

Seed abortion in an animal dispersed species, *Syzygium cuminii* (L.) Skeels (Myrtaceae): The chemical basis

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In *Syzygium cuminii*, an animal dispersed species, invariably only one of the 30 ovules develops to maturity. Arathi¹ and Arathi *et al.*² hypothesized that the abortion could be mediated by an intense intra-fruit sibling rivalry to gain dispersal advantage. They showed that aqueous extract of dominant seeds causes the abortion of healthy subordinate seeds while extracts of maternal tissue such as fruit coat tissue do not. In this paper, we show that the abortion is caused by the inhibition of resource uptake by the subordinate seeds. Extracts of the dominant seeds significantly inhibited the uptake of radioisotope labelled sucrose by healthy subject seeds, when compared to extracts from maternal tissue (fruit coat tissue) and unfertilized ovules. Inhibition was also caused by the diffusates from the dominant seeds. Thin layer chromatography and indirect ELISA indicated the presence of indole compounds in the diffusate. HPLC analysis indicated the presence of a single retention peak in the diffusate with a substantial overlap with that of indoleacetic acid and contained most of the functional chemical causing the abortion. We propose that the chemical involved in the abortion of seeds might be a small molecular weight, highly diffusible indole compound.

INTRAFRUIT seed abortion is a widespread phenomenon in plants¹⁻¹⁵. Several proximate causes of seed abortion have been proposed based on resource limitation¹⁶⁻¹⁹, lack of pollination and fertilization²⁰⁻²³ and developmental lethals^{15,24}. However, these explanations do not satisfactorily explain the consistent patterns of seed abortion observed in several species over locations and seasons^{1-5,13,25,26-29}, nor do they explain several ecological correlates of seed abortion such as that with the dispersal mode of the species, breeding systems, habit, etc.^{4,5}.

Ganeshiah and Uma Shaanker⁴ and Uma Shaanker *et al.*⁵ proposed that seed abortion can be viewed as a consequence of intra-fruit competition among the developing sibs to maximize their fitness. Thus in species in which the fruits are dispersed as entire units through wind, water or animals, siblings would be selected to kill others and be the lone survivor to gain dispersal advantage through increased wing loading^{4-7,26-29} or,

high pulp to seed mass ratio^{1,2,5,8,10,25}. They showed that this view is consistent with several patterns of seed abortion observed, as well as with the ecological correlates of seed abortion.

Recently, a few studies have addressed the physiological and biochemical basis of such intra-fruit sibling rivalry^{1,2,4,5,25-27}. Ganeshiah and Uma Shaanker⁴ and Mohan Raju *et al.*²⁷ showed that in a wind-dispersed species *Dalbergia sissoo*, where predominantly only one of the four or five ovules develops to maturity, the dominant among the seeds produces a chemical which kills the rest of the subordinate seeds. A similar mechanism of seed abortion has recently been reported in the animal-dispersed species, *Syzygium cuminii*, where only one out of 30 ovules develops to maturity^{1,2,25}. Here we explore the underlying mechanism of seed abortion in *Syzygium cuminii* and investigate the nature of chemical interaction between the dominant and subordinate seeds.

Syzygium cuminii (L.) Skeels (Myrtaceae), an animal dispersed species, is widely distributed in India and is mostly grown for its timber and for its fleshy berries. The tree bears flowers on cymose inflorescence during May and August. Of the 25 to 30 ovules in the flower, only one develops to maturity. The study was conducted using trees in the Botanical Garden, University of Agricultural Sciences, GKVK Campus, Bangalore, India, during 1991-1993.

Eight to ten days after fertilization, about four to eight of 30 ovules grow distinctly larger than others. These are called 'dominant seeds'. A dominance hierarchy is also observed among these developing seeds and the most dominant among them eventually matures into a seed. The remaining smaller seeds, which we refer to as 'subordinate' seeds gradually get sclerotized in about 10 days and are called 'aborted seeds'. Young seeds two to three days after fertilization, when dominance hierarchy has not been yet visibly set in, were used as 'subject seeds' in assay experiments. We use the term seeds for ovules, because these are fertilized and have developing embryos in them.

Equal quantities (3.0 mg fresh weight) of dominant and aborted seeds and of fruit coat tissue (of one-week old fruits as control) were ground separately in 5 mM MES (morpholinoethane sulfonic acid) buffer (pH 6.5) and then transferred to eppendorf tubes and centrifuged at 5000 rpm for 10 minutes. The extract was made up to a known volume and 10 µl of it was dispensed into clean cavity slides. Twenty subject seeds from one or two-day old fruits were transferred to each cavity slide and incubated at 70% relative humidity and at 30°C for 6 h. A control wherein subject seeds were incubated in 10 µl of the buffer was also maintained. After 6 h, 10 µl of 25 µCi ¹⁴C-labelled sucrose (uniformly labelled sucrose obtained from BARC, Bombay; specific activity 65 mCi/mole) was added to each of the cavity slides

and the subject seeds incubated. After 12 h, the seeds were removed and thoroughly washed in several changes of distilled water until there was no radioactivity in the bathing medium. The seeds were then ground in 2.0 ml of Bray's solution^{26,27}, and their radioactivity measured using a liquid scintillation counter and expressed as counts per minute (cpm) per seed or per mg fresh weight.

Equal quantities (3.0 mg fresh weight) of dominant and aborted seeds and of fruit coat tissue were incubated in a known volume of double distilled water for 12 h. The diffusate was collected by centrifuging the contents in eppendorf tubes at 5000 rpm for 5 minutes and the volume made up to 40 μ l using 5 mM MES buffer (pH 6.5). The diffusates thus collected were assayed for their effect on the uptake of labelled sucrose by the subject seeds as described in the earlier experiment.

For thin layer chromatography (TLC) analysis, diffusate from 100 dominant seeds was collected in 50 μ l of double distilled water. 20 μ l of the sample was used for spotting in silica gel TLC. Standards used were tryptophan, phenylalanine, indole, indoleacetic acid, indole acetonitrile and tyrosine. The solvent system used was isopropanol:ammonia:water in the ratio of 8:1:1. After the solvent was run for about 4/5th of the plate, they were allowed to dry in an oven at 60°C and were sprayed with 0.05 M FeCl₃: 5 per cent perchloric acid mixture. The colour developed in the sample as well as the relative flow (Rf) values were compared with that of the standards.

For high performance liquid chromatography (HPLC) analysis, diffusates obtained from the dominant seeds were subjected to HPLC analysis set at a wavelength of 280 nm at which indole compounds are detected. 20 μ l of the diffusate (collected from 80 dominant seeds) was loaded on a HPLC column (Water Associates, liquid chromatograph model 440) containing silica gel as separating material and eluted using a solvent mixture of methanol, acetic acid and water in the proportion of 30:1:69 parts respectively³⁰. Standard IAA was run for comparison and the peak retention time was recorded. C-18 reverse phase column was used for IAA detection.

Table 1. Effect of extracts of dominant and aborted seeds, fruit coat tissue and unfertilized ovules on the uptake of labelled sucrose by subject seeds

| Treatment | Absolute uptake (cpm/ovule) | % inhibition over control |
|--------------------|-------------------------------|---------------------------|
| Control | 312.5 \pm 34.5 ^a | — |
| Dominant seeds | 124.3 \pm 21.4 ^e | 58.7 |
| Aborted seeds | 172.3 \pm 32.5 ^d | 43.7 |
| Fruit coat tissue | 204.3 \pm 24.5 ^c | 34.0 |
| Unfertilized ovule | 243.4 \pm 14.6 ^b | 18.0 |

Two-way ANOVA; $P < 0.01$; Critical difference at 5% = 34. Each value is a mean of three replicates and in each replicate at least 20 subject seeds were maintained. Means followed with dissimilar superscripts are significantly different from each other.

Table 2. Effect of diffusates from dominant and aborted seeds and fruit coat tissue on the uptake of labelled sucrose by subject seeds

| Treatment | Absolute uptake (cpm/ovule) | % inhibition over control |
|-----------------------------------|--------------------------------|---------------------------|
| Control | 141.73 \pm 4.86 ^a | — |
| Diffusates from dominant seeds | 43.05 \pm 2.41 ^d | 69.6 |
| Diffusates from aborted seeds | 118.85 \pm 6.33 ^b | 16.1 |
| Diffusates from fruit coat tissue | 88.25 \pm 4.4 ^c | 37.7 |

Two-way ANOVA; $P < 0.0001$; Critical difference at 5% = 15.62. Each value is a mean of three replicates each containing at least 20 subject seeds. Superscripts represent significance and decreasing order of ¹⁴C sucrose uptake.

For assaying the labelled sucrose uptake by subject seeds, 100 μ l of the diffusate (obtained from 400 dominant seeds) was loaded on the HPLC. Using a fraction collector, a total of fifteen 1-ml fractions were collected corresponding to a total run time of 15 min. The fractions so collected were pooled as follows: i) fractions corresponding to 1 to 3 minutes and 6 to 15 minutes, which did not show any absorbance at 280 nm were pooled and ii) fractions corresponding to 4 and 5 minutes which exhibited a peak absorbance were pooled. The pooled fractions were lyophilized to evaporate the methanol fraction. The residue was redissolved in 5 mM MES buffer (pH 6.5), and the volume made up to 40 μ l in each case and used in ¹⁴C-sucrose uptake assay as described in earlier experiments.

For the quantification of indole compounds in the diffusate collected from dominant and aborted seeds, unfertilized ovules and fruit coat tissue, 8 mg (by fresh weight) of dominant and aborted seeds, unfertilized ovules and fruit coat tissue were used for diffusate collection as described earlier and the final volume made up to 250 μ l. Indirect ELISA for indoleacetic acid and indole compounds was conducted to quantify the indole compounds in different tissues³¹.

The extract of dominant seeds caused significantly higher inhibition in the uptake of labelled sucrose by subject seeds compared to that from aborted seed, unfertilized ovule and fruit coat tissue (Table 1, two-way ANOVA, $P < 0.01$), suggesting that the dominant seeds might mediate the abortion of the subject seeds by the production of chemical(s) that adversely affects the resource mobilization into them.

Aqueous diffusates from dominant seeds also caused significantly greater inhibition in the uptake of labelled sucrose than that from other tissues (Table 2, two-way ANOVA, $P < 0.0001$). The diffusate from the dominant seeds caused 69.6 per cent inhibition while that from aborted seeds reduced the uptake only by 16.1 per cent. Thus the chemical responsible for the inhibition in up-

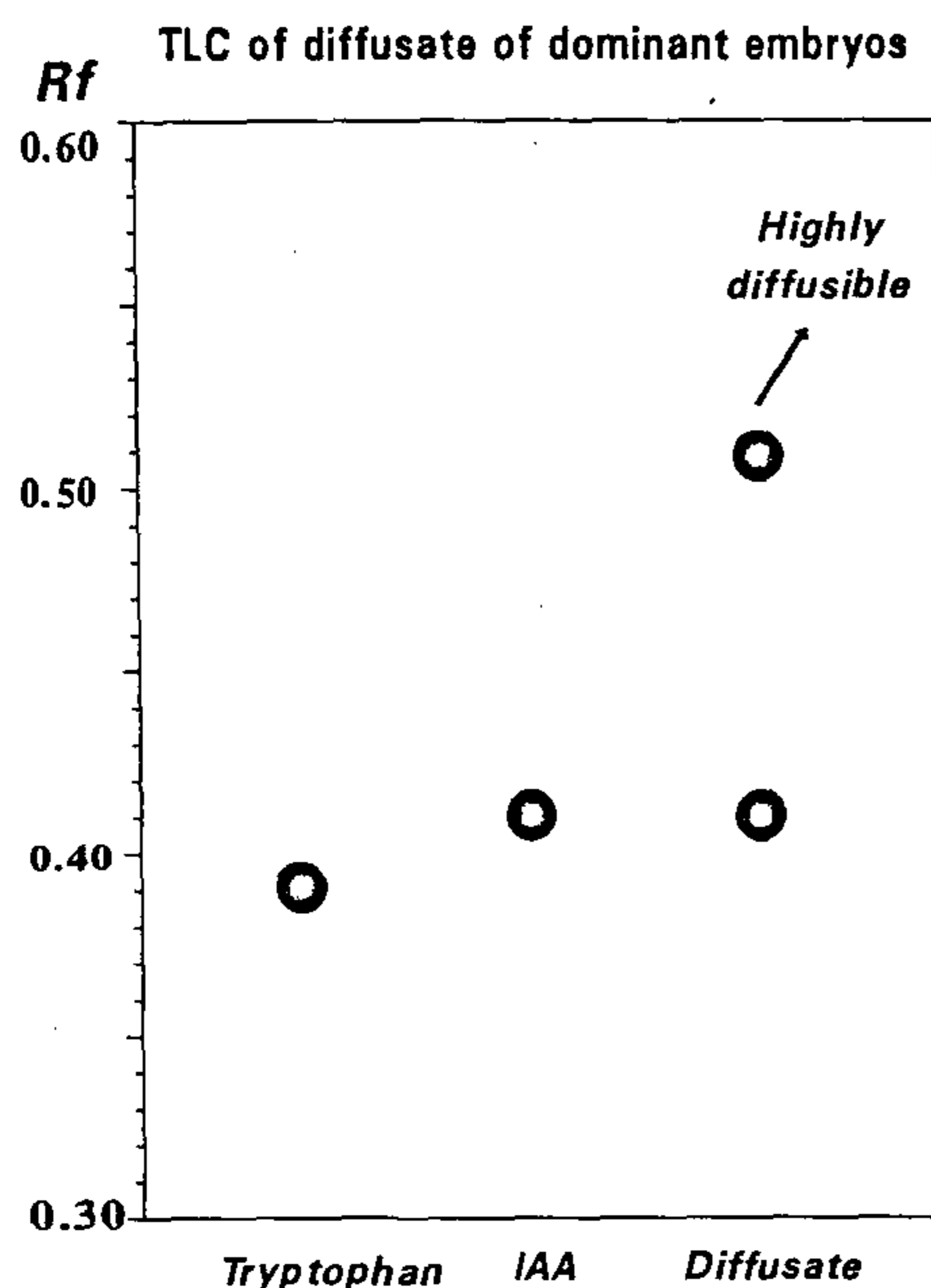


Figure 1. Thin layer chromatography of diffusate of dominant seeds (among the standards only that of tryptophan and indoleacetic acid (IAA) are shown. Notice the highly mobile indole spot obtained from the diffusate of dominant seeds. R_f = relative flow).

take of labelled sucrose by the subject seed appears to be highly diffusible.

TLC of the diffusates from the dominant seeds revealed the presence of IAA (R_f of 0.41) along with a highly diffusible substance (R_f of 0.51; Figure 1). Other standards used in the experiment such as phenylalanine, indole, indoleacetic nitrile and tryptophan were not found in the diffusate of the dominant seeds.

HPLC analysis of the diffusate of dominant seeds at a detection wavelength of 280 nm revealed the presence of a single peak with a retention time of 3.55 min; the retention time of standard IAA was 4.7 min (Figure 2). However, a substantial overlap between the retention time of the diffusate with that of standard IAA suggests that the former also contains indole compounds.

^{14}C -uptake assay conducted with the HPLC eluates showed that the fractions corresponding to the peak retention time (3.55 min) caused significantly higher inhibition (94%) in the uptake of labelled sucrose by the subject seeds compared to that caused by rest of the fractions (63%, Table 3). Indirect ELISA showed that the diffusate from dominant seeds had the highest amount of indole compounds compared to that from other tissues; dominant seeds contained nearly 950 picomoles per mg fresh wt., while fruit coat tissue had only 445.17 picomoles per mg fresh wt. (Table 4).

Our study indicates that abortion of seeds in *Syzygium cuminii* could be due to the production of a diffusible

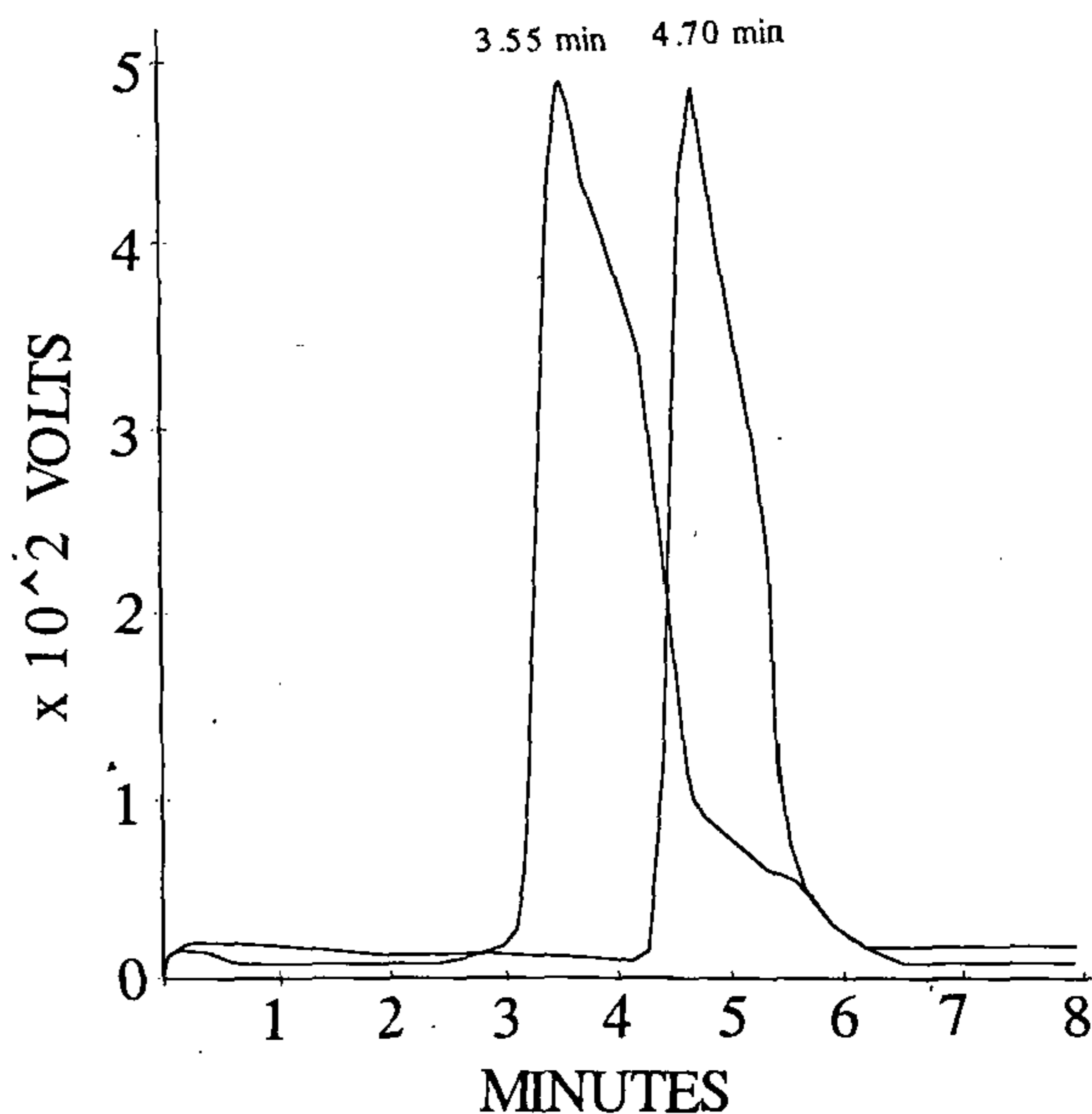


Figure 2. High performance liquid chromatography profile of the diffusate of dominant seeds. Peak corresponding to 3.55 min is the diffusate; that corresponding to 4.7 min is of indoleacetic acid.

Table 3. Effect of different HPLC fractions of diffusate from dominant seeds on the inhibition in the uptake of labelled sucrose by subject seeds

| Fraction no. | cpm/seed | % inhibition |
|---------------------|---------------------------|--------------|
| Control | 532.1 ± 29.4 ^a | — |
| Peak (4 and 5) | 36.0 ^c | 94.0 |
| Rest (1-3 and 6-15) | 189.9 ^b | 63.0 |

Two-way ANOVA; $P < 0.001$; Critical difference at 5% = 38.4. A minimum of four replicates each containing at least 20 subject seeds were maintained. Superscripts represent significance and decreasing order of ^{14}C sucrose uptake.

Table 4. Indole content in different tissues of *Syzygium cuminii* fruit

| Tissues | Indole content (Picomoles per mg fresh weight) | SE (±) |
|---------------------|--|--------|
| Dominant | 950.33 ^a | 45.7 |
| Aborted seeds | 410.89 ^c | 29.1 |
| Fruit coat tissue | 440.85 ^b | 40.2 |
| Unfertilized ovules | 271.76 ^d | 22.1 |

Two-way ANOVA; $P < 0.0001$; Critical difference at 5% = 30.19. Superscripts represent significance and decreasing order of ^{14}C sucrose uptake.

indole compound by the dominant ovules that inhibits resource uptake by the other ovules. Aqueous extract and diffusate from dominant seeds significantly inhibited the uptake of labelled sucrose by subject seeds

compared to those from the fruit coat (maternal tissue) or the aborted seeds. It has been shown earlier that the chemical factor might be heat resistant and non-proteinaceous^{1,2}. Because of its high diffusibility, the chemical might be a low molecular weight compound.

Analysis of the diffusate using TLC, HPLC and indirect ELISA points, to the presence of indole compounds in the extract and diffusate of dominant seeds. HPLC fractions corresponding to the peak retention time (3.5 min) overlapped substantially with that of indoleacetic acid and resulted in maximum inhibition of the uptake of labelled sucrose by the subject seeds. Similar mechanism involving the production of indole chemical by the dominant ovules that eventually starve the subordinate ovules has been reported by Mohan Raju *et al.*²⁷ in the wind-dispersed species, *Dalbergia sissoo*.

Based on these, we propose a general model to explain the abortion of ovules in plants (Figure 3). The model assumes that, to begin with, all ovules in an ovary are identical and are equally likely to mature as seeds. However, temporal differences in their fertilization sets in a dominance hierarchy in their development and the early fertilized among them dominate in drawing resources from the maternal parent¹². These dominant seeds in turn gain a head start in the synthesis of growth hormones including diffusible auxins and also probably related indole compounds. This may a) facilitate greater mobilization of resources into them resulting in a positive feedback aggravation of their dominance^{12,32-34} and b) inhibit the basipetal movement of diffusible indole compounds from the other (later fertilized) ovules due to primegenic dominance³⁵. Bangerth³⁵ proposed the theory of primegenic dominance to explain the development of dominance hierarchy among fruits and hence abortion of fruits in an inflorescence. According to this hypothesis, transport of diffusible auxin basipetally by the first-formed fruits prevents its diffusion from the remaining later-formed fruits. Such inhibition in the movement of diffusible auxin from the later-formed fruits has been shown to lead to one or both of the following consequences: (i) a poor differentiation of the sieve elements connecting these fruits resulting in poor nourishment and in extreme cases to the abortion of fruits³⁶; (ii) build-up of high levels of endogenous auxin in the fruits, which in turn might trigger the synthesis of ethylene which then arrests the further growth and development of the tissue^{37,38}. We propose that a similar mechanism of action could lead to the abortion of later fertilized ovules within a fruit.

Ethylene-mediated seed abortion has been reported by several workers. In apple, application of ethylene increased seed abortion while the ethylene action inhibitor, amino vinyl glycine, decreased seed abortion³⁹. In *Dalbergia sissoo*^{26,27} and *Syzygium cumini*^{1,2,25}, inhibition of ethylene action by silver thiosulphate (STS) re-

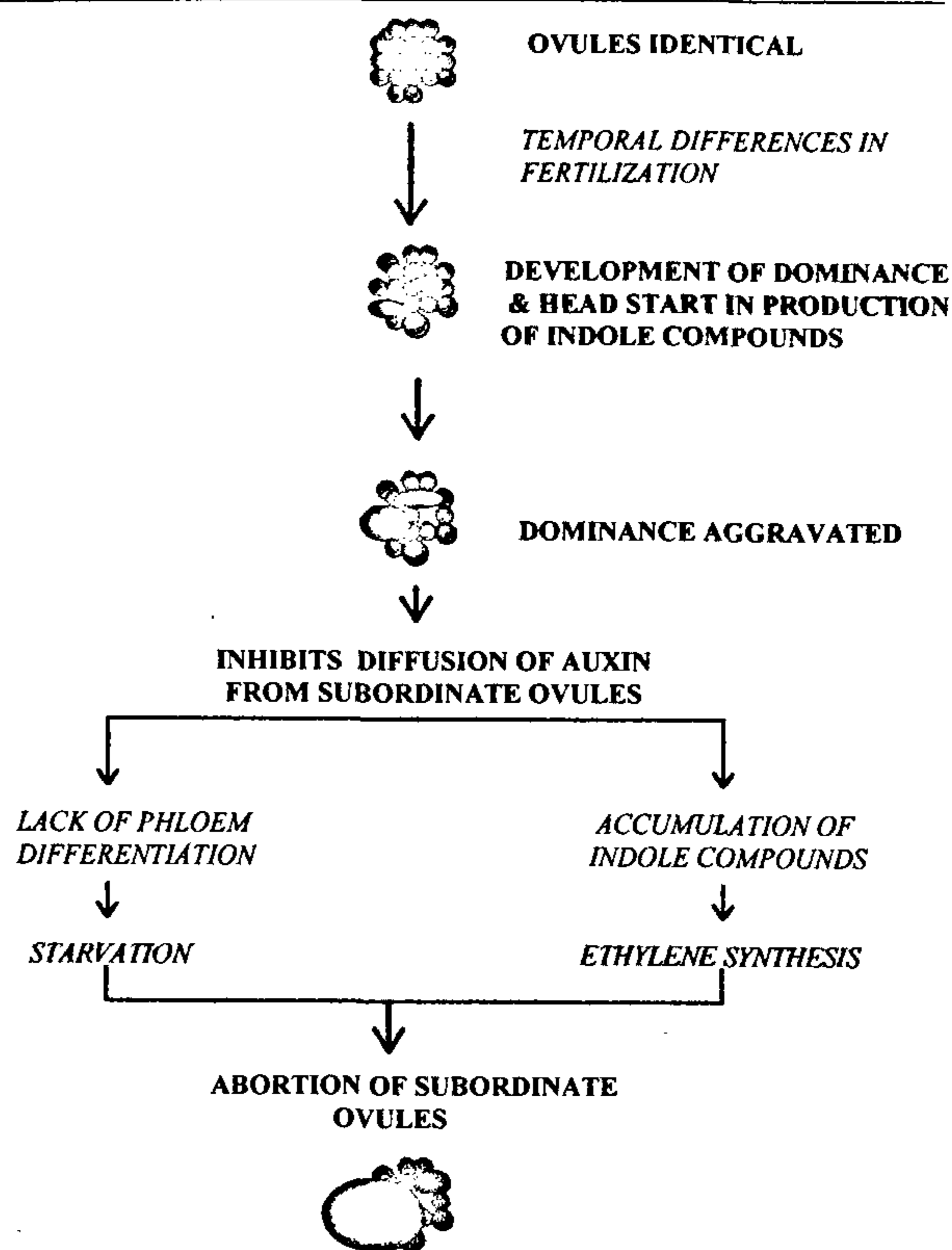


Figure 3. A general model of seed abortion in plants. For details, see text.

stored the uptake of labelled sucrose otherwise inhibited by extracts of dominant seeds; in *Syzygium cumini*, seed abortion was shown to decrease with application of STS to developing fruits^{1,2}.

This model is consistent with the observed results that extracts and diffusates of dominant seeds significantly inhibit the uptake of labelled sucrose by the subordinate seeds. Further, the model is unique because it explains the generation of dominance hierarchy. Thus, both the development and death of seeds could be brought about by a single factor involving the production of diffusible indole compounds.

Additionally, seed abortion could also be brought about by the production of 'death' chemicals by the dominant ovules that kill the other ovules. One such death hormone, 3-chloro-indoleacetic acid⁴⁰, produced in developing pea seeds is shown to induce senescence of vegetative tissue to which it is applied and is implicated also in fruit abortion. It is likely that the first fertilized dominant seeds produce this chemical and abort the remaining later-fertilized ovules.

Thus, the results of the present study suggest that differential development and abortion of seeds in fruits may be mediated by the production and diffusion of chemical factors, probably indole derivatives, by the

dominant seed which starves the rest of the seeds leading to their death. It is interesting to note that a similar mechanism operates in the widely-separated genera, *Dalbergia sissoo*^{26,27} suggesting a possible common mechanism of seed abortion across plant systems.

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