GERMINATION STUDIES ON ARID ZONE PLANTS

V. The Nature and Role of Germination Inhibitors present in Leaves of *Prosopis juliflora*

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In the arid and semi-arid areas of Rajasthan *Prosopis juliflora* is found in diverse growth forms from dense thickets of small shrubs to large trees. It was observed that very few plants come up within the community of these trees and the ground was covered with a thick layer of leaf litter. Investigations reported here indicate that the inhibitors present in the leaves of *P. juliflora* affect both the processes of germination and growth of seedlings. The magnitude of germination inhibition, however, varied in different species. The inhibitors restrict both shoot and root growth but the latter seems to be more affected. The leaf litter of *P. juliflora* community contained inhibitors but the results suggest that inhibitors present in leaves do not accumulate in the soil to inhibitory concentrations. It seems that inhibitors contained in the litter together with the physical effects of litter may regulate the natural regeneration within the community. Paper chromatography followed by bio-assay of the acid ether fraction of aqueous leaf extract indicated the presence of two inhibitors around 0·6 and 0·75 respectively. The substance around 0·6 Rf caused large inhibition but the activity of the other compound was relatively less.

Introduction

Widespread occurrence of germination-inhibiting substances in the plants of arid and semi-arid areas has an important bearing on the natural regeneration of vegetation in these areas. These substances have been found in the seeds and propagative organs (Sroelov 1940; Went 1948, 1949; Koller and Negbi 1955, 1959; Larner *et al.* 1959; Oppenheimer 1960; Lahiri and Kharabanda 1963), as well as in other plant parts, not strictly associated with seeds but which come in contact with them in soil after the seeds are shed (Evenari 1949, Yardeni and Evenari 1952, Zemit 1954 as quoted by Toole *et al.* 1956). In this connection it has been reported that phytotoxic substances in the leaf litter of certain plants do not permit the regeneration of other species in that area. Went (1952) observed that litter, shed by *Salvia mellifera,* inhibits the germination of various other species. Similarly, Donner (as quoted by Oppenheimer 1960) found that fresh oak leaves or those of the

previous season not only obstruct the development of the seedlings mechanically but contain germination-inhibiting substances. Investigations of Larner and Evenari (1961) suggested that the inhibitors contained in the leaves along with the physical effects of the leaf litter may control the vegetation under *Eucalyptus rostrata*.

In the arid and semi-arid areas of Rajasthan *Prosopis juliflora* is found in diverse growth forms from dense thickets of small shrubs to large trees. Several communities of this species were examined around Jodhpur. It was striking to observe that excepting for a few plants of *Tephrosia purpurea*, *Dactyloctenium sindicum* and *Peristrophe bicalyculata*, giving a percentage ground cover of only 0·18 to 0·25, the ground was almost devoid of vegetation and it was covered by a thick layer of leaf litter, varying between 500 g and 1000 g per sq. metre in different sites. In view of the foregoing observations it was speculated that germination inhibitors may be present in the leaves. Our preliminary studies (Lahiri 1965) indicated that water extracts of leaves have germination-inhibiting properties. About the same time Sankhla *et al.* (1965) also observed that aqueous extracts of leaves inhibit germination of wide varieties of seeds. Therefore a study was undertaken to elucidate the nature and mode of action of these inhibitors with special reference to their impact on the vegetation under the strands of *P. juliflora*.

Material and Methods

Fresh leaves, leaf litter and soil were collected from two *P. juliflora* communities—one near the filter station and the other near the Umed Saga Tank, both located within a radius of 10 miles from Jodhpur. Seeds which have been used for germination and growth tests have been mentioned in the appropriate place where results are reported.

Germination of seeds was tested on moist filter paper (with 5 ml extract or water) or on soil contained in plastic boxes. Seedlings were raised on filter paper collar, dipping in 20 ml water or in extract, kept in boiling tubes. Volume was kept constant in all cases by adding requisite amount of water (on weight basis) at 24 hours' intervals. Conditions given for germination and growth tests were 32 ± 1 °C and continuous darkness. Watering and other manipulations were done under dim yellow-green safe lamp. Germination tests on 12 species were conducted with three replications of 100 seeds under each treatment. Germination percentages were converted to corresponding angles $\text{Arcsin}\sqrt{\text{percentage}}$ and significance of the results were assessed by analysis of variance. Growth observations are based on 10 plants and the significance of the differences were adjudged by *t*-test wherever necessary. In all cases experiments have been repeated at least three times.

For the extraction of the inhibitors weighed quantities of leaves (fresh, dry or leaf litter) were blended with water and the brei was left for 20 hours

at 2±1 °C. Thereafter the brei was filtered under suction or centrifuged when necessary to get the clear extract. The resultant extract was then diluted to make it 10 per cent. This concentration was slightly higher than the lowest concentration (6·6 per cent) in which complete germination inhibition by *P. juliflora* leaf extract has been reported (Sankhla *et al.*) to occur in responsive species.

The soil extract (1 : 5) was prepared by shaking the requisite quantity of soil in water for three hours.

The acid ether fraction of the aqueous extract was prepared by following the technique reported earlier (Lahiri and Audus 1960). The ether extract was reduced under low pressure and aliquots were applied on strips of Whatman No. 1 paper for descending chromatography in iso-propyl alcohol: ammonia : water (8 : 1 : 1) solvent system. Developed chromatograms were cut into 20 equal segments. These segments were shredded into small pieces and eluted in 4 ml of water for 20 hours at 2±1 °C in Petri dishes (5 cm dia.). Seeds of *Panicum antidotale* (100 seeds) were germinated in these eluates on filter paper and 10 Petri dishes with distilled water served as control. Final germination in the eluates expressed as percentage of the mean of the controls has been indicated in the histogram. Fiducial limits were calculated from the germination percentages of controls and areas below the fiducial limits indicate the positions of inhibitors.

Results

In the preliminary experiment, germination of the seeds of certain annual and perennial plants was tested in 10 per cent water extract of fresh leaves. Seeds germinating in distilled water served as controls. The results of analysis of variance (Table I (Appendix)) of the data indicate that the treatment has a high order of significance and the differences between the species are also highly significant. Significant interaction signifies that the magnitude of germination inhibition in the leaf extract was different in different species. These facts are very apparent from Table I where the mean angular values for germination percentages have been presented for the two treatments. It may also be noted that the extent of germination inhibition was relatively more in the seeds of perennial species than in the annuals although the differences between the control and treated remained highly significant in most cases. However, in the case of *C. psoraloides*, the difference between the control and treated is significant only at 5 per cent level (C.D. at 5 per cent= 6·30).

In order to determine the effect on the growth of seedlings, germinated seeds of *P. typhoides* were raised in the extract of fresh leaves of *P. juliflora* and seedlings raised in water served as control. The results presented under Table II indicate that the leaf extract significantly inhibited the growth of

both coleoptile and root. However, the root growth inhibition was relatively more.

It was further observed (Table III) that the inhibitors contained in the leaves remain active when the leaves have dried up. As in the case of fresh leaves, the extract of dry leaves caused relatively more inhibition of the root

TABLE I

Mean angular values of germination percentages of the seeds of different species in 10 per cent aqueous leaf extract of P. juliflora *and in distilled water*

	Species	Percentage germination transformed into Arcsin$\sqrt{\text{percentage}}$			S.Em	C.D. at 0·1 per cent level
		In water	In extract	Mean		
1.	*Prosopis juliflora*	56·83 (70·1)	21·90 (13·9)	39·25 (40·2)	±1·60	7·874
2.	*Prosopis spicigera*	60·01 (75·01)	21·58 (13·5)	40·79 (42·7)		
3.	*Albizzia lebbek*	66·42 (84·0)	33·20 (30·00)	49·81 (58·3)		
4.	*Acacia senegal*	66·42 (80·0)	17·18 (8·7)	41·80 (44·4)		
5.	*Panicum antidotale*	64·93 (82·0)	30·28 (25·4)	47·60 (54·5)		
6.	*Lasiurus sindicus*	55·00 (67·1)	19·27 (10·9)	37·13 (36·4)		
7.	*Cenchrus ciliaris*	57·0 (70·3)	38·60 (38·9)	47·80 (54·9)		
8.	*Phaseolus aconitifolius* ..	84·24 (99·0)	64·89 (81·9)	74·56 (93·9)		
9.	*Phaseolus radiatus*	70·65 (89·0)	58·10 (72·1)	64·37 (81·3)		
10.	*Cyamopsis psoraloides* ..	60·06 (75·1)	53·73 (65·0)	56·89 (70·1)		
11.	*Halianthus anus*	90·00 (100·00)	66·53 (84·1)	78·26 (95·8)		
12.	*Pennisetum typhoides* ..	73·26 (91·7)	52·87 (63·5)	63·06 (79·5)		
	Mean	87·07 (84·8)	39·84 (41·0)	–	For cell values: S.Em ±12·25 C.D. at 0·1 per cent level 11·215	
	S.Em	±0·65				
	C.D. at 0·1 per cent level ..	3·199				

* Values within brackets indicate corresponding percentage values.

TABLE II

Growth of seedlings of P. typhoides *in the extract (10 per cent) of fresh leaves of* P. juliflora *after 120 hours at 32 ± 1 °C and in darkness. Results are based on 10 observations. ± indicates S.E.*

Observations	In water (A)	In extract (B)	Differences of means	*t*
1. Length of coleoptile in cm ..	9·4 ± 0·19	2·2 ± 0·74	A–B = 7·2	9·47*
2. Length of root in cm	12·6 ± 0·8	1·4 ± 0·42	A–B = 11·2	12·4 *

* Significant at 1 per cent.

growth in comparison to that of the coleoptiles. These experiments were repeated several times and in all cases similar results were obtained. This confirmed that the substances contained in the leaves inhibit both germination and growth of seedlings.

TABLE III

Growth of seedlings of P. typhoides *in the extract of dry leaves of* P. juliflora. *Other particulars are same as in Table II*

Observations	In water (A)	In extract of dry leaves (B)	Differences of means	*t*
1. Length of coleoptile in cm ..	4·40 ± 0·37	2·0 ± 0·57	A–B = 2·4	4·0*
2. Length of root in cm	8·60 ± 1·22	3·7 ± 0·63	A–B = 4·9	3·7*

* Significant at 1 per cent.

However, it was necessary to ascertain the impact of the inhibitors at the germinative phase and growth phase separately since an indication was obtained that the magnitude of germination inhibition is relatively less in the annuals. For this purpose an experiment was conducted with the following four different conditions:

1. W/W—germinating as well as growing medium was water.
2. E/W —germinating medium was extract and the growing medium was water.
3. W/E —germinating medium was water and growing medium was extract.
4. E/E —germinating as well as growing medium was extract.

Seeds of annual species like *P. aconitifolius*, *P. radiatus* and *P. typhoides* were used in this experiment along with one perennial species, *P. antidotale*

TABLE IV

Growth of shoot and root of germinated seeds of four species under various germinating and growing conditions. Results are based on 10 observations. ± indicates S.E. For further explanation see the text

Species	Time allowed for germination in hr.	Time allowed for seedling growth in hr.	W/W (control)		E/W		W/E		E/E	
			Shoot length cm	Root length cm	Shoot length cm	Root length cm	Shoot length cm	Root length cm	Shoot length cm	Root length cm
1. *Phaseolus aconitifolius*	48	72	2·6±0·29	1·82±0·72	3·20±0·27	2·67±0·78	0·0	0·0	0·0	0·0
2. *Phaseolus radiatus*	48	72	2·45±0·31	1·11±0·54	3·31±0·27	2·24±0·35	0·0	0·0	0·32±0·25 (20)*	0·20±0·09 (70)
3. *Pennisetum typhoides*	24	24	1·09±0·27	2·03±0·19	1·00±0·08	2·61±0·42	0·0	0·0	0·0	0·0
4. *Panicum antidotale*	48	72	2·67±0·15	1·15±0·21	2·93±0·21	1·50±0·1	1·01±0·20	0·0	1·15±(100)	0·13±(60)

* Percentage of seeds where extension of shoot and root was noted.

for comparison. In all cases germinated seeds from the germinating medium (water or extract) were transferred to water or extract for the study of the growth of seedlings. The results have been presented under Table IV. The time allowed for germination was kept as 48 hours in most cases, so that the imbibition may be complete and large number of germinated seeds may be available for the transfer to the growing medium.

It may be observed that in most cases the growth of shoot and root was relatively more under the E/W treatment in comparison with the control (W/W). It is possible that the inhibitors within the embryo cells were diluted to a promontory range by subsequent uptake of water at the growing phase. However, the differences with the control have not been found to be significant. When the germinated seeds (in water) were transferred to extract, there was no growth of shoot and root except in *P. antidotale* where coleoptile growth was noted. But in this case growth inhibition was very marked. Similarly, seeds which were soaked and germinated in extract (W/W) showed pronounced growth inhibition of seedlings, in cases where certain seeds (i.e. in *P. radiatus* and *P. antidotale*) showed extension of shoot and root. However, in *P. aconitifolius* and *P. typhoides*, none of the germinated seeds showed any growth of plumule and radical. These observations suggested that these inhibitors separately affect the germination and growth processes. Moreover, it seems that these growth inhibitors have the capacity to suppress the growth of seedlings of species which showed relatively less germination inhibition in the leaf extract.

In the next stage of the work, attempts were made to determine the impact of these inhibitors on the regeneration of plants within the community. For this purpose it was necessary to determine whether the inhibitors are present in the soil and leaf litter beneath the trees. Therefore, samples of leaf litter, combined sample of surface soil and leaf litter and soil from about 2-3 cm below the surface were collected from the two communities mentioned earlier under 'Material and Methods'. Seedlings of *P. typhoides* were grown for 120 hours in the extract of these samples with seedlings growing in water as control. The mean growth of coleoptile and root in the different extracts has been presented under Table V.

The results indicate that the inhibitors do not accumulate in the soil. In other experiments soils from deeper layers (10 cm and 30 cm) were also tested and their extracts did not cause growth inhibition of seedlings. The surface soil always contain some litter in decomposed or partially decomposed state and therefore in this case the extract of a combined sample of surface soil and leaf litter has been tested. Only one such sample caused root growth inhibition which could be due to the presence of inhibitors in the litter of this sample. The extract of the leaf litter of both the sites, however, caused significant root growth inhibition and the extract from one site also showed inhibition of

TABLE V

Mean growth of coleoptile and root of P. typhoides *after 120 hrs. in extracts of leaf litter (10 per cent), leaf litter and surface soil (10 per cent) and soil (1 : 5). Results are based on 10 observations. ± indicates S.E.*

Site of the community	In water (control)		Soil		Surface soil and leaf litter		Leaf litter		Differences of means	*t*
	Length of coleoptile cm A	Length of root cm B	Length of coleoptile cm C	Length of root cm D	Length of coleoptile cm E	Length of root cm F	Length of coleoptile cm G	Length of root cm H		
1. Near the filter station	4·43 ±0·37	8·60 ±1·22	6·11 ±0·92	5·68 ±1·37	5·65 ±1·04	3·63 ±0·73	2·01 ±0·57	3·67 ±0·63	B–D = 2·92 B–F = 4·97 A–G = 2·42 B–H = 4·93	1·59 N.S. 3·52* 3·56* 3·60*
2. Near the Umed Sagar Tank	4·43 ±0·37	8·60 ±1·22	5·20 ±0·99	9·13 ±1·59	5·35 ±0·84	5·76 ±0·83	3·53 ±0·66	3·96 ±0·92	B–F = 2·84 A–G = 0·9 B–H = 4·64	1·93 N.S. 1·18 N.S. 3·05*

* Significant at 1 per cent level ; N. S. not significant.

coleoptile growth. Almost similar results were obtained when this experiment was repeated.

In order to study the emergence behaviour of seeds under the conditions of *P. juliflora* community, soil and leaf litter from the community near the filter station were arranged in polythene containers as found in nature. In one case seeds of *P. typhoides* were kept just below the soil surface with the litter on top (*c.* 2 cm thick) and in the other case seeds were kept on the top of the litter. Along with these, seeds were sown also in the soil collected from the community in separate containers. Soil collected from the farm of this Institute served as control. Water was added to all to assure the supply of moisture for germination. Not a single plant showed emergence when the seeds were placed on the leaf litter, although about 50 per cent emergence was noted when the seeds were placed just below the soil surface. However, in soil where no litter was present, about 88 per cent emergence was noted which was comparable with the control (90 per cent). These observations further confirmed that the inhibitors contained in the leaves do not accumulate in the soil. But it seems that when the seeds are on the leaf litter, sprouting and subsequent growth of seedlings may not be possible.

In order to determine the nature of these inhibitions, attempts were made to separate them by paper partition technique. Preliminary trials indicated that germination tests with the seeds of *P. antidotale* may be used for the bio-assay of the inhibitors from paper chromatograms. The results of the bio-assay of the acid-ether fraction of the leaf extract (from 30 g tissue) have been illustrated in Fig. 1. It may be observed that a large zone of inhibition occurs between 0·5 and 0·7 Rf. There is also an indication of the presence of a weak inhibitor at 0·75 Rf. Small promotions of germination was noted around 0·05, 0·35 and 0·85 Rf.

Discussion

The widespread occurrence of germination inhibitors in different plant parts is now a well-known fact and Evenari (1949) has listed about 100 species of plants in which these inhibitors have been detected. The inhibitors contained in the *P. juliflora* leaves have been found to inhibit germination but the magnitude of inhibition significantly varies in different species. It further seems that the germination inhibition is relatively more in the annuals than in the perennials although more detailed studies are needed to confirm this fact.

It is known (Toole *et al.* 1956) that some germination inhibitors are rather specific for germination process in contrast to the antecedent respiration or many of the subsequent changes in seedlings. Moreover, these specific inhibitors influence the cell elongation of roots. Mayer and Evenari (1952) demonstrated 26 derivatives of coumarine, fall closely in the same order as

inhibitors of radical emergence of lettuce and as inhibitors of wheat root elongation. Inhibitors of *P. juliflora* leaves have been found to affect separately the germination process and the growth of shoot and root. Even the species which showed relatively less germination inhibition in the leaf extract, displayed marked growth inhibition of their germinated seeds. It was further observed with the seedlings of *P. typhoides* that the root growth inhibition was more pronounced than the inhibition of coleoptile growth. These evidences suggest that these substances are possibly specific inhibitors. Again it was noted that seeds of four species which were pretreated in extract show consistent growth promotion of seedlings (particularly of roots) in water. However, significant differences with the control (in water) could not be established perhaps due to the short duration of growth.

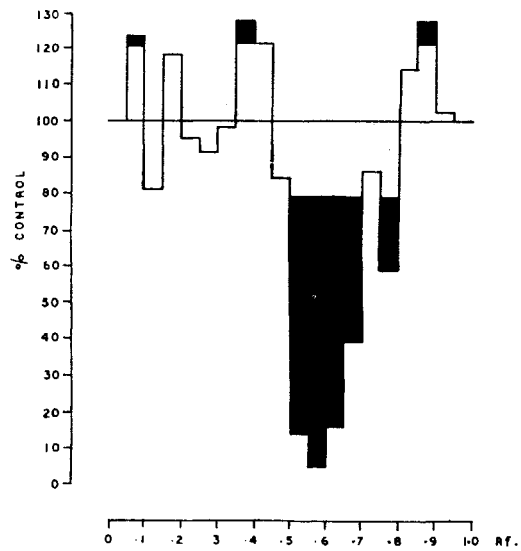

FIG. 1. Histogram illustrating the bio-assay results of the chromatogram of acid-ether fraction from the aqueous extract of 30 g leaf tissue of *P. juliflora*.

The impact of these inhibitors on the regeneration of vegetation beneath the *P. juliflora* trees is of considerable significance since these are established with ease in arid and semi-arid areas. Results of this investigation indicate that the large quantities of the leaf litter which accumulate beneath the trees contain these inhibitors, presumably the substances being of stable nature. They are water soluble but they do not seem to accumulate in the soil in inhibitory concentrations. Larner and Evenari (1961) also observed that the inhibitors in the litter of *Eucalyptus rostrata* do not accumulate in soil beneath the trees. Evidences furnished here suggest that in *P. juliflora* community regeneration of plants may not be successful if the seeds fall on the thick layer

of leaf litter due to direct action of these inhibitors on the germination and growth processes. Seeds present just below the soil surface may show some germination but this will be again restricted due to the action of the inhibitors on growth as well as the mechanical hindrances of the litter. Effects of shade, competition for moisture and the influence of certain other factors which may have indirect effect on the establishment of plants have not been investigated.

Chromatographic investigations indicated the presence of two germination inhibitors at close Rf values (0·6 and 0·75), one appearing to be biologically more active than the other. Coumain has a mobility around 0·6 Rf in this solvent system and one of these inhibitors may be a coumarine. It is further known that inhibitor-β complex, which also moves to about the same Rf, constitutes a number of substances, one of which is the coumarine, scopoletin (Housley and Taylor 1958). It may be possible that large zone of inhibition around 0·6 Rf may be due to the presence of more than one substance with close or similar Rf values. However, detailed investigations may be necessary to characterize the chemical nature of these compounds.

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References

Donner, A. (1959). Changes in composition of natural pasture vegetation under the influence of shading Velonea oak trees. M.Sc. thesis, Agric. Faculty, Hebrew University, Rehovot, Israel. Quoted by Oppenheimer, H. R. (1960), in Adaptation to Drought: Xerophytism. Plant-water relationships in arid and semi-arid conditions. UNESCO (Paris), p. 105.

Evenari, M. (1949). Germination inhibitors. *Bot. Rev.*, **15**, 153.

Housley, S., and Taylor, W. C. (1958). Studies on plant-growth substances VI. The nature of inhibitor-β in potato. *J. exp. Bot.*, **9**, 458.

Koller, D., and Negbi, M. (1955). Germination regulating mechanisms, V: *Colutea istria*. *Bull. Res. Coun. Israel*, **5**, 73.

———— (1959). The regulation of germination in *Oryzopsis miliacea*. *Ecology*, **40**, 20.

Lahiri, A. N. (1965). Annual report of the Central Arid Zone Research Institute of 1965-66.

Lahiri, A. N., and Audus, L. J. (1960). Growth substances in the roots of *Vicia faba*. *J. exp. Bot.*, **11**, 341.

Lahiri, A. N., and Kharabanda, B. C. (1963). Germination studies on arid zone plants II. Germination inhibitors in the spikelet glumes of *Lasiurus sindicus*, *Cenchrus ciliaris* and *Cenchrus setigerus*. *Ann. of Arid Zone*, **1**, 114.

Larner, H. R., Mayer, A. M., and Evenari, M. (1959). The nature of germination inhibitors present in the dispersal units of *Zygophyllum dumosum* and *Trigonella arabica*. *Physiologia Pl.*, **12**, 245.

Larner, H. R., and Evenari, M. (1961). The nature of germination inhibitors present in leaves of *Eucalyptus rostrata*. *Physiologia Pl.*, **14**, 221.

Mayer, A. M., and Evenari, M. (1952). The relation between the structure of coumarin and its derivatives, and their activity as germination inhibitors. *J. exp. Bot.*, **3**, 246.

Oppenheimer, H. R. (1960). Adaptation to Drought: Xerophytism. Arid Zone Research XV. Plant-water relationships in arid and semi-arid conditions. Review of research. UNESCO (Paris), p. 105.

Sankhla, N., Baxi, D., and Chatterjee, U. N. (1965). Eco-physiological studies on arid zone plants I. Phytotoxic effects of aqueous extract of Mesquite, *Prosopis juliflora* Dc. *Curr. Sci.*, **34**, 612.

Sroelov, R. (1940). Germination inhibitors of *Sinapis alba* and other seeds when enclosed in their fruit. *Palest. J. Bot.*, Jerusalem, **2**, 33.

Toole, E. H., Hendricks, S. B., Borthwick, H. A., and Toole, V. K. (1956). Physiology of seed germination. *Ann. Rev. Plant Physiol.*, **7**, 299.

Went, F. W. (1948). Ecology of desert plants I. Observations on germination in the Yoshna tree National Monument, California. *Ecology*, **29**, 242.

——— (1949). Ecology of desert plants II. The effect of rain and temperature on germination and growth. *Ecology*, **30**, 1.

——— (1952). The effects of rain and temperature on plant distribution in the desert. *Proc. International Symp. on Desert Research*, Jerusalem, p. 232.

TABLE I (APPENDIX)

Analysis of variance of the data of germination percentages (transformed into Arcsin$\sqrt{\text{percentage}}$) of the seeds of different species in water and in the leaf extract of P. juliflora

Sources of variation	D.f.	M.S.S.	F.
1. Species	11	1157·0295	75·23*
2. Treatment	1	13362·8552	654·24*
3. Interaction	11	229·1460	14·90*
4. Error	48	15·309	–

* Significant at 0·1 per cent.