Eco-Physiological Studies on *Zornia diphylla* Pers. with Reference to Adaptive Seed Dormancy

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Summary. Seeds of *Zornia diphylla* possess a complex type of dormancy which is of great survival value for the species. Seeds show polymorphism. The optimum temperature for germination explains the cause of its distribution in the tropics. The grazing and scraping promotes the vegetative growth.

Zornia diphylla Pers. is a common leguminous species found in the grassland and forest habitats of Gorakhpur. Its distribution is everywhere in the tropics. It is distributed in plains from Himalaya to Ceylon up to 4,000 feet[2]. Role of legumes in nitrogen economy of soil is well known, but *Zornia diphylla*, because of its use in inducing sleep in children, has proved to be of more significance[3].

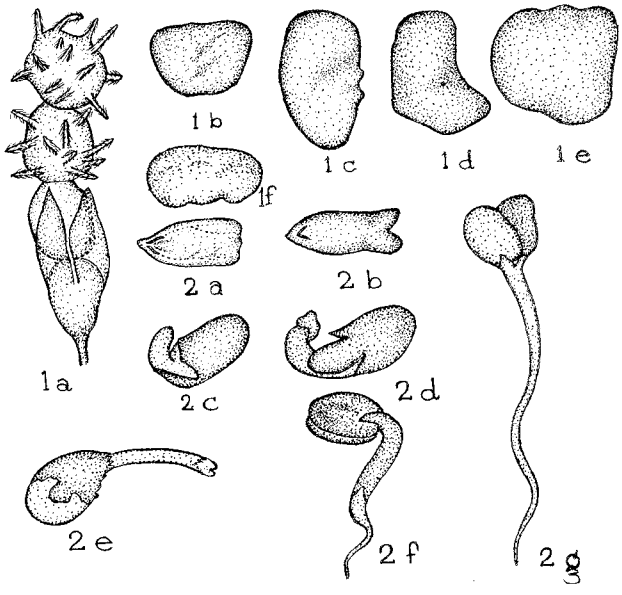

Fig. 1. a) Lomentum fruit with glochidiate prickles; b) subreniform seed; c) oblong seed; d) sickle-shaped seeds; e) sub-quadrangular send and f) reniform seed.
Fig. 2. a) Rudiment embryo; b) mature embryo; c–g) stages of epigeal germination.

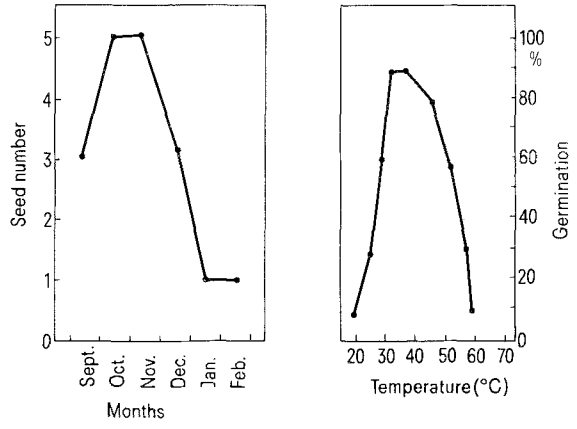

Fig. 3. Average seed output per fruit in different months.
Fig. 4. Percentage germination at different temperatures.

It is a rainy season, prostrate, suberect or procumbent annual herb. The seeds start germination in the first week of July and continue until mid of August. The plant shows intense flowering and fruiting from the middle of September to November, although surviving plants continue flowering even up to the first week of January.

Fruit is compressed lomentum having several glochidiate prickles which point backward and forward (Figure 1). Each fruit has 1–5 seeds, and it varies in length from 6 to 14 mm and breadth from 2 to 3 mm. The entire fruit in the young stage is protected by boat-shaped concealing bracts but on maturation gradually emerges out. The maturation of seeds is always in basipetal order. The average seed output per fruit is maximum from mid October to mid November, and later on it decreases[4] (Figure 3). The dispersal of mericarps occurs by the grazing animals. The glochidial prickles help in the attachment to the fur coat of cattle. The seeds are light to dark brown with dark spots.

Germination. To study germination behaviour, fresh seeds were kept under moist filter papers in a pair of petri dishes. It was kept in darkness, diffused light and direct light, but none of the seeds responded to the above treatment. To know the cause, the treatments were made: 1. Scarification by sulphuric acid; 2. high temperature treatment; 3. low temperature treatment; 4. treatment of high temperature followed by low temperature; 5. treatment of low temperature followed by high temperature; 6. washing of seeds in running water to remove inhibitors, if any; 7. treatment with chemicals – thiourea, urea, calcium nitrate, ammonium nitrate, IAA, and GA_3. All these treatments did not produce success in germination. However, embryos were dissected out. It is interesting to record that fresh seeds have a rudiment embryo in which the plumular part is undeveloped (Figure 2a). After 6 months storage, the seeds show up to 40% germination. Washing of these seeds for 3 days in running water results into 90% germination. Hence embryo dissection after the storage of seeds reveals that they require about 6 months for maturation (Figure 2b). However, rudiment embryo and presence of unknown chemical inhibitor/s is recorded in the seeds[5–9].

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The germination is of epigeal type (Figures 2c–g). The seeds stored for 6 months were allowed to germinate at different temperatures. The result indicates that for germination the temperature range is 20–55 °C with an optimum of 30–36 °C (Figure 4). The seedlings appear in July when there is optimum temperature and moisture. This explains the cause of its distribution everywhere in the tropics during the rainy season.

Primary dormancy is of survival value for the species, as is indicated by its prevalence among wild plants[10–13]. Winter and summer are the dry part of the year and they are unfavourable for the growth of *Zornia diphylla*. Fresh seeds of the species are available in October–November, but they show marked dormancy as they do not germinate in any condition. The primary dormancy in freshly harvested seeds is due to rudiment embryo like Orchids, Ginkgo, Holly[5]. These incomplete seeds reach post-harvest maturation after passing through winter and summer seasons. Hence on the arrival of the rainy season, the seed gets released from its dormant phase, firstly due to the formation of complete embryo and secondly due to leaching of unknown chemical inhibitor/s in the rain water. Hence for survival over the unfavourable winter and summer seasons, *Zornia diphylla* possesses complex type of dormancy which does not permit its germination in fresh seeds. This behaviour is of great ecological significance[6,7].

The grassland and forest habitats of Gorakhpur have intense biotic activity of cattle and man. Grazing and scraping are special features. Monthly herbal removal by scraping is observed to stimulate shoot production in the species which is a character of non-palatable species of grassland[8]. The reason for good growth for this non-palatable species may be due to complete absence of palatable species[9].

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Structure of Furasterin, a Chlorinated Metabolite from the Fungus *Phialophora asteris* (Downson) Burge et Isaac[1]

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Summary. Furasterin, 4-chloro-7-hydroxy-5-methoxy-2-methyl-benzofuran, a new naturally occurring mono-chlorinated fungal metabolite was isolated from *Phialophora Asteris* (Downson) Burge et Isaac. Its possible relationship with the biosynthetical pathway of the fungitoxic dichlorinated fungal metabolite cryptosporiopsin is discussed.

In the scope of our work on different *Phialophora* vascular pathogens on plants[2], we have now studied *Phialophora asteris* (Downson) Burge et Isaac[3]. Mycological findings proved its close relationship with *P. asteris* f. sp. *helianthi*[4]. The latter species was earlier found to produce (–)cryptosporiopsin[5], the enantiomer of the fungitoxic and antibiotic product cryptosporiopsin[6,7] (Id, Scheme) Product Id was earlier isolated by MCGAHREN et al.[6], and STRUNZ et al.[7] from *Sporormia affinis* (Sacc.) Bomm and Rous and *Cryptosporiopsis* sp., respectively. Our aim was to prove a chemotaxonomic relationship between *P. asteris* f. sp. *helianthi* and *P. asteris* (Downson) Burge et Isaac. The present paper describes the isolation and identification of a chlorinated metabolite from the latter species. Its possible relationship to the biosynthesis of cryptosporiopsin is discussed. The fungus was similarly grown in liquid stationary cultures as was earlier described for *P. asteris* f. sp. *helianthi*[5]. Growth of the organism was somewhat slower (total period 24 days). The isolation of metabolites was identical to the method applied for (–)cryptosporiopsin[5].

Thin layer chromatography (using Merck Fertigplatten SiO_2; chloroform-methanol 5%) proved the presence of a substance with a similar Rf-value as (–)cryptosporiopsin (Rf 0.72). The unknown product (II b or c, Scheme) was purified by preparative thin layer chromatography in a final yield of 0.1 mg from each liter of culture medium. Gas chromatography (3% OV–17, isothermally at 160 °C) proved the product to be at least 95% pure, the remaining percentage being composed of several minor quantities of impurities.

Mass spectrometry revealed the molecular weight 212 with a typical intensity ratio between the ions M^+ and $(M + 2)^+$ of 3:1, indicative for the presence of a single isotopic chlorine atom. Loss of 36 mass units from the fragment at *m/e* 169 with simultaneous disappearance of the typical isotope fragment at *m/e* 133 + 2 further supported the mono-chlorinated nature of the unknown compound. At first instance, the above findings strongly suggested a close relationship between the unknown product and cryptosporiopsin (Id). The latter product with molecular weight 264 could yield – after replacement of one chlorine atom by hydrogen and additional dehydration – a product possessing the correct molecular weight 212 in agreement with the molecular formula obtained from exact mass measurements, $C_{10}H_9ClO_3$. However, the presence of the methylester group was not confirmed by the mass spectrum. Typical fragmentation by loss of 59 mass units and a strong fragment ion at *m/e* 59, as observed in the spectrum of Id, were lacking from the spectrum of the unknown.

The 100 MHz ^{1}H-NMR-spectrum measured in CCl_4 showed an OMe-resonance at δ 3.9 ppm in a very similar region as in the spectrum of Id, however, the typical resonances belonging to the allylic side-chain were not

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