $p = 0.0001$ | | $F_{(1,492)} = 14.77$, $p = 0.0001$ | | $F_{(1,491)} = 3.48$, $p = 0.0627$ | | $F_{(1,492)} = 2.11$, $p = 0.1474$ | | $F_{(1,491)} = 0.30$, $p = 0.5857$ | | $F_{(1,491)} = 68.73$, $p < 0.0001$ | |

In the present study, all the morphological, agronomic and biochemical traits in harvested plants were highly variable, as described in previous reports (Kreft, 2005; Wills and Stuart, 1999). Kreft (2005) indicated that only a small portion of the large variability could be explained by environmental and cultural conditions, with the inter-individual differences being the main source of variability. Our data support his findings. *E. purpurea* is a cross-pollinated plant and tends to be self-incompatible (Li, 1998). Therefore, the large variability in its morphological and agronomic traits is not unexpected. It appears that a continuous mass selection is a necessity to reduce the heterogeneity in these morphological and agronomic traits within the cultivated *E. purpurea* populations.

As shown in Table 1, the spring-grown plants produced more flowers than autumn-grown plants. The greater flower setting in the spring crop is not surprising because *E. purpurea* is an intermediate-day plant and the flowering percentage is greater under photoperiods of 13–15 h (Runhle *et al.*, 2001). The photoperiods for the 2004 spring season were around 13 h, but they dropped to about 11 h in the 2003 and 2004 autumn seasons (Figure 2B). The relatively lower mean daily temperatures recorded during the autumn in comparison with the spring might also limit the growth and development of *E. purpurea* plant to some extent (Figure 2A). However, it should be noted that the monsoon (May to June), and typhoons (July to September), which occur frequently in Taiwan, would be two environmental risks affecting the successful growth and development of *E. purpurea*, if the growers intend to cultivate these plants during the spring.

The data in Table 1 further demonstrated that the seedling-transplanted plants (2003 autumn season) appeared to grow better and produce more biomass than *E. purpurea* plants regenerated from rhizomes (2004 autumn season). However, this could also be a result of higher temperatures in 2003 autumn compared to 2004 autumn. Thus, for optimal production, the first harvest of the aerial parts of autumn-grown plants should be carried out at the end of the season (around December), and then allow the plants to regenerate from rhizomes in the coming spring. However, the rhizome-regenerated plants should be ploughed up and replaced with newly grown seedlings at the end of summer. In contrast, Kreft (2005) recommended that plantations should be ploughed up and replanted every three years.

Phenolic substances extracted from aerial parts of *E. purpurea* plants are very efficient antioxidants, which have been suggested for the treatment of various types of illness (Thygesen *et al.*, 2007). In the present study, crop season was also found to affect the content of total phenolics in leaf and flower tissues (Tables 2 and 3). The environmental factors that might affect the accumulation of phenolics are still unknown. Because *E. purpurea* plants are native to temperate regions (Mistríková and Vaverková, 2007), growing and developing in the relatively cool conditions (Figure 2B) and low humidity (autumn crop season) may be beneficial to the expression of phenolic compounds differentially, particularly in the leaf and flower portions of the plants.

Cichoric acid is one of the most important markers affecting the market quality of *E. purpurea* (Thygesen *et al.*, 2007). Kreft (2005) found that *E. purpurea* grown in Slovenia contained cichoric acid at 11 and 16 mg $g^{-1}$ dry weight (DW) in flower and

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leaf tissues of the plant. The contents of cichoric acid in *E. purpurea* grown in Germany have been reported to be 13 mg $g^{-1}$ DW in leaves and flowers. The Australian-grown *E. purpurea* flowers and leaves contain 30–38 and 4–15 mg $g^{-1}$ DW, respectively (Wills and Stuart, 1999). In all cases, the levels of cichoric acid pound in our study were much higher than their results. The higher cichoric content in leaves and flowers of *E. purpurea* would give commercial cultivation of this medicinal plant in Taiwan a greater competitive edge over other *E. purpurea* growing regions.

In conclusion, the present results indicate that the biomass and caffeoyl phenols production of *E. purpurea* plants are indeed influenced by the growing season. Autumn-grown plants produce more caffeoyl phenols, particularly cichoric acid and caftaric acid, in their leaves and flowers than spring-grown plants. Spring-grown *E. purpurea* plants produced more biomass possibly due to their greater flowers and stem yields than those of autumn-grown plants. Our results indicate that to grow *E. purpurea* at the end of the summer and subsequently harvest aerial parts of the plants in the autumn, and then harvest the rhizome-regenerated plants again in next spring is technically feasible in Taiwan. This unique cultural practice, which allowing the growers to harvest *E. purpurea* plants containing high caffeoyl phenols content twice annually, would give commercial cultivation of *E. purpurea* in Taiwan a great competitive advantage over other *E. purpurea*-growing regions. However, the introduction of *E. purpurea* on a commercial scale requires a more homogenous plant population. In this regard, a consecutive mass selection and purification programme to breed an environmentally suitable population should be continued in Taiwan.

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