THE INTER-RELATION OF CATALASE, RESPIRATION, AFTER-RIPENING, AND GERMINATION IN SOME DORMANT SEEDS OF THE POLYGONACEAE

by

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INTRODUCTION

Mature fruits of some species of the Polygonaceae have dormant embryos which must undergo a period of after-ripening before germination can take place. The studies reported in this paper have to do with dormancy, after-ripening, germination and especially with the relation between the so-called enzyme catalase and the intensity of respiration during these periods and also with their significance in indicating changes in the metabolism of the fruits of the Polygonaceae studied.

MATERIALS AND METHODS

Fruits of Polygonum scandens, P. pennsylvanicum, P. aviculare, P. acre, P. virginianum, P. lapathifolium, and Rumex crispus were collected in October, 1931 at Manhattan, Kans. The fruits were dried, cleaned and with the aid of a seed blower the lighter weight and immature fruits were eliminated. The remaining fruits were then tested by cutting thru two hundred to make sure that they contained embryos. Fruits were stored dry at laboratory temperatures

and in a refrigerator at 3° C. Fruits were also planted in soil in pots, one half of which were placed in the greenhouse and the other half out of doors. Fruits were taken from time to time from dry storage and placed on moist cotton in petri dishes. These were placed at different temperatures ranging from 3° to 30° C. and after varying periods of treatment were tested for germination, respiration, and catalase.

Fruit coats were removed by treatment for five minutes in concentrated H_2SO_4. The fruits were then washed thoroughly and the ovary wall rubbed off. Due to the structure of the seed it was impossible to remove the seed coat without injury to the embryo.

The apparatus and methods used in determining catalase were the same as described and illustrated by Appleman (2), Davis (17), and others. Care was taken to maintain a constant rate of shaking for all tests and temperature conditions in the laboratory were practically constant. In each case 25 fruits were ground in the morter and neutralized with $CaCO_3$. The pulverized grindings were then washed into the shaking bottle with 10 cc. of distilled water. Five cc. of neutralized Dioxygen was added to the separatory

funnel and after the apparatus was completely adjusted, introduced into the shaking bottle. Readings were taken at 5 and 10 minute intervals. The figures in the tables are averages of the 10 minute readings.

Measurements of the gaseous exchange in respiration were made with a respirometer such as that devised by Harrington and Crocker (27). In each test 50 seeds were placed in the apparatus and the test run for 48 hours, care being taken to keep the temperature constant. Sodium hydroxide was used in the absorption of the CO_2 and the volume of the oxygen absorbed and the CO_2 given off was calculated according to the formula used by Sherman (42), Harrington and Crocker (27), and others.

Germination tests were made at room temperature on moist cotton in petri dishes.

STRUCTURE

As the enveloping membranes have a direct relation to the rate of after-ripening and germination, a brief description of the morphology of the fruits will be given.

According to Woodcock (44) the seed of the Polygonums at maturity is enclosed in a thick hard coat, consisting of

the cutinized epidermal layer and the compressed inner layers of the ovary. The integuments have become closely compressed and their crushed remains form a thin layer surrounding the embryo and endosperm. Just inside the integuments is a thin aleurone layer. The embryo occupies a cavity just beneath the aleurone layer in an angle of the seed and free from the mealy endosperm. The two inner layers cannot be removed without separating the embryo from the endosperm as there is no other physical bond keeping them together. The embryo cannot develop without nutrition so experiments have necessarily been limited to those seeds surrounded by the fruit coat and those with the fruit coat removed, leaving the seed coat intact.

AFTER-RIPENING AND GERMINATION

Dormancy in seeds has been studied extensively and may be due to any one or a combination of the following causes:

1. Membranes such as hard seed and fruit coats may retard or prevent the intake of water and oxygen or may by mechanical strength prevent the swelling and expansion of the embryo and so delay germination for a season or for many years (7, 8, 9, 25, 30).

2. Immature embryos are another cause of dormancy. The seed, when it is shed, contains an embryo which has not reached maturity and several weeks under germinating conditions may elapse before it becomes sufficiently developed to grow (9).

3. Dormant embryos are to all appearances mature but must undergo a change or period of after-ripening before they will germinate (5, 13, 16, 19).

In the seeds of the Polygonaceae reported in this paper, the embryo is dormant at maturity. Rumex crispus is an exception in that a large per cent of the fruits germinated immediately at room temperature. However, the remaining fruits required varied periods of treatment before germination took place. The fruit coat and probably the seed coat and aleurone layer are instrumental in retarding after-ripening and preventing germination. The removal of the fruit coats greatly shortened the period for after-ripening, those with the fruit coats intact requiring three times as long as those with the fruit coat removed. Also the rate of respiration in the seeds with the fruit coats removed was considerably higher than those with the fruit coats intact, indicating a greater availability of the oxygen supply.

Fully after-ripened embryos did not all germinate even when placed under favorable conditions for growth and if left at high temperatures reverted into dormancy. The failure of these embryos to grow was probably due to the restricting membranes surrounding them as germination took place in most cases when the fruit coats were removed.

Davis (19) has shown that in fruits of the ragweed the seed coat and nucellar membrane are more effective than the fruit coat in retarding the exchange of gasses and it is likely that the embryos of the Polygonaceae would react more rapidly if they could be freed from all enveloping membranes.

Fruits in water after 24 hours at room temperature absorbed 30 per cent of their air dry weight and after 48 hours absorbed only a two per cent increase over the 24-hour period. This is a rather low intake of water and may, to some extent, affect the period of after-ripening.

Considerable experimentation has been conducted on different methods of overcoming dormancy in seeds. In the case of hard seed or fruit coat restrictions, scarification, treatment with chemicals, alternate freezing and thawing, and the application of high air pressures are some of the methods commonly used (14, 24, 29, 39). Dormant embryos have been found to after-ripen most favorably when placed

at from 3° to 6° C. in a saturated condition (5, 11, 16, 23). Other seeds such as Bermuda grass and tobacco are light sensitive (21, 34). In some cases a period of dry storage at ordinary temperatures is sufficient to overcome dormancy.

The following methods were used to overcome dormancy in the seeds studied.

Dry Storage

Room temperature was found just as satisfactory for storage as 3° C. This has also been found true of many other seeds (1, 11). There was no complete after-ripening in dry storage at either laboratory temperature or at 3° C. during the period of the experiment, but as the time of storage was prolonged there was a noticeable increase in the maximum temperature at which after-ripening would take place. The periods of after-ripening on moist cotton at 3° to 6° C. also decreased in length as the time of storage increased. Extended dry storage has also been found to be helpful in the after-ripening of dormant seeds of the Rosaceae (5, 13, 23).

In Soil

There was no germination in pots placed in the greenhouse, indicating that no after-ripening had taken place under those conditions. Fruits in pots out of doors showed a small percentage of germination and all grew simultaneously about the last of April, there being no subsequent germination. Since the fruits were covered with a thin layer of soil the oxygen supply was probably much less than that available to fruits on moist cotton in petri dishes which may account for the lower percentage of germination. If the fruits do not germinate by a certain time in the spring, they apparently lose their opportunity for that season and lie dormant until a later favorable season. This may be due to the lack of oxygen as the higher temperatures occurring in summer make the oxygen demand greater for respiration and its restriction by enveloping membranes would tend either to prevent germination or to throw the embryo into a secondary dormancy. It is true that on newly plowed ground many weed seeds are brought to the surface and grow as oxygen becomes more available.

The difference in time of germination of many seeds may be due to variation in the permeability of the membranes as

some seeds of the same species remain inactive much longer than others. In the Polygonaceae the period of after-ripening varied for the different species as is shown in Tables 1 and 3. It also varied for different fruits of the same species, this character being most marked in Rumex crispus, Polygonum pennsylvanicum, P. aviculare, and P. lapathifolium. In Rumex crispus some seeds germinated at maturity, others required different lengths of treatment at low temperatures, and some never germinated during the time of the experiment. Rumex crispus is generally reported as light sensitive (21). Polygonum scandens, P. virginianum, and P. acre after-ripened more evenly, germination occurring simultaneously in all the seeds not restricted by the outer coats.

Davis (unpublished) has found in experiments with Velvet Leaf, Abutilon theophrasti, that 100 seeds submerged in water showed a wide variation in permeability. A few seeds have germinated each year until only 4 remain after 22 years. Others have found this variation in permeability of the seed coats in vetch, Holcus, and many wild species (22, 30, 43).

Saturated at 3° to 6° C.

Temperatures of 3° to 6° C. were found to be the optimum for after-ripening in all the species. Polygonum scandens reacted more favorably to the lower extreme and P. pennsylvanicum reacted better at 6° C. Polygonum scandens transferred from dry storage to moist cotton at 3° C. on May 1, germinated 85 per cent in 31 days and when the fruit coats were removed, germinated 95 per cent in 7 days. These seeds would not germinate without treatment at low temperatures. A period of dry storage decreased the length of the period required for after-ripening. The data in Tables 1, 2, and 3 are averages for the year.

Saturated at 9° to 12° C.

After a period of from 4 to 6 months in dry storage some after-ripening took place after 98 days at 9° and 12°C., 14 per cent of the fruits germinating.

Saturated at 18°, 20°, 30°, and 20° to 30° C. Alternating

No after-ripening took place at these temperatures. Fruits partly after-ripened and placed at 30° C. for 1 to 2 months and then returned to low temperatures required a much

longer period to after-ripen than fruits taken directly from dry storage, indicating that the period at high temperature not only overcame the effects of the time at low temperatures, but also rendered the embryo more dormant. This may be due to the increased respiratory demands at high temperatures. At the higher temperatures the readily oxidized food materials may be used in respiration as fast as they are formed, while at the lower temperatures necessary for after-ripening, the reduced respiratory activity allows them to accumulate in sufficient quantities to initiate growth (12, 15, 18, 26).

Treatment with Fruit Coats Removed

Seeds of Polygonum scandens with the fruit coat removed reacted in much the same manner as those with fruit coat intact but response was always more rapid. At high temperatures swelling occurred in the cotyledons and hypocotyl, breaking open the seed coat but not germinating normally thru the micropyle. This was evidently due to imbibition as there was no development of the radicle.

The following tables show the percentage of germination and the length of time required for after-ripening under these different conditions in the species studied.

Table 1.--The percentages of germination and periods of after-ripening required in fruits of Polygonum scandens.

Stor. Med.	Temperature	No. tests	No. fruits per test	No. days	Total per cent germ.
Saturated	3° C.	10	100	75	78.4
Saturated	6° C.	10	100	92	52.4
Saturated	9° to 12° C.	6	100	98	14.0
In soil	Open field	1	50	181	12.0
In soil	Greenhouse	1	50	181	0
Saturated	18°, 20°, 30° & 20° to 30° C. alternating	10	100	181	0
Dry seed	Lab. cond.	2	100	278	0
Dry seed	3° C.	2	100	181	0

Table 2.--The percentages of germination and periods of after-ripening required in seeds (fruit coats removed) of Polygonum scandens.

Stor. Med.	Temperature	No. tests	No. seeds per test	No. days	Total per cent germ.
Saturated	3° to 6° C.	10	100	21	90
Saturated	12° C.	2	100	28	35
Saturated	18°, 20°, 30° C.	6	100	60	0
Dry seed	Lab. cond.	2	100	20	0

Table 3.--The percentages of germination and the periods of after-ripening required for fruits of different species of the Polygonaceae.

Species	Stor. cond.	No. tests	No. fruits per test	Total days	Total per cent germ.
Rumex crispus	Dry in lab.	2	100	40	65
	Sat. at 3° C.	2	100	92	85
	Outdoors in soil	1	50	181	0
Polygonum pennsylvanicum	Sat. at 3° C.	9	100	172	52
	Sat. at 6° to 9° C.	3	100	150	84
	Sat. at 12° C.	2	100	150	9
	Outdoors in soil	1	50	181	10
P. aviculare	Sat. at 3° C.	5	100	150	100
	Outdoors in soil	1	50	181	8
P. virginianum	Sat. at 3° C.	9	100	91	88
	Outdoors in soil	1	50	181	2
P. acre	Sat. at 3° C. soil	3	100	32	75
	Outdoors in	1	50	181	10
P. lapathifolium	Sat. at 3° C.	5	100	33	88
	Outdoors in soil	1	50	181	4

CATALASE

Since Loew (32) first extracted catalase from the leaf of the tobacco plant, much study has been done to find its exact relation and function in the plant and animal kingdoms where it has been found to occur almost universally. It has been called an enzyme but differs from a true enzyme in that it is used up in the reaction.

Appleman (3, 4), Crocker and Harrington (10), Burge (6), Davis (19), and others have satisfactorily shown that catalase is directly correlated with respiration in a great many cases. Ranjan and Mallik (37) believe that they are not always correlated and that catalase is directly related to the monosaccharide content, especially to hexose formation. Rhine (38) and Lantz (31) found that catalase and respiration do not follow the same curve. Morgulus (33) has shown that there is no correlation between respiration and catalase in frogs. Harvey (28) showed that a blue green alga growing in a hot spring contained no catalase and Kywasch and Kywasch (40) showed that certain anaerobic bacteria do not contain catalase, both pointing out that catalase must not be essential to respiration in anaerobic forms but associated with aerobic respiration. Shaffer (41)

suggests that catalase acts as a brake against excessive oxidation. Catalase has been shown to be an indicator of viability and related to growth (17, 20, 35, 36).

Polygonum scandens was selected for detailed study as fruits of the other species were small and were found to behave similarly. In Polygonum scandens there was a gradual rise in catalase as after-ripening progressed, increasing very rapidly when germination began. Fruits at 30° C. became more dormant and showed an initial increase and then a marked decrease in catalase. Fruits at 12°, 16°, and 20° C. seemed to be at a standstill since the catalase remained more or less constant for long periods after the initial increase. At 3°, 6°, and 9° C. the catalase gradually increased until after-ripening was completed. (Figures 1 and 2.) The catalase was greatest at 3° and 6° and lowest at 30° C. At intervening temperatures the catalase varied only slightly from a point about half way between the two extremes. (Table 4.) Dry fruits showed a decrease in catalase as the season advanced, even tho they apparently were partially after-ripened. (Table 4.) This may be due to a drying out of the fruits as there seems to be a relation existing between catalase and the moisture content of the fruits. Those kept moist at 20° C. thruout the

storage period showed no loss of catalase. When the fruit coats were removed the initial catalase dropped in the dry seeds but increased rapidly when they were soaked, being much higher and in general reacting more quickly than in those with the fruit coats intact. (Figure 2.)

Color

In preparing fruits of Polygonum scandens for catalase determinations, it was found that the fruit coats contained a salmon-colored pigment which appeared to disintegrate after a period of saturation at high temperatures. Soaked seeds showed a more intense salmon color when ground than did dry seeds. The following data show that the disintegration of the pigment was faster as the temperature increased. Dry seed showed no disintegration. Saturated fruits at different temperatures showed the following color reactions:

From 3° to 6° C. The pigment was a bright salmon for 30 days, then faded slightly and after 167 days it became a pale salmon.

Twelve degrees C. A bright salmon for 15 days, pale for 15 days, and after 30 days, colorless.

From 18° to 25° C. A bright salmon for 5 days, pale for 20 days, and after 25 days, colorless.

Thirty degrees C. A bright salmon for 4 days, pale for 8 days, and after 15 days, colorless.

Table 4.---Catalase activity of Polygonum scandens placed in a saturated condition at different temperatures.

	Fruits		Seeds
Temperature	cc. of O_2 : winter average	cc. of O_2 : spring average	cc. of O_2 : spring average
3° to 6°	31.9	22.3	15.7
9° to 25°	26.3	20.4	14.0
30°	22.1	17.5	19.1
Dry seed	24.5	18.5	15.0

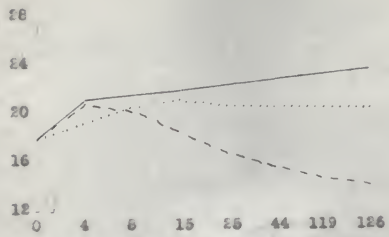

Fig. 1. Catalase activity in fruits of Polygonum scandens after varying periods of treatment in a saturated condition at different temperatures.

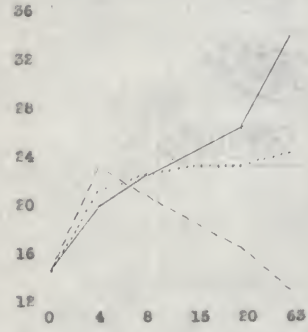

Fig. 2. Catalase activity in seeds of Polygonum scandens after varying periods of treatment in a saturated condition at different temperatures.

Horizontal - number of days treated.
Vertical - cc. of O_2 liberated in 10 minutes.
_____ 3° to 9° C. ..... 12° to 20° C. ---- 15° to 30°C.

RESPIRATION

In *Polygonum scandens* the rate of respiration varied directly with the temperature as has been generally reported. (Table 5.) At a constant temperature respiration followed a curve similar to catalase, showing a gradual increase at temperatures favorable for after-ripening and increasing rapidly after germination began, but at high temperatures where the dormancy became more pronounced, the rate of respiration decreased. (Figures 3 and 4.) The seeds with the fruit coat removed showed a greater respiratory activity than those with fruit coat intact. These facts indicate that a definite correlation exists between catalase and respiration. Both activities showed a parallel relation to after-ripening, germination, and viability in dormant seeds of the Polygonaceae.

The higher the temperature the greater the respiration and the greater the demands on the reserve of the seed, but after remaining at 30° C. for some time, these fruits seemed to adjust themselves and the respiration was cut down, accompanied by a decrease in catalase. This reduction in respiratory activity possibly enables the seed (tho in a stage of deeper dormancy) to retain its viability longer in

nature, since the food supply would be less rapidly exhausted by the demands of respiration. This adaptation has also been found in the ragweed, apple and other seeds (18, 19, 26).

Table 5.--Respiratory activity of Polygonum scandens placed in a saturated condition at different temperatures.

Temperature	Fruits			Seeds		
	O_2	CO_2	CO_2/O_2	O_2	CO_2	CO_2/O_2
6° C.	.47	.35	.74	.78	.68	.90
12° C.	.55	.45	.82	.93	.84	.90
18° C.	.67	.60	.90	.98	.87	.89
30° C.	.83	.72	.87	1.09	1.00	.92

Fig. 3. The compared rate of respiration in fruits and seeds of Polygonum scandens after varying periods of treatment at 6° C.

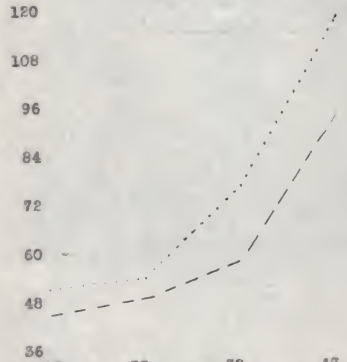

Fig. 4. The compared rate of respiration in fruits and seeds of Polygonum scandens after varying periods of treatment at 30° C.

Horizontal - number of days treated. Vertical - cc. of O_2 absorbed in 48 hours. ___fruits. ..... seeds.

SUMMARY

1. The embryos of fruits of the Polygonaceae studied are dormant when mature.

2. The optimum temperature for after-ripening is 3° to 6° C. Fruits treated in a saturated condition at these temperatures for several months grew 80 to 100 per cent when removed to the higher temperatures suitable for germination. There was a gradual increase in catalase and respiration thruout the period of after-ripening abruptly increasing as germination started.

3. Fruits did not after-ripen in dry storage either at laboratory temperatures or at 3° C. after a period of 278 days, but the dry storage considerably shortened the time required for after-ripening in a saturated condition at low temperatures.

4. Fruits in pots out of doors grew from 2 to 12 per cent after 181 days. There was no germination of fruits in the soil in the greenhouse after 181 days, indicating that no after-ripening took place.

5. Fruits placed at 30° C. showed a rapid increase in catalase and respiration during the first week. This was followed by a decided decrease in catalase and respiration

which became more or less constant at the end of one or two months. When removed from high temperatures to 3° C., they were found to require 120 days for after-ripening, showing that the dormancy had become more pronounced at high temperatures.

6. After a period of from 4 to 6 months in dry storage, some after-ripening took place after 98 days at 9° and 12°C., 14 per cent of the fruits germinating.

7. The period required for after-ripening in fruits of Polygonum scandens (false climbing buckwheat) became shorter as the dry storage period was lengthened. Fruits required 51 to 75 days for after-ripening at 3° to 6° C., but after the fruit coats were removed, after-ripening was completed in 7 to 21 days. Catalase and respiration increased with the rate of after-ripening in seeds with the fruit coat removed.

8. The seed and fruit coats are restricting membranes in relation to the intake of oxygen and water.

9. Dormancy was unevenly distributed, some seeds requiring a longer time to after-ripen than others of the same species.

10. Fruit coats of Polygonum scandens contained a salmon-colored pigment which disintegrated completely when

the seeds were moist at high temperatures.

11. Dry seed showed a decrease in catalase after several months of storage which may likely be due to a decreased moisture content of the seeds.

12. Catalase activity was greatest at temperatures of 3° to 9° C. and lowest at 30° C., while the rate of respiration was lowest at 3° to 9° C. and highest at 30° C., but at a given temperature catalase and respiration were correlated, following a similar curve in direct relation to after-ripening and germination.

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