

# Early Events in Maize Seed Development<sup>1</sup>

## 1-METHYL-3-PHENYL-5-(3-[TRIFLUOROMETHYL]PHENYL)-4-(1H)-PYRIDINONE INDUCTION OF VIVIPARY

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### ABSTRACT

Preharvest sprouting or vivipary is induced in developing maize (*Zea mays*, inbred Tx 5855 and Va 35) seeds by fluridone, a pyridinone inhibitor of carotenoid biosynthesis. Fluridone has a maximal effect on vivipary at 11 days after pollination (DAP) and little effect at 13 DAP in the inbred maize line Tx 5855. Abscisic acid partially reversed the chemically induced vivipary. Though the precise mechanism of fluridone-induced vivipary is unknown, these results indicate that there are important developmental changes occurring at 11 DAP which reversibly commit the immature embryo to vivipary or dormancy.

The induction of seed dormancy is an important stage of seed development which enables a plant species to survive periods of severe environmental stress. Dormancy is caused by various internal factors, *e.g.* hormonal status or energy charge (12). This is contrasted with quiescence which is caused by external factors, *e.g.* anoxia, water drought. Without the onset and maintenance of dormancy there is the continued development of the embryo, otherwise referred to as 'vivipary' or 'preharvest sprouting.' Our current understanding of the onset of dormancy in maize seed is principally based on the characterization of several independent genes which control vivipary (7-11, 13). There are at least two classes of viviparous genes (9-11): type I includes those genotypes which have reduced seed color and normal responsiveness to ABA; and type II includes those with normal seed color and reduced responsiveness to ABA.

In this report, we describe a chemical system for induction of vivipary in maize and identify a developmental stage during seed maturation when vivipary or dormancy is determined.

### MATERIALS AND METHODS

Inbred maize (*Zea mays*) lines Tx 5855 and Va 35 were planted and maintained at the Texas A & M University Farm using normal cultivation practices and conditions with supplemental irrigation when needed. All plants were hand-pollinated and the date recorded. For comparison of several chemicals or a time-course experiment, all the plant material used in a particular experiment was pollinated on the same day. This minimized any

possible temperature-dependent effects with material that was pollinated over a several-day period. At the appropriate DAP<sup>4</sup>, the husk leaves were cut and pulled back to expose the developing seeds. After the remaining silks were removed, solutions were sprayed or painted on the seeds. Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4-(1H)-pyridinone) was applied in 1% (v,v) acetone-water. ABA was applied as a 1 mM aqueous solution. The husk leaves were gently refolded over the seeds, and the ear was rebagged, dated, and labeled. In all experiments reported here, the seeds were allowed to fully mature in the field until the stems and leaves had died, or at about 45 to 50 DAP.

From five to ten ears were used for each treatment date. Ears which were damaged by insects or diseases were not used. After harvesting the ears, the seeds were removed from the ear and seeds separated from viviparous ones. Figure 1 shows the typical range of response seen with fluridone-treated seeds.

### RESULTS

Fluridone and other pyridinone, and pyridazinone, compounds, are known to inhibit the desaturation reactions converting phytoene to phytofluene in algae and in higher plants (1, 6). Seeds treated with fluridone at 5 to 7 DAP appeared white at maturity whereas those seeds treated later in relation to the pollination date appeared increasingly yellow (Fig. 1). Under our

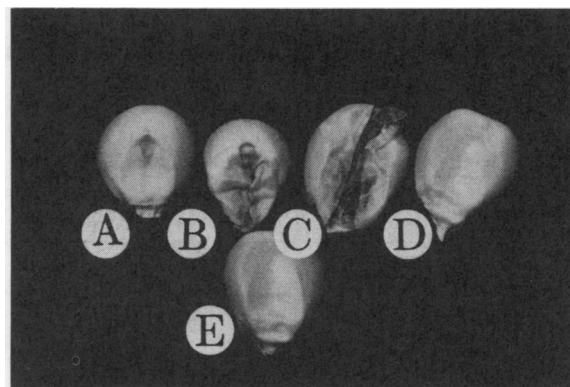


FIG. 1. Fluridone-treated seeds of inbred maize Tx 5855. Developing seeds were typically treated with 100  $\mu\text{g/ml}$  fluridone from 5 through 17 DAP, harvested at 45 to 50 DAP when plants were completely dried, and husked from the ears. The typical germside view of the seeds treated at (A) 5 to 7 DAP, (B) 7 to 9 DAP, (C) 9 to 11 DAP, (D) 15 DAP or later, and (E) not treated. Viviparous seeds have a visibly dark, enlarged plumule extending to or emerging from the tip of the seed (A, B, or C). Dormant seeds have a white scutellar region without any visible plumule (D or E).

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<sup>4</sup> Abbreviation: DAP, days after pollination.

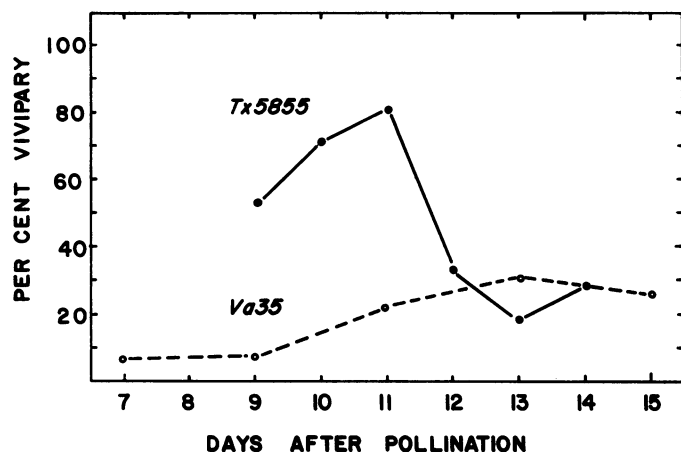


FIG. 2. Influence of fluridone on expression of vivipary in inbred maize Tx 5855 and Va 35. Developing seeds were treated with 100  $\mu$ g/ml fluridone from 9 through 14 DAP in Tx 5855 and from 5 through 15 DAP in Va 35. All treated seeds were harvested after 45 to 50 DAP, husked, and sorted out as dormant or viviparous. From 150 to 950 seeds were scored for each treatment in Tx 5855, and from 55 to 199 seeds in Va 35. Dormancy, or no vivipary, was seen at seed maturity when 11 DAP seeds were treated with either water (321 seeds) or 1% (v/v) acetone in water (239 seeds). No viviparous seeds were observed among several thousand wild type seeds that were scored in each of the two inbred lines.

Table I. Pyridazinone and Pyridinone Induction of Vivipary in Va 35 Seeds

Developing seeds were treated with 100  $\mu$ g/ml solutions of the above chemicals dissolved in 1% (v/v) acetone.

Treatment	No. of Seeds	
	Dormant	Viviparous
Fluridone	61	17
Pyrazon <sup>a</sup>	203	0
Norflurazon <sup>b</sup>	734	0
Flurazon <sup>c</sup>	376	0

<sup>a</sup> 5-Amino-4-chloro-2-phenyl-3-(2H)-pyridazinone.

<sup>b</sup> 4-Chloro-5-(methylamino)-2-( $\alpha,\alpha,\alpha$ -trifluoro-*m*-tolyl)-3-(2H)-pyridazinone.

<sup>c</sup> 4-Chloro-5-(dimethylamino)-2-( $\alpha,\alpha,\alpha$ -trifluoro-*m*-tolyl)-3-(2H)-pyridazinone.

Table II. ABA Reversal of Vivipary Induced by Fluridone in Developing Seeds of Inbred Maize Tx 5855

Fluridone Treatment <sup>a</sup>	ABA Treatment <sup>b</sup>	Avg. Vivipary in Ears <sup>c</sup>
DAP		%
9	None	53.8
9	10	54.5
9	11	51.6
9	12	70.6
9	13	54.2
9	14	50.5
9	15	25.6

<sup>a</sup> Fluridone treatment was done as described in Table I.

<sup>b</sup> ABA was applied in a 1  $\mu$ M solution in water.

<sup>c</sup> Only ears with over 50 seeds were used in this experiment.

growing conditions, seed color is apparent from 11 to 13 DAP. Fluridone application after this time did not significantly alter the color of the mature seed. Phytoene absorbs predominately UV light, and is the primary carotene accumulating in white,

Table III. Analysis of Variance of ABA Reversal Data Including Orthogonal Comparisons of 15 DAP and Control Groups

Percentage data were transformed to  $y = \arcsin \sqrt{y}$ .

Source of Variation	Degrees of Freedom	Mean Square	F
Among ABA treatments	6	0.0751	1.916 NS
15 DAP vs. all others	1	0.4231	10.793 <sup>a</sup>
Control vs. all others	1	0.0176	NS
Within ABA treatments	20	0.0392	

<sup>a</sup> Comparison significant at 0.01 probability level.

fluridone-treated seeds (7).

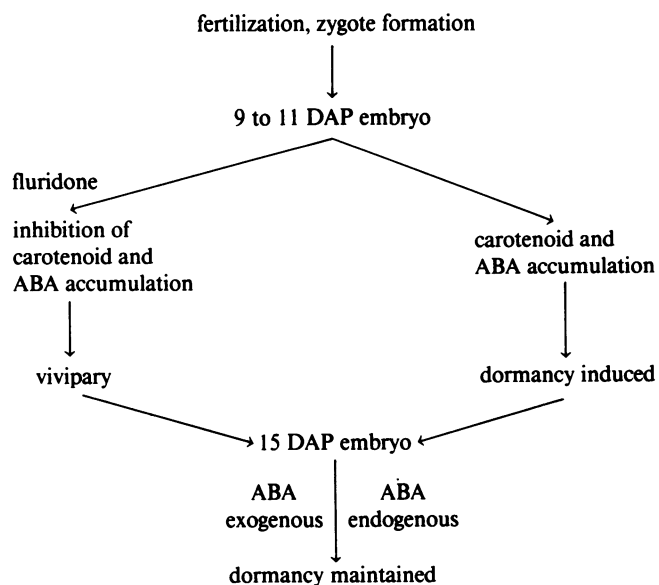
In addition to its effect on seed color, fluridone altered the developmental pattern of the embryo. The plumule developed prematurely if fluridone was applied at 9 DAP, with greatest response (80% for viviparous seeds) at 11 DAP (Fig. 2) in Tx 5855. After 11 DAP, there was a rapid decline in the extent of vivipary among the treated seeds. Using a second inbred maize line, Va 35, a similar experiment showed that, unlike Tx 5855, fluridone was less effective in inducing vivipary and had maximum effectiveness at 13 DAP. These qualitative and quantitative differences may reflect varietal differences or possibly environmental differences since Va 35 was pollinated 1 week earlier than Tx 5855.

In comparing various inhibitors of carotenoid biosynthesis, treatment with the pyridazinone compounds pyrazon, norflurazon, and flurazon, which also inhibit carotenoid biosynthesis (1, 4, 6, 7), did not affect seed color or induce vivipary in corn (Table I). These differences in response may be caused by differences in penetration, or in metabolism of these chemicals by the developing seed. Thus, of the inhibitors tested, only fluridone, a pyridinone compound, will inhibit carotenoid biosynthesis and induce vivipary during maize seed development.

Application of the phytohormone ABA to developing seeds previously treated with fluridone can affect the level of vivipary at the time of harvest. Tables II and III show that ABA was effective in reducing vivipary in fluridone-treated seeds only when applied at 15 DAP. Thus, during maize seed maturation, there is a developmental period when fluridone will induce vivipary, such as from 9 to 12 DAP in Tx 5855, and this is followed by a more narrowly defined period at 15 DAP, when ABA can partially reverse this vivipary.

## DISCUSSION

Fluridone treatment mimics all the pleiotropic effects of the 'type I' vivipary mutants in maize, *i.e.* vivipary, normal responsiveness to ABA, reduced seed color, and white seedling leaves. An interrelationship between these various phenotypic characteristics has been suggested from other work as well. Seed dormancy has long been associated with ABA content (12). Carotenoids and ABA have been linked via xanthoxin, a pH-neutral, ABA-like substance found in a wide variety of plant materials and produced from carotenoids (2, 5). Finally, fluridone-treated maize seedlings show a gibberellic acid hypersensitivity that is indicative of low ABA content (3). We propose the following working hypothesis to summarize our results with the developing maize seed:



During the first 9 DAP, there is zygote formation, followed by rapid development of the embryo and endosperm tissues. From 9 to 11 DAP, there is the induction of dormancy by internal factors, probably ABA. If fluridone is present, there is inhibition of carotenoid accumulation followed by vivipary. At 14 DAP, dormancy is maintained by high endogenous or exogenous ABA levels.

There is no direct evidence to explain the mechanism by which fluridone will induce vivipary. Whether the fluridone effect is caused by concomitant inhibition of carotenoid and ABA biosynthesis, by indirect effects on membranes, or in as yet undefined ways, remains to be determined.

These fluridone studies reveal a stage in the developmental pattern of maize seeds not evident from previous studies using genetic mutants. In genetically induced vivipary, the viviparous state is not detectable until 15 DAP when differences in seed color are noticeable or at 25 DAP when plumule emergence is apparent. In fluridone-induced vivipary, treatment of the seed at

9 to 11 DAP reversibly alters the subsequent developmental pattern of the plumule. More subtle changes have undoubtedly occurred at the biochemical and physiological levels, the consequences of which are not apparent until many days later. This chemical system complements the genetic system for studying vivipary, in providing larger quantities of biological material for biochemical studies as well as in providing greater experimental control in defining events of seed development.

Though we still do not understand the precise mechanism of induced vivipary, this experimental system will make possible more detailed studies on the branchpoint between vivipary and dormancy in seed development as well as on the temporal and biochemical nature of interrelationships between carotenogenesis, dormancy, and ABA metabolism.

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