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Irrigation strategies to optimise water use efficiency and production in *Polygonum tinctorium* Ait., a new indigo delivering crop

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Abstract. *Polygonum tinctorium* Ait. (dyer's knotweed) is an annual plant of the Family *Polygonaceae*, very popular in Japan and China, where it has been employed for large-scale indigo production until 19th century. Today there is increasing interest toward this species as new crop for indigo production but until now, no research has been carried out on its irrigation requirements. With the aim to assess the crop coefficient (Kc), the seasonal crop water requirement (CWR) and the effects of irrigation on vegetative production and indigo yield, six irrigation levels (T100, T80, T60, T40, T20 that received a seasonal water amount equivalent to 100, 80, 60, 40, 20 % of ETc and a rain-fed control T0) have been compared in a randomised block design experiment with four replications. The trials have been carried out in Central Italy during two growing seasons characterized by exceptionally rainy (2002) and dry summer conditions (2003) in comparison with the typical ones. Results outlined that the concentration per unit of leaf weight of the indoxyl indigo precursor indican, determined by HPLC-ELSD, was not influenced by irrigation, whereas it was increased by temperatures and light intensity. On the other hand irrigation significantly influenced seasonal plant dry and fresh yield as well as indigo production in both years. A significant decrement of yield was observed with T0 and T20 treatments in comparison with T40, T60, T80 and T100. Irrigation rates higher than 40%ETc did not enhance significantly plant and indigo production. The seasonal CWR recorded from April/May to October 2003, corresponding to 4767 m^3 ha^{-1}, was significantly higher (+98%) than 2002. Kc values differed significantly with the crop growth stage reaching the maximum value of 0.7-0.8 at full vegetative development when the plants were ready to be harvested for the first time at the beginning of July. The maximum Kc values of 0.5-0.6 were regained at beginning of September before the crop second harvest.

Keywords. *Polygonum tinctorium* – Indoxyl β-D-glucoside – Irrigation – Kc – ETc – Crop – Yield.

Stratégies d'irrigation pour optimiser l'efficacité d'utilisation de l'eau et la production de Polygonum tinctorium Ait., une nouvelle culture pour la production de l'indigo

Résumé. *Polygonum tinctorium Ait. est un plant annuel de la famille de Polygonaceae, très populaire en Japon et Chine, où elle a été utilisée pour la production à grande échelle d'indigo jusqu'au 19ème siècle. Aujourd'hui l'intérêt augmente vers cette espèce pour la production d'indigo, mais jusqu'au moment, aucune recherche n'a été effectuée sur ses conditions d'irrigation. Pour le but d'évaluer le coefficient de culture (kc), le besoin de l'eau (CWR) et les effets de l'irrigation sur la production de l'indigo, six niveaux d'irrigation (T100, T80, T60, T40, T20, qu'ont reçu une quantité saisonnière d'eau équivalente a 100, 80, 60, 40, 20% de ETc, et T0 sans irrigation) ont été comparés. Les épreuves ont été effectuées en Italie centrale pendant deux ans caractérisées par les états particulièrement pluvieux (2002) et secs (2003) en comparaison des états typiques. Les résultats ont décrit que la concentration par unité du poids de feuilles du précurseur d'indigo indican, déterminé par HPLC-ELSD, n'a pas été influencée par irrigation tandis qu'elle a été augmentée par les températures et intensité de la lumière. D'autre part l'irrigation a influencé de manière significative le rendement sec et frais, et aussi la production d'indigo en les deux années. On a observé une décroissance significative de rendement avec les traitements T0 et T20 en comparaison de T40, de T60, de T80 et de T100. Niveaux d'irrigation plus haut que 40% ETc n'a pas augmenté de manière significative la production du plant et d'indigo. Le CWR saisonnier enregistré à partir d'avril-de mai à l'octobre 2003, correspondant à 4767* m^3 ha^{-1}*, était sensiblement plus haut (+98%) que 2002. Les valeurs de Kc ont différé de manière significative avec l'étape de croissance atteignant la valeur maximale de 0.7-0.8 au plein développement végétatif au début de juillet. Les valeurs du maximum Kc de 0.5-0.6 ont été regagné au début de septembre avant de la deuxième récolte.*

Mots-clés. *Polygonum tinctorium – Indoxyl β-D-glucoside – Irrigation – Kc – ETc – Culture – Rendement.*

I – Introduction

Indigo used in the dyeing industry mainly for denim, is currently synthesized from by-products of fossil fuels. However, several plants are able to synthesize indigo precursors such as *Polygonum tinctorium* Ait. (dyer's knotweed) an annual plant of the Family *Polygonaceae*, very popular in Japan, China and Russia, where it has been employed for large-scale indigo production until 19th century. The plant has large dark bluish-green leaves which contain some glycosides as secondary metabolites, the major one is a colourless glucoside called indican (indoxyl β-D-glucoside). When the plant cells are put in water, indican is extracted and it is degraded to indoxyl and glucose. A dimerization of this indoxyl by air oxidation follows and indigo is formed, which is commonly used as a blue dye since ancient time (Minami, 1997). Today there is increasing interest toward this species as new crop for indigo production in Europe but until now, no research has been carried out on its irrigation requirements.

Aim of the present study was to analyze the crop coefficients (Kc), the crop water requirement (CWR) and the response to irrigation of this new crop grown under field conditions.

II – Material and methods

1. Field trials

Trials were conducted during the two growing seasons 2002 and 2003 at the Experimental Centre of DAGA-University of Pisa (Pisa countryside, Central Italy 43°41' N; 10°23' E; altitude 5 m a.s.l). *P. tinctorium* seeds were sown in paper pots on March and incubated in germination cabinets under controlled air temperature (20°C) until transplanting in the field in the spring (8th May 2002 and 18th April 2003). The plants were transplanted at 4th true leaf stage with 30 cm inter row and 30 cm intra row distances and a crop density of 120.000 plant ha^{-1}. Soil was a typical Xerofluvent of the low Arno river plain, characterized by a superficial water table 120 cm deep in dry conditions. At the beginning of the experimental season, soil was sampled along the 0-30 cm profile and physical and chemical characteristics as well as wilting point and field capacity were measured (Table 1).

Table 1. Chemical and physical characteristics of the soil used for the field trial in 2002 and 2003. Bulk density was averaged on 0-30 cm soil layer.

Parameter	Unit	2002	2003
Sand (2-0.05 mm)	%	36.8	25.8
Silt (0.05-0.002 mm)	%	45.5	46.2
Clay (<0.002 mm)	%	17.7	28.1
pH		8.0	7.9
Organic matter	(%)	1.7	1.3
Total nitrogen	(g kg^{-1})	1.2	1.2
Available phosphorus	(mg kg^{-1})	4.8	15.3
Exchangeable potassium	(mg kg^{-1})	112.2	105.8
Field capacity	% weight	21.0	21.5
Permanent wilting point	% weight	9.6	9.6
Bulk density	g cm^{-3}	1.3	1.3

The field used for the experiments had been previously cultivated with wheat. The soil was ploughed to a depth of 35 cm in November 2001 and 2002 and ploughing was followed by a superficial disk harrowing in March to a fine tilth to prepare the sowing bed.

Throughout the two experimental periods plants were maintained under identical fertilisation conditions. Mineral fertiliser was applied before planting at rates of 100/100/100 kg ha^{-1} of N/P_2O_5/K_2O. Further 50 kg N ha^{-1} were supplied after the first and the second harvest of the leaves. Weeds were mechanically controlled by hand weeding. No diseases and insects occurred.

2. Irrigation treatments

During the first week of growth, water was supplied in equal amounts to all plots to facilitate post-transplanting recovery. Subsequently, six irrigation levels (T100, T80, T60, T40, T20 that received a seasonal water amount equivalent to 100, 80, 60, 40, 20% of ETc and a rain-fed control T0) were compared in a randomised block design experiment with four replications. Each plot was 12 m^2 size with 144 plants per plot. The crop evapotraspiration (ETc) during the growing season was estimated by two microlysimeters while monitoring the climatic parameters and the phenological crop development (Bertolacci and Megale, 1991). The microlysimeters consisted of two prismatic containers (1.20 m x 1.20 m x 0.50 m deep) buried in the soil within the crop layout, leaving two centimeters emerging from the ground. Plants growing in the inside area were therefore perfectly integrated in the crop, thus avoiding advection. The portion of crop confined in the microlysimeters was water fed from a proper artificial water table, placed at the bottom of the containers that were equipped with an automatic device for management and control. The device ensured prompt water replenishment for daily implementation of the automated drip irrigation system, in order to deliver water to the crop at a rate matching water consumption, i.e. water amounts equivalent to 100% ETc rate. Daily meteorological data and daily ETc data were automatically collected and recorded. The water was delivered daily by an automated drip irrigation system equipped with a pressure-compensated and non-leakage dripper line, with emitter flow rate of 2.3 $l \cdot hr^{-1}$ and emitter spacing of 30 cm. To calculate the reference evapotranspiration (ET0), climatic parameters were monitored by a meteorological station and daily measures taken by a Class A pan evaporation placed near the experimental field. ET0 was estimated by the following equation: ET0 = Kp Epan [ET0 = evapotraspiration for grass reference crop, mm/day; Kp = pan coefficient by Doorenbos and Pruitt (1977); Epan=pan evaporation, mm/day]. ETc was calculated by adding any rainfall of significance to the microlysimetric daily water requirement. Therefore, ETc represents the maximum crop evapotraspiration. The ratio between ETc and ET0 within time intervals gives the crop coefficient Kc [Kc = ETc/ET0]. The seasonal crop water requirement (CWR) (m^3 ha^{-1}), ETc (mm day^{-1}) and Kc were evaluated.

3. Environmental parameters

Changes in air temperature, rainfall, global radiation and photosynthetically active radiation (PAR) were recorded throughout by using a weather station, properly equipped to the purpose. Cumulative sums of PAR (mE m^{-2}; 1E = 1 moles of photons) and global radiation (KJ m^{-2}) on hourly and daily basis from sowing to harvesting were calculated by a data logger Campbell CR10X. Sensors were mod. Rg19 by Silimet Quantum Sensor system.

4. Plant productive determinations

During the crop cycle the phenological crop development was followed. Plants were hand-cut at 10 cm above soil level at the beginning of the flowering phase when they had reached their maximum height. Subsequent harvests were taken when inter-row closure was complete and maximum height had been regained. In 2002 *P.tinctorium* plants were harvested on July 4th for the first time, and on October 3rd for the second time; in 2003 plants were harvested on July 5th, September 3rd and October 28th for the first, second and third time respectively. Measurements made on individual harvests (leaves, stems, and total fresh and dry plant yield in t ha^{-1}) were summed to estimate crop seasonal yield. Samples were taken from an area of 5 m^2 on each plot excluding the plants on the two outer rows of each plot.

5. HPLC indican analysis and indigo quantification

Ten leaf samples from each field experimental plot were taken before harvesting. Leaf discs (1cm diameter) were obtained from the central part of the leaf (excluding veins) and immediately transferred into a glass tube with deionised water in a 1:10 weight/volume ratio. Indican water extraction was carried out at 100°C in a boiling bath for 7 min. Leaf water extracts were diluted 1:10 (v/v) with water and 20 ml aliquots injected into the HPLC system (Jasco PU980) coupled with an Evaporative Light Scattering Detector (ELSD 2000, Alltech), according to Angelini *et al.* (2003 and 2004). The theoretical indigo amount obtainable from the complete reaction of indoxyl was predicted by stoichiometric calculations. The method, fully described by Angelini *et al*. (2003), allowed a sensitive and reproducible resolution of samples in a short running time (5 min).

6. Statistical analysis

All variables were analyzed by ANOVA using a randomized block experimental design to test the significance of differences associated to irrigation treatments separately for each year. Significantly different means were separated at 0.05 probability level by Last Significant Difference (LSD) test (Gomez and Gomez, 1984).

III – Results and discussion

1. Weather conditions

Total rainfall per month and monthly mean air temperature in 2002 and 2003 are presented in Figure 1. The two growing seasons were characterised by contrasting rainfall distributions during spring and summer in comparison with long-term trend. Considerable variability in rainfall amount and distribution was observed among the two years (468 mm and 59 mm from April to October in 2002 and 2003 respectively) and in comparison with the typical long-term trend (426 mm from April to October). In particular, 2003 was characterised by a very dry summer, with rainfall amount significantly lower than the year before and in comparison with the long-term trend. Summer 2002 (June to August) was exceptionally rainy, with a total rainfall of 164.4 mm against 6.3 mm in 2003 and 113.4 mm for the long term. Mean air temperatures showed the typical long-term trend. The mean monthly temperatures increased from March to the end of July, and decreased thereafter. The peak value in 2003 was higher than in 2002 (33°C vs 29 °C respectively) due to higher maximum temperature values. Cumulative daily PAR (Σ mean values per month in mE m^{-2}) and cumulative Global Radiation (Σ mean values per month in KJ m^{-2}) are reported in Figure 2. PAR showed the typical increasing trend from April to July, thereafter it decreased slowly until September. In 2002 weather conditions were unstable throughout summer, with no sustained periods of high irradiance. As a consequence global radiation and PAR summer values in 2002 were lower than in 2003.

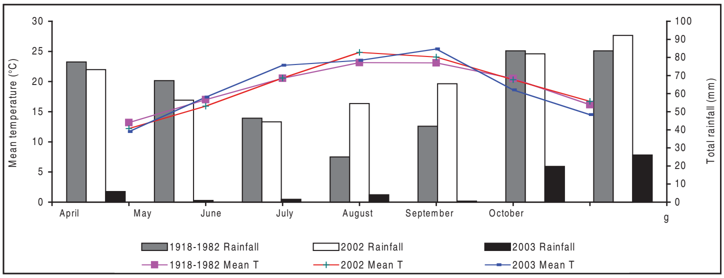


Figure 1. Total rainfall (mm) and mean air temperature (°C) from April to October in 2001 and 2002 growing seasons, in comparison with long term 1918-1982 data for the same site.

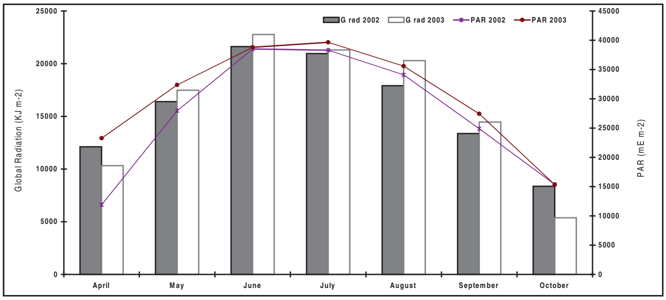


Figure 2. Mean monthly values of Global Radiation (KJ m^{-2}) and Photosynthetically Active Radiation (PAR mE m^{-2}; 1E = 1 moles of photons) measured from April to October in 2002 and 2003 growing seasons.

In Figure 3 the trend of ET0 daily values from April/May to October 2002 and 2003 is reported. The values recorded are those typical of the Tyrrhenian coast with peak values at the end of spring and summer when dry windy and sunny conditions occurred. It is evident that ET0 values in 2003 were always higher than 2002.

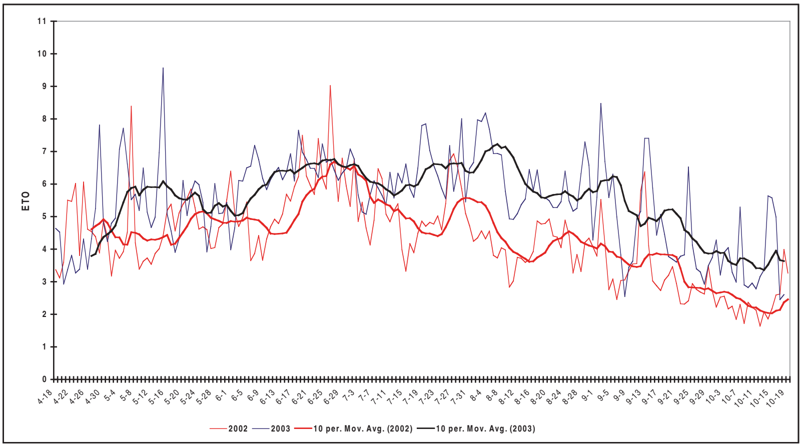


Figure 3. Trend of ET0 (mm/day) daily values from April/May to October 2002 and 2003. Data averaged every 10 days (moving average) represented by the bold lines.

2. Seasonal crop water requirement (CWR), crop evapotraspiration (ETc) and crop coefficients (Kc)

The seasonal CWR from April/May to October differed significantly between the two years being significant higher (+97%) in 2003 than in 2002, the wetter season (4767 *vs* 2413 m^3 ha^{-1}).

The seasonal trend of ETc calculated in the two seasons, is showed in the Figure 4.

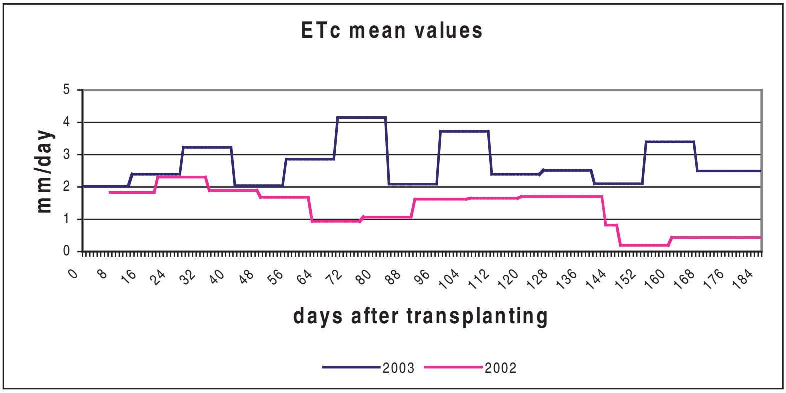


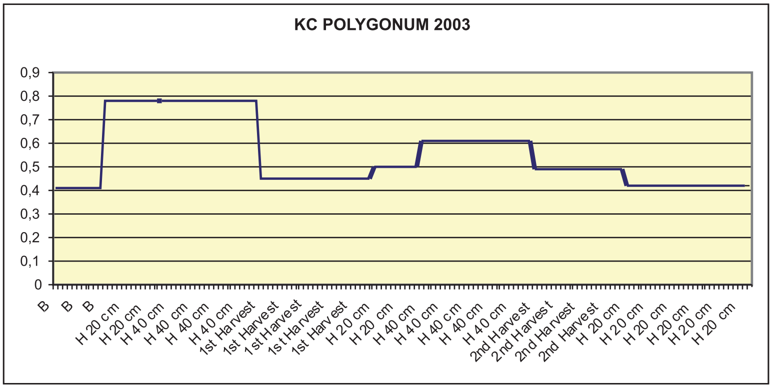
Figure 4. ETc mean values (mm /day) in 2002 and 2003 seasonal course. Values were averaged over 14 days.

To evaluate the ETc and the crop coefficients in the different phases of development, it's useful to keep in mind the development of *Polygonum tinctorium*. This species begins the vegetative development with beginning of branching about 45 days after sowing, thereafter the main stem lengthens, reaching 20 cm height about three months after sowing and 40 cm height about three and half months after sowing. In this phase a large amount of leaves were formed. The stems are free of indigo precursor, which is confined to the leaves. For this reason harvesting is best done before the end of the vegetative growth period. Therefore the best time for the first harvest is reached when the ratio between leaves and stalks is about 1:1 when the crop rows are closed by the developing *Polygonum* plants.

Subsequently to the first harvest, the plant re-growths and develops new stems and leaves. The plant production after every harvest has the tendency to decrease during the season, mainly as consequence of the diminution of day-length, PAR as well as air temperatures.

The ETc values were different in the two years according with the different trend of the ET0. During the 2002 growing season, the medium values of daily ETc fell in the range 1.0-2.3 mm/day, except in the last month of the season, when they decreased to 0.2 mm/day. This behaviour can put in relation with the unusual 2002 cloudy and rainy summer conditions. In particular the abundant rainfall took frequently the soil to the field capacity, so plants had much available water and consequently the water supply to the microlysimeters was limited. On the contrary, the 2003 ETc reached the maximum values over 4 mm/day, due to the high sunshine and temperatures during summer with the minimum values of about 2 mm/day immediately after each harvesting (Figure 4).

The trend of Kc values measured in the two experimental seasons is reported in Figure 5.



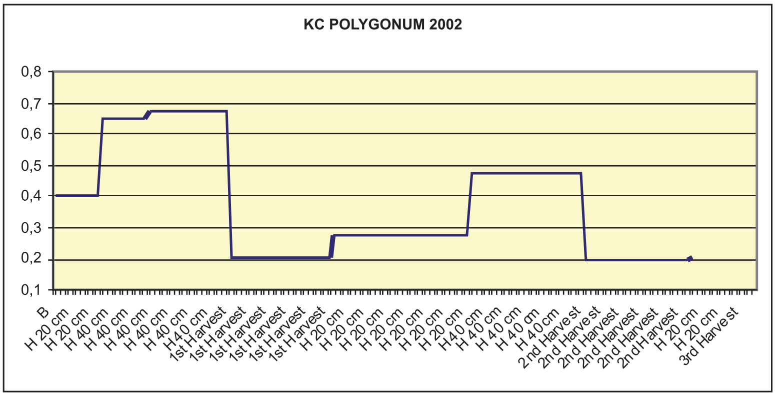


Figure 5. Crop Coefficients (Kc) in the different *P.tinctorium* phenological phases in 2002 and 2003 growing seasons. B= Beginning of stem branching; H 20 cm= plant with stem height of 20 cm; H 40 cm= plant with stem height of 40 cm.

Kc values varied by crop growth stage reaching the maximum value of 0.7/0.8 at full vegetative development when the plants were ready to be harvested for the first time. The plants reached this stage with full soil cover at the beginning of July. Cutting creates a ground surface with less than 10% vegetation cover. Therefore the plant re-growth and the maximum Kc values of 0.5/0.6 were regained at beginning of October (2002) or September (2003) before the crop second harvest. The Kc of the first growth cycle are higher than those of the second growth cycle due to the lower temperature that reduced crop growth activity. The higher values were found in the 2003 driest growing season.

Regarding the final yield of indigo it is a result of two major factors: the indigo content of the leaves, and the yield of leaves. The first of these two factors is increased by temperatures and light intensity reaching the highest values in the leaves before the first harvest in July and the lowest ones before the harvest in October (Table 2). Furthermore indigo precursor indican was in general not affected/enhanced by water stress conditions. In fact the long drought spell observed in 2003 summer did not significantly affect indigo content per unit leaf fresh weight, showing values which were not significant different between plants grown without irrigation and those fully irrigated (Table 2). These results with field-grown *Polygonum* crops echo earlier experiments (Campeol *et al*., 2006) in which it was shown that the indigo concentration per unit of leaf weight in *Polygonum* is increased by exposure to higher light intensities prior to harvest.

Table 2. Effect of different irrigation levels on mean (standard deviation) leaf indigo concentration (g kg^{-1} FW) in the different harvests accomplished in 2002 and 2003 growing seasons.

Irrigation Treatments	2002		2003		
	4th July	24 Oct.	7th July	3rd Sept.	24th Oct.
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
T_0	12.16 a (0.20)	7.22 (0.20)	12.7 (0.53)	11.6 (0.38)	5.19 (0.53)
T_{20}	11.74 ab (0.09)	7.39 (0.25)	12.2 (0.56)	12.0 (0.35)	5.16 (0.55)
T_{40}	10.97 bc (0.10)	7.12 (0.10)	13.2 (0.65)	12.2 (0.33)	5.26 (0.89)
T_{60}	9.98 d (0.02)	7.05 (0.09)	13.7 (0.39)	12.3 (0.17)	5.09 (0.49)
T_{80}	11.08 bc (0.18)	7.83 (0.10)	12.8 (0.54)	11.9 (0.55)	4.95 (0.71)
T_{100}	10.80 cd (0.24)	7.27 (0.20)	12.5 (0.39)	12.3 (0.52)	4.97 (0.65)
Mean	11.12	7.31	12.85	12.05	5.01
Significance	*	N.S.	N.S.	N.S.	N.S.

Mean values within each column followed by the same letter are not significantly different for P<0.05 probability level according to LSD test. NS=Not Significant according to F test by ANOVA analysis.

The second factor, i.e. the yield of leaves, depends crucially on number of harvests taken in a season, which in turn depends, among other factors, on the rate of foliage re-growth between harvests. Environmental conditions, mainly temperature and water availability affect strongly yields of foliage and indigo production per hectare. Two and three harvests per year, from July to October were possible in year 2002 and 2003 respectively. Seasonal whole plant yield was higher in 2002, due to higher rainfall as well as higher air temperatures than 2003. Amounts of fresh leaves, representing the actual economic yield, were also greater in 2002 with a potential indigo yield of up to 298.7 kg ha^{-1} (Table 3).

Table 3. Effects of different irrigation levels on mean (standard deviation) fresh whole plant, leaf (t FW ha^{-1}) and indigo (kg ha^{-1} FW) productions on Polygonum tinctorium.

Irrigation Treatments	2002(1)			2003(2)		
	Plant	Leaf	Indigo	Plant	Leaf	Indigo
	t ha^{-1}	t ha^{-1}	kg ha^{-1}	t ha^{-1}	t ha^{-1}	kg ha^{-1}
T_0	48.2 c (3.6)	23.4 c (2.7)	214.6 b (16.6)	26.2 b (5.5)	10.7 c (2.1)	110.2 b (19.3)
T_{20}	71.8 b (6.7)	31.9 b (2.8)	293.2 a (18.4)	32.6 b (4.3)	15.6 bc (2.3)	158.8 b (15.7)
T_{40}	90.1 a (8.6)	36.8 a (3.4)	324.4 a (15.0)	55.6 a (6.2)	24.6 ab (3.3)	268.2 a (16.2)
T_{60}	90.8 a (3.2)	39.7 a (2.2)	327.1 a (14.8)	66.3 a (4.7)	29.3 a (4.1)	318.0 a (15.0)
T_{80}	84.5 ab (6.6)	35.1 ab (2.5)	325.2 a (18.3)	64.0 a (9.6)	27.4 a (4.2)	281.7 a (17.2)
T_{100}	89.8 a (7.3)	36.2 ab (2.7)	313.9 a (17.7)	73.0 a (11.9)	29.1 a (4.7)	307.0 a (18.7)
Mean	79.2	33.8	298.7	52.9	22.8	240.6
LSD 0.05	12.82	4.9	39.9	18.95	9.28	94.3

(1) Harvest dates: 04 July; 03 October 2002; (2) Harvest dates: 07 July; 3 September; 28 October 2003

Mean values within each column followed by the same letter are not significantly different for P<0.05 probability level according to LSD test.

Even if plant dry yield was not statistically different between the two years, averaging 14.25 t ha^{-1}, a lower production of dry leaves was observed in the driest 2003 growing season (Table 4). Furthermore, irrigation significantly influenced seasonal plant dry and fresh yield (t/ha) as well as indigo production (kg/ha) in both years (Table 3 and 4). In particular in the driest 2003 growing season plants grown in T0 showed over 64% and 63% plant and leaf fresh yield reduction in comparison with T100 as a consequence of the very stressful conditions which occurred in July and August 2003. A significant decrement of dry yield was observed with T0 and T20 treatments in comparison with T40, T60, T80 and T100 (Table 4). Irrigation rates higher than 40%ETc did not affect significantly plant and indigo production.

Table 4. Effect of different irrigation levels on mean (standard deviation) dry whole plant and leaf productions (t DW ha^{-1}) on *Polygonum tinctorium*.

Irrigation Treatments	2002[1]		2003[2]	
	Plant	Leaf	Plant	Leaf
	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}
T_0	9.80 c (3.6)	4.66 d (2.7)	7.89 b (5.5)	2.85 b (2.1)
T_{20}	13.22 b (6.7)	5.82 c (2.8)	10.32 b (4.3)	3.72 b (2.3)
T_{40}	16.40 a (8.6)	6.86 a (3.4)	15.58 a (6.2)	6.71 a (3.3)
T_{60}	15.60 ab (3.2)	6.84 ab (2.2)	18.19 a (4.7)	7.23 a (4.1)
T_{80}	14.92 ab (6.6)	5.97 bc (2.5)	15.86 a (9.6)	5.99 a (4.2)
T_{100}	16.51 a (7.3)	6.61 abc (2.7)	16.72 a (11.9)	6.16 a (4.7)
Mean	14.40	6.13	14.09	5.44
LSD 0.05	2.86	0.89	1.78	0.88

[1] Harvest dates: 04 July; 03 October 2002; [2] Harvest dates: 07 July; 3 September; 28 October 2003

Mean values within each column followed by the same letter are not significantly different for P<0.05 probability level according to LSD test.

IV – Conclusion

P.tinctorium has the morphology of a marsh plant characterized by a rather superficial root development, which is responsible for great sensitivity to water stress. Therefore, *P.tinctorium* appears to be more productive in not limiting water conditions, thus making appropriate irrigation plans (*i.e.* 40%ETc corresponding to 1907 m^3 ha^{-1} in the driest season) necessary to achieve sustainable high yields.

References

Angelini, L., Campeol, E., Tozzi S., Gilbert, K.G., Cooke, D.T., John, P., 2003. A new HPLC-ELSD method to quantify indican in *Polygonum tinctorium* L. and to evaluate β-glucosidase hydrolysis of indican for indigo production. *Biotechnol Prog*, 19. pp. 1792-1797.

Angelini, L.G., Tozzi, S., Nassi o Di Nasso, N., 2004. Environmental factors affecting productivity, indican content and indigo yield in *Polygonum tinctorium* Ait., a subtropical crop grown under temperate conditions. *J Agr Food Chem*, 52, 25. pp. 7541-7547.

Bertolacci, M., Megale, P.G., 1991. Automazione degli impianti irrigui con microlisimetri. *Colture Protette*, 6. pp. 25-28.

Campeol E., Angelini L.G., Tozzi S., Bertolacci M., 2006. Seasonal variation of indigo precursors in *Isatis tinctoria* L. and *Polygonum tinctorium* Ait. as affected by water deficit. *Environ Exp Bot*, 58. pp. 223-233.

Doorenbos, W., Pruitt, W.O., 1977. *Guidelines for predicting crop water requirements*. Rome, Italy: Fao. (Irrigation and drainage paper, 24 rev.).

Gomez, K. A., Gomez, A. A., 1984. Two factor experiments. In: *Statistical procedures for agricultural research*. 2nd ed. New York: Wiley J. and Sons. pp. 84-130.

Minami, Y., Takao, H., Kanafuji, T., Miura, K., Kondo, M., Hara-Nishimura, I., Nishimura, M., Matsubara, H., 1997. β-glucosidase in the indigo plants: intracellular localisation and tissue specific expression in leaves. *Plant Cell Physiol*, 38, 9. pp. 1069-1074.

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