Effect of artificially induced stress conditions on the growth of the medicinal plant *Hypoxis hemerocallidea*

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Received 23 September 1994; revised 14 December 1994

H. hemerocallidea corms are a rich source of hypoxoside which is used medically. Certain aspects of cultivation of this plant have been investigated in this study. Herbicide treatments were undertaken as weeds pose a problem in cultivated lands. It was found that 2,4-D amine and glyphosate killed the *H. hemerocallidea* plants. Paraquat at low concentrations allowed good growth and yielded a good hypoxoside content. The control plants, however, grew the best and produced the most hypoxoside. *H. hemerocallidea* apparently prefers poor soils with little nutrients. High levels of nitrogen, phosphorus and potassium are needed initially to produce a good biomass. Once the plants have been established the fertilizer treatment can be discontinued. Best hypoxoside production was achieved when nitrogen levels were low. This finding is in line with the fact that these plants naturally grow well in poor soils. The plants survived and grew best in a clay/shale soil rather than on either a sandy or a grey prismic columnar soil. The hypoxoside content of plants grown on the clay/shale soil was the highest.

Die kormus van *H. hemerocallidea* is 'n ryk bron van hipoksosied wat as medisyne gebruik word. Sekere aspekte van die verbouing van die plant is ondersoek. Van die onkruiddoders wat getoets is, het glifosaat en 2,4-D amien die plante gedood. Parakwat teen lae konsentrasies het goeie groei en hipoksosied-produksie tot gevolg gehad. Die kontroleplante het die heel beste gegroei en die meeste hipoksosied geproduseer. *H. hemerocallidea* vereis skynbaar arm grond met 'n lae voedingstatus. Hoë vlakke van stikstof, fosfor en kalium word aan die begin benodig om die plante 'n aanvaarbare biomassa te laat bereik. Sodra gevestig, moet bemesting gestaak word. Die hoogste hipoksosied-produksie is verkry wanneer stikstofvlakke laag was. Hierdie bevinding is in ooreenstemming met die feit dat die plante natuurlik goed groei in arm grond. Die plante het beter gegroei en oorleef in klei/skalie-grond as in sanderige of grys prismatiese grond. Die hipoksosiedgehalte van plante gekweek op eersgenoemde grondtipe was ook die hoogste.

Keywords: Fertilizer, herbicide, hypoxoside, *H. hemerocallidea*, environment.

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Introduction

Corms of *Hypoxis hemerocallidea* Fisch. & Mey, of the family Hypoxidaceae, are a rich source of hypoxoside (Drewes *et al.* 1984), a phenolic diglucoside with a diarylpentane-type structure (Marini-Bettolo *et al.* 1982). This compound is considered to be one of the active constituents in the corms which are used for medicinal purposes. It is reputed to alleviate symptoms of prostatic hypertrophy and urinary tract infections (Warren 1972). Members of the genus have long been used by South African people as herbal medicines. Currently, plants are harvested from the wild. It is unknown to what extent this plant can be cultivated to maximize hypoxoside production. Aspects that require investigation are: which locations are best suited for cultivation; how growth is affected by herbicides; and how nutrient stress and soil type may affect their survival, growth and secondary metabolite production. These questions were addressed during this investigation using both field and greenhouse trials.

Materials and Methods

Plant material

Cloned *H. hemerocallidea* plants were produced *in vitro* (Page & van Staden 1984; van Staden & Bayley 1988). Following micropropagation the plants were transferred to 7.5-cm pots in a 1:1 soil:compost mix. These cloned plants were used for all experiments. The ages of the plants used for different trials varied as experiments commenced at different times. For statistical analysis the Anova Tukey test was used.

Effect of N, P, and K

Modified Hoagland's solutions (Hoagland & Snyder 1933) (prepared in 15-l black aspirators and of which the pH was adjusted to 6.8) containing 100% and 10% N, P and K, in a 2×2×2 factorial design, were applied as 8 different treatments to cloned *H. hemerocallidea* plants. The plants were transferred to 15-cm pots containing acid-washed sand and grown under greenhouse conditions. Plants were placed in a randomized block design and were destructively harvested after 3, 12 and 21 months, respectively. All corm material was freeze-dried, its dry mass recorded and subsequently ground to homogeneous powders which were analysed for hypoxoside as described below. Five replicates were used for each treatment.

Effect of herbicides

Plants that were 10 months old were used in a greenhouse trial. The herbicides tested were paraquat (®Gramoxone) at concentrations of 1.6 and 0.3 ml l^{-1} (v/v) and 2,4-D amine (2,4-D) at concentrations of 4.9 and 2.7 ml l^{-1} (v/v), respectively. The experimental plants were planted into 15-cm pots one month prior to spraying. The visible effects of the herbicidal treatment and the survival rate of the plants were recorded. The plants were destructively harvested at 3-monthly intervals. After harvest the leaves, corms and roots were freeze-dried, and their dry masses recorded. The hypoxoside content of the corms was determined. Five replicates were used for each treatment.

In the case of the field trial, the experimental plants were 20

months old. Six months before herbicide application the plants were transplanted into the experimental field at Pietermaritzburg. Five replicates were used for each treatment. The herbicides tested were paraquat (®Gramoxone) at concentrations of 1.6 and 0.3 ml l^{-1} (v/v) and glyphosate (®Roundup) at a dosage of 1.5 ml l^{-1}. The experimental plot was sprayed with the respective herbicides every 3 months and plants were harvested every 6 months. Plants were destructively harvested and the same parameters were recorded as for the greenhouse trial.

Effect of planting site

Plants used in this trial were 3 months old. They were planted in randomized blocks at Dargle (29 30 AC), Eston (29 30 DC) and Pietermaritzburg (29 30 CB), respectively, three different areas in respect to climate, soil type and aspect. The plants were planted at different seasons throughout the year and their survival recorded. Twelve plants were used for each treatment.

Hypoxide extraction and quantification

The method used for the extraction and analysis of hypoxoside was modified from those of Page (1984) and Bayley (1989). The corms were freeze-dried using liquid nitrogen, and 0.5 g of the homogeneously ground freeze-dried powders were extracted in 40 ml 80% ethanol in the dark at 23°C for 4 h. The extracts were filtered, washed with 40 ml 80% ethanol and then dried *in vacuo* at 30°C. The extracts were redissolved in 0.5 ml 80% ethanol and loaded onto Merck silica gel fluorescent 60 F_{254} TLC plates. Hypoxoside was used as the standard. The TLC plates were then double-developed for 1 h each in a solvent containing butan-2-ol:benzene:distilled water:methanol (4:3:2:1 v/v, upper phase). The developed plates were air-dried, and the UV absorbent bands co-chromatographing with authentic hypoxoside were removed and eluted with 40 ml 80% ethanol. The extracts were filtered, washed with 40 ml 80% ethanol, reduced to dryness *in vacuo*, redissolved in 0.5 ml 80% ethanol and then passed through a 0.22-µm Millipore solvent filter.

The filtered samples were then analysed for hypoxoside by HPLC using an isocratic programme of acetonitrile:water (20:80). A Spectra Physics instrument fitted with a Hypersil (4.6 × 250 mm) 5µ ODS, C18 reverse-phase column was used. The yield of hypoxoside was calculated from a standard curve prepared by using known amounts of authentic hypoxoside.

Results and Discussion

Effects of N, P and K

The *H. hemerocallidea* plants maintained their geophytic growth

Table 1 The effect of different nitrogen, phosphorus and potassium combinations on the dry weight and hypoxoside content of *H. hemerocallidea*

N, P, K combinations[a]			Time after planting (months)	Mean corm dry mass (mg)[b]	Mean hypoxoside level (µg g^{-1})[b]
N	P	K		57 plants harvested at planting	23 plants harvested at planting
high	high	high	3	81	1296
			12	227	635
			21	320 c	674 a
high	high	low	3	89	334
			12	203	652
			21	255 bc	1083 a
high	low	high	3	87	1030
			12	163	964
			21	255 ab	1082 ab
high	low	low	3	54	286
			12	145	914
			21	78 a	155 a
low	high	high	3	87	325
			12	302	1970
			21	206 c	1668 ab
low	low	high	3	108	1027
			12	229	2300
			21	254 bc	5202 bc
low	high	low	3	82	353
			12	212	2850
			21	283 c	3272 abc
low	low	low	3	82	513
			12	187	1388
			21	224 bc	2971 c

[a] high: Modified Hoagland's solution containing 100% of the element specified.
low: Modified Hoagland's solution containing 10% of the element specified.

[b] Treatments with the same letters do not differ significantly at $P < 0.05$. The letters are for fertilizer treatments over the 21-month period, not for age.

pattern under greenhouse conditions. Leaf senescence occurred in autumn with new growth taking place in spring. At the commencement of the experiment, the corms were small and they contained only 23 $\mu g\ g^{-1}$ hypoxoside (Table 1). As was to be expected, the corms increased in weight with time. This increase was most consistent and pronounced when the plants received only 10% of the normal N present in Hoagland's medium. When N was applied at full strength (high), then low levels of P and K were apparently limiting and corm growth was severely reduced. This was also the case for hypoxoside production. When N was fed at the lower level, the effect of low P and K was not pronounced. A low level of N did not greatly reduce corm biomass accumulation. It did, however, significantly increase hypoxoside production. The production of hypoxoside was highest with both low N and P or low N and K. It is well known that secondary plant products increase when plants are grown under stress conditions. The soils in which these plants grow naturally in the environs of Pietermaritzburg are very low in nitrogen. The application of N, P and K at high levels resulted in the best biomass production with time. A large initial biomass increase may be important in cultivation as it may provide the corm volume in which to subsequently store secondary products (Bayley 1989).

Table 2 The effect of paraquat and 2,4-D on the growth and survival of *H. hemerocallidea* plants maintained under greenhouse conditions

Time after herbicide application (days)	Concentration of paraquat ($ml\ l^{-1}$)		Concentration of 2,4-D ($ml\ l^{-1}$)	
	0.3	0.8	2.7	4.9
1	100% necrosis	100% necrosis	No visible effect	No visible effect
4	50% resprouting	50% resprouting	12.5% chlorosis of the midrib of the leaves	65% chlorosis of the midrib of the leaves
10	80% resprouting	80% resprouting	Death of all plant components	Death of all plant components
14	100% resprouting	100% resprouting	Dead	Dead
90	100% survival	100% survival	Dead	Dead

Table 3 The effect of the paraquat on the growth and hypoxoside levels of *H. hemerocallidea* plants under greenhouse conditions

Time after herbicide application (months)	Herbicide treatment	Fresh mass (g)[a]			Dry mass (g)[a]			Hypoxoside level ($\mu g\ g^{-1}$)[a]
		Leaf	Corm	Root	Leaf	Corm	Root	
0	—	0.7	1.1	1.8	0.11	0.29	0.13	383
3	Control	3.9 a	2.6 a	12.6 a	0.81 a	0.82 a	1.45 a	49 a
	0.3 $ml\ l^{-1}$	4.0 a	2.0 a	3.6 b	0.49 ab	0.32 b	0.21 b	197 b
	1.6 $ml\ l^{-1}$	2.0 a	1.8 a	3.0 b	0.27 b	0.31 b	0.20 b	79 ab
6	Control	2.8 a	3.8 a	15.9 a	0.67 a	1.07 a	1.45 a	1078 a
	0.3 $ml\ l^{-1}$	7.2 a	6.7 a	9.0 a	1.05 a	1.65 a	0.61 a	931 ab
	1.6 $ml\ l^{-1}$	2.1 a	3.6 a	7.3 a	0.35 a	0.66 a	0.42 a	275 b

[a] Treatments with different letters are significant at $P < 0.05$.

Table 4 The effect of paraquat and glyphosate on growth and hypoxoside levels of *H. hemerocallidea* plants under field conditions

Time after planting	Herbicide treatment	Mean dry mass (g)[a]			Mean fresh mass (g)[a]			Hypoxoside level ($mg\ g^{-1}$)[a]
		Leaf	Corm	Root	Leaf	Corm	Root	
0	Control	9.6	3.4	8.2	1.75	0.79	0.78	91
12	Control	5.9 a	12.1 a	27.0 a	3.50 a	3.29 a	2.94 a	1368 a
	Low paraquat	1.1 b	5.1 b	6.9 b	0.46 b	1.24 b	0.73 b	433 b
	High paraquat	0.4 b	3.6 b	4.7 b	0.07 b	0.63 b	0.45 b	213 b
	Glyphosate	0.3 b	3.1 b	3.4 b	0.12 b	0.65 b	0.39 b	203 b
18	Control	174.4 ae	34.4 a	27.7 a	28.16 a	9.97 a	3.45 a	1365 a
	Low paraquat	43.5 ab	16.9 a	19.2 a	7.06 ab	2.69 a	2.14 a	1205 ab
	High paraquat	1.2 ab	5.9 a	4.8 a	0.24 b	0.71 a	0.56 a	660 be
	Glyphosate	1.2 a	5.3 a	1.9 a	0.35 ab	0.98 a	0.45 a	268 c

[a] Treatments with different letters are significant at $P < 0.05$.

Production of hypoxoside may be optimized using a strategy of initial fertilization only.

Effect of herbicides

The morphological appearance of the plants kept in a greenhouse following herbicide application is summarized in Table 2. The application of paraquat brought about rapid chlorosis of the plants. They were, however, not killed and all plants resprouted within 14 days. The effect of 2,4-D manifested itself slower but was more lasting. All these plants eventually died and rotted.

At low concentrations of paraquat the production of hypoxoside was least affected (Table 3). Initially a decrease in hypoxoside content between 0 to 3 months of growth was observed. By 6 months the corms had recovered considerably both with respect to growth and hypoxoside production. Energy was probably used in the re-establishment of the leaves, rather than the production of hypoxoside. After 6 months the plants treated with low levels of paraquat had largely recovered and the levels of hypoxoside started to increase. A higher level of paraquat (1.6 ml l^{-1}) had a detrimental effect on growth and hypoxoside production that was still obvious 6 months after treatment.

Spraying plants in the field with glyphosate had little effect for the first 7 days. By 14 days the leaves, however, showed signs of necrosis. With paraquat those *Hypoxis* plants sprayed with a high level showed 80% necrosis within the first day and 100% necrosis by day 4. Resprouting had occurred by day 21. Those sprayed with low concentrations of paraquat were necrotic within 2 days after spraying. Reshooting, however, occurred by day 7. The low paraquat showed the greatest response. Plants sprayed with glyphosate had the lowest survival. The control plants which were not sprayed and had to compete with the weeds grew the best (Table 4).

The hypoxoside content was the highest in the control plants. Glyphosate-treated plants produced the least hypoxoside. High levels of paraquat adversely affected hypoxoside levels. With low paraquat, hypoxoside production was better but did not match that of the untreated controls (Table 4).

From these herbicide trials it is clear that the herbicides tested not only reduced growth of *H. hemerocallidea* but also reduced hypoxoside production. The untreated plants grown with weeds were not detrimentally affected.

Effect of planting site on survival and growth

Plant survival at each harvest was recorded at the three planting sites. Plants at the Dargle site did not survive beyond the second harvest (1992-10-01) (Table 5). This was largely due to the heavy grey prismic/columnar type soil which expands and contracts during summer and winter. This resulted in the crushing of the contractile roots in dry conditions and flooding during wet periods. The fleshy contractile roots are situated at the lower half of the corm and are easily damaged. Plants at Eston survived until the sixth harvest (1993-10-01). Initially the survival was good but then decreased until no plants were left by 1994-04-01. The sandy soil found here does not retain water well and the plants had to survive long periods of drought. At the Pietermaritzburg site the survival rate was high (Table 5). The Pietermaritzburg site had a clay/shale soil which is the type of soil in which *H. hemerocallidea* grows naturally. Environmental condi-

Table 5 Survival (%) of *H. hemerocallidea* plants grown at three different environmental sites

Planting date	Season	Harvest date	Plant age (months)	Survival		
				PMB [a]	Eston	Dargle
91-05-01	Autumn	91-08-01	3	100	100	91.7
91-07-01	Winter	91-04-01	3	83.3	91.7	41.7
91-05-01	Autumn		6	83.3	91.7	58.3
92-01-02	Summer	92-04-01	3	83.3	91.7	–
91-10-01	Spring		6	83.3	83.3	–
91-07-01	Winter		9	100	83.3	–
91-05-01	Autumn		12	66.6	91.7	–
92-01-02	Summer	92-10-01	9	83.3	66.6	–
91-10-01	Spring		12	100	58.3	–
91-07-01	Winter		15	83.3	33.3	–
91-05-01	Autumn		18	66.6	25.0	–
92-01-02	Summer	93-04-01	15		16.6	–
91-10-01	Spring		18	83.3	41.6	–
91-07-01	Winter		21	66.6	16.6	–
91-05-01	Autumn		24	66.6	25.0	–
92-01-02	Summer	93-10-01	21	66.6	16.6	–
91-10-01	Spring		24	83.3	33.3	–
91-07-01	Winter		27	83.3	33.3	–
91-05-01	Autumn		30	83.3	25.0	–
92-01-02	Summer	94-04-01	27	83.3	–	–
91-10-01	Spring		30	83.3	–	–
91-07-01	Winter		33	83.3	–	–
91-05-01	Autumn		36	16.6	–	–

[a] PMB = Pietermaritzburg

tions at the different planting sites clearly played a role in the survival of the plants. When considering cultivation and commercial exploitation this aspect will require careful consideration.

Acknowledgements

The University of Natal Research Fund is thanked for financial support.

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