SEED GERMINATION IN POLYGONUM

A Thesis

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INTRODUCTION

Seed dormancy, a common phenomenon in many species of plants, allows seed from a given year to produce seedlings over several subsequent years. The selective evolutionary advantage of dormancy is particularly significant in weedy species of temperate environments where a device allowing the preservation of a species through the adverse conditions of winter is desirable. It permits weedy populations such as Polygonum (smartweeds) to successfully compete in often disturbed areas, e.g., where land is cleared for building or agricultural purposes, or where natural disturbances, such as high water, are common.

Seed dormancy in Polygonum has been attributed to the hard, impermeable pericarp and an after-ripening requirement (Justice, 1941; Ransom, 1935; and Timson, 1965). Timson (1965) believed that the after-ripening process involved an enzyme with a low optimum temperature which attacked the pericarp from within, thus facilitating water entry. Removal of the pericarp with sulfuric acid or treatment at low temperature under moist condition enhanced germination of many weedy species of Polygonum (Justice, 1941; Ransom, 1935; and Timson, 1965). In addition, Timson (1966) showed that germination in P. convolvulus can be obtained quite easily using de-apexed seed and treating with gibberellic acid, thiourea, uracil, thymine, adenine, guanine, or cytosine.

The present study was undertaken to determine laboratory methods of germinating certain weedy species of Polygonum commonly found in the area of Emporia, Kansas.

METHOD AND MATERIALS

Seed Source

All study seeds of *Polygonum lapathifolium* L., *P. bicorne* Raf., *P. pensylvanicum* L., *P. punctatum* Ell., and *P. scandens* L. were collected in the late summer of 1966 and 1967. They were harvested, air-dried, and stored either at 4 C or room temperature. Voucher specimens for each seed lot collection of 1967 (Table I) were housed in the herbarium of the Kansas State Teachers College.

Germination Tests

The dormancy-breaking ability of the following were tested: stratification, X-ray exposure, ultraviolet light exposure, continued darkness, electric current (AC), scarification by de-apexing, and solutions each of: adenine, cytosine, guanine, thymine, α-amylase, a dinitrophenol (DNP), gibberellic acid (GA), indole acetic acid (IAA), or thiourea.

Because of the variation in germination percentages of different seed lots of any one species, only one lot per experiment was used unless a limited seed supply made this impossible. Seeds were prepared for the tests by removing the persistent perianth and testing for inviability*. Clean, theoretically viable seed was planted in plastic petri dishes half-filled with white silica sand

* Incompletely formed seeds crushed easily.

TABLE I. Dates, lot numbers, and locations of the 1967 harvest.

Species	Lot No.	Location*	Harvest Date
P. lanathifolium	1	Cimarron Co., Okla.	24 Sept.
	6		29 Sept.
	16		25 Oct.
	21		27 Oct.
P. biocrne	5		29 Sept.
	12	Sequoyah Co., Okla.	7 Oct.
	14		20 Oct.
	17		25 Oct.
	18		25 Oct.
	19		25 Oct.
	22		27 Oct.
	23		27 Oct.
	13013		27 Sept.
P. pensylvanicum	7		29 Sept.
	9		29 Sept.
	11		29 Sept.
	13		20 Oct.
	15		22 Oct.
	24		27 Oct.
	13008		27 Sept.
	13018		13 Oct.
P. punctatum	10		29 Sept.
	20		27 Oct.
P. scandens	8		29 Sept.

* Location is Lyon County, Kansas, unless stated otherwise.

saturated with the test solution or distilled water. The petri dishes were affixed with rubber dish seals and then placed in a Sherer-Gillett growth chamber set on a 16-hr light period (27 C) and an 8-hr dark period (16 C). Initial tests consisting of ten seeds each were made and those which indicated stimulated germination were repeated in quadruplet using 30 seeds per plate. Seeds were considered germinated only upon positive geotropic response by the radicle as "false germination" was common. Data from only those tests indicating increased germination were presented.

Permeability Tests

Water permeability of intact seed of P. biennis was compared to permeability of seed scarified by cracking the pericarp. Duplicate samples each of 30 intact or cracked seeds were submerged in distilled water at room temperature and at hourly intervals were removed, dried on paper toweling, weighed, and replaced in fresh water.

Inhibition by Polygonum Seed Extract

The presence of a cold temperature-labile inhibitor in the seed of Polygonum was tested for by observing the effect of seed extract upon germination of psyllium (Plantago lanceolata L.) seed. Seed extracts of P. biennis, P. pensylvanicum, and P. scandens were made by grinding 2 g of seed with 50 ml of distilled water in a Waring

blender, filtering, and placing one-half of each either at 4 C or room temperature for 48 hr. Quadruplet batches of 50 psyllium seeds were planted in petri dishes containing filter paper disks saturated with 5 ml of one of the extract halves or distilled water and then affixed with rubber dish seals. These were placed in a Sherer-Gillett growth chamber set on a 16-hr light period (27 C) and an 8-hr dark period (16 C) and germination counts were made from the fifth through the ninth day after planting.

RESULTS AND DISCUSSION

Dormancy in seeds of Polygonum appeared to be, at least in part, dependent upon seed structure. Anatomically the five species of Polygonum seed studied were quite similar. The fruit (achene), referred to here as the seed, consisted of a pericarp, integuments, the remains of a nucellus, an aleurone layer, a starchy endosperm, and an embryo (Fig. 1). The aleurone layer, located in the outer part of the endosperm (Justice, 1941), completely surrounded the endosperm and embryo. The nucellus consisted of a mass of crushed cells at the base of the seed. Each of the species studied exhibited two integuments. The embryo was pressed into one angle of the seed and curved in much the same shape as the surrounding integuments and pericarp, and was oriented within the seed with its radicle tip at the seed's apex.

Water Permeability

Cracked seed of P. biconne absorbed water at a faster rate and in greater amount than did intact seed of this species. The cracked seeds initially absorbed water an average of approximately 0.8 mg per seed per hr as compared to 0.4 mg per seed per hr by intact seeds (Fig. 2). At the end of the 37 hr test period, cracked seeds had gained approximately 1.6 mg per seed and intact seeds, approximately 1.4 mg per seed. The t-test showed this difference to be significant at the 5% confidence level.

FIGURE 1. Achenes of Polygonum
A to B, P. lapathifolium (intact and longitudinal section); C to E, P. pensylvanicum (intact, longitudinal section and intact apical view); F to H, P. bicorne (intact, longitudinal section and intact apical view); I to J, P. punctatum (intact and longitudinal section); K to L, P. scandens (intact and longitudinal section). a, pericarp; b, embryo; c, endosperm; d, integuments. Because all species had similar anatomical structure, only P. scandens was labeled.

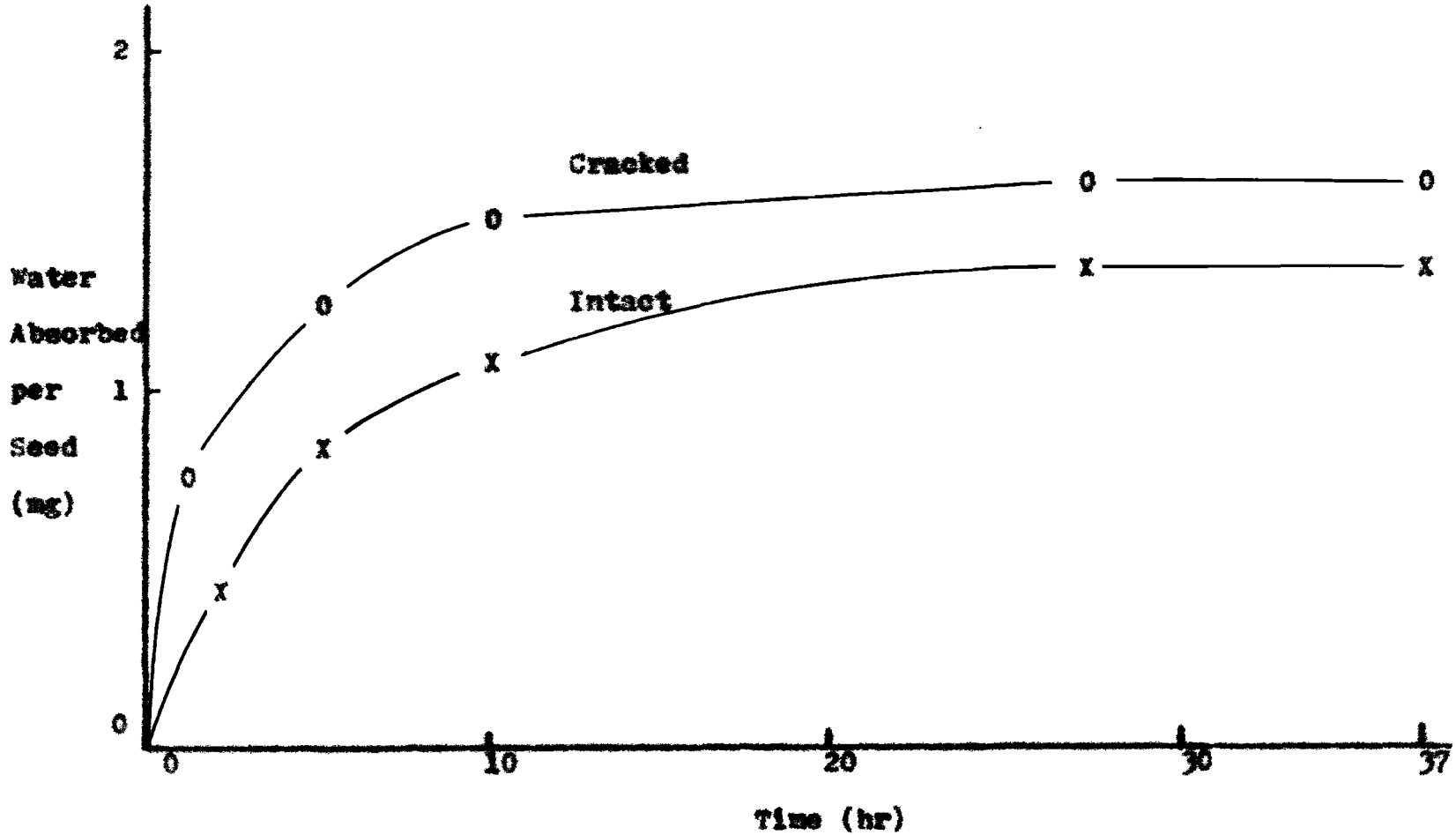

FIGURE 2. Water absorption rates of cracked and intact P. bicorne seed. (Values are averages of 60 seeds.)

Neither the intact nor the cracked seeds germinated following water absorption, thus the difference in water absorption, though statistically significant, was not the determining factor of germination. It seemed likely that the pericarp mechanically restricted radicle protrusion subsequent to water imbibition. In this sense, water imbibition may be "limited" by the pericarp and integuments. If this were the case, removal of the pericarp covering the radicle tip should allow protrusion and subsequent germination.

Scarification by De-apexing

De-apexing of Polygonum seed effectively removed the barrier to the radicle and increased germination (Table II), thus substantiating the theory of mechanical restriction of embryo growth by the pericarp. De-apexing of the seed resulted in 80 and 86% germination in P. punctatum and P. lapathifolium respectively. Both P. biaorne and P. pensylvanicum seeds exhibited 20% germination after de-apexing and had exhibited no germination prior to de-apexing. P. scandens did not germinate following de-apexing.

TABLE II. Germination of 3-month old seed of the 1967 harvest as effected by de-apexing.

Species	Germination (%)
P. lapathifolium	86
P. bicorne	20
P. pensylvanicum	20
P. punctatum	80
P. scandens	0

If the pericarp were the only barrier to germination, high percentages of germination would be expected as a result of de-apexing. This did occur in *P. punctatum* (*P. lapathifolium* did not exhibit a high degree of dormancy prior to de-apexing). Seeds of *P. bicorne*, *P. pensylvanicum*, and *P. scandens*, however, appeared to possess additional barrier or barriers to germination. The phenomenon of "false germination" which occurred frequently in *P. scandens* and to a lesser degree in *P. bicorne* and *P. pensylvanicum* suggested that perhaps certain compounds were present which prevented mitotic growth after the radicle extended, or alternatively, that stimulatory compounds had not as yet been synthesized in adequate concentration for germination. It was possible that radicle protrusion was simply the result of water imbibition and little or no actual cell growth had occurred.

Inhibition by Polygonum Seed Extract

Water extract of Polygonum seed was stimulatory rather than inhibitory to psyllium seed germination. Untreated seed extracts of P. biaorna, P. pensylvanicum, and P. scandens effected psyllium seed germination of 89, 96, and 84% respectively, whereas those extracts given a cold treatment effected germination of 85.5, 92.5, and 91% respectively. Psyllium seed germination of 83.5% was obtained in the control plates (Table III).

TABLE III. Germination of psyllium seed as effected by water extract of Polygonum seed.

Extract Species	Treatment	Daily Germination (mean per cent) Day: 4	5	6	7	9
P. biaorna	Cold	2.5	65.0	84.0	84.5	85.5
	None	17.5	81.0	87.0	88.0	89.0
P. pensylvanicum	Cold	12.0	73.0	89.0	90.0	92.5
	None	39.0	80.5	90.0	93.0	96.0
P. scandens	Cold	73.0	86.5	90.5	90.5	91.0
	None	59.0	69.5	76.5	80.0	84.0
Control	None	30.0	74.0	81.5	82.0	83.5

These results may indicate that germination stimulatore rather than inhibitore was the critical factor to germination. Further, P. pensylvanicum (1967 harvest) seed extract was more stimulatory than either P. biaorna or P. scandens (1966 harvest). This may indicate that

storage at room temperature was more conducive to synthesis of germination stimulators than was storage at 4 C, or alternatively, that a diluted concentration of inhibitors caused the stimulation, since it was shown that low concentrations of inhibitors may be stimulatory to seed germination (Evanari, 1949).

Light Requirement

Light was not a requirement for germination of de-apexed Polygonum seed. Stratified, de-apexed seed of P. hispidum and P. pensylvanicum germinated in continuous darkness at 83.3 and 91.6% respectively (Table IV). Germination of unstratified, de-apexed seed of these species (76.6 and 90% respectively) in continuous darkness was not significantly less. Due to the age (10 months) of the seed used, proof of a negative light requirement in fresh Polygonum seed was not to be assumed since light requirements are known to be lost with storage (Niethammer, 1927, and Mayer and Poljakoff-Mayber, 1963).

TABLE IV. Germination of stratified and non-stratified 10-month-old Polygonum seed of the 1967 harvest as effected by continuous darkness.

Treatment	Mean Germination ± SE and Per Cent Germination*	
	P. hispidum	P. pensylvanicum
Stratified (36 hr)	25.0 ± 0.7 (83.3)	27.5 ± 0.6 (91.6)
Non-stratified	23.0 ± 1.1 (76.6)	27.0 ± 1.0 (90.0)

* Per cent germination shown in parentheses.

Ageing and Germination

Germination ability increased during storage at room temperature. P. biporus seed exhibited increased germination percentages due to storage at room temperature from 23.3% at 3 months storage, up to 70.8% at 9 months storage, and P. pensylvanicum seed germination increased from 13.3 to 90% in the same period (Table V).

TABLE V. Per cent germination of Polyporus seed stored dry for 3 and 9 months at room temperature as effected by de-apexing in combination with various periods of stratification at 4 C.

Storage Period	Stratification Period	Per Cent Germination P. biporus	P. pensylvanicus
3 months	None	23.3	13.3
	24 hr	36.6	16.6
	48 hr	46.6	26.6
	1 wk	30.0	36.6
	1 mo	56.6	56.6
9 months	None	70.8	90.0
	24 hr	85.5	91.6
	48 hr	89.1	85.0
	1 wk	88.3	91.6

This phenomenon, after-ripening, has been reported in other dormant seeds as well (Mayer and Poljakoff-Mayber, 1963) and has been considered to be due to certain physiological changes which occurred during storage at room temperature. Certain of these physiological changes in ageing seeds have been studied in detail. Seed content of sugars, amino acids, inorganic phosphate,

starch, and insoluble protein were shown to vary with age in certain species of clover and rye (Ching and Schoolcraft, 1968). Likewise, relative concentrations of germination inhibitors and stimulators in seeds were shown to change during storage (Luckwill, 1952). It was not known exactly what physiological changes occur in Polygonum during storage, but it was probable that such changes occurred resulting in the loss of dormancy. It was interesting to note that seeds stored dry at 4 C remained dormant. This low temperature in dry condition must function in slowing seed metabolism and thus the changes that occurred at room temperature were repressed.

Stratification of De-apexed Seed

Stratification of de-apexed Polygonum seed enhanced germination. Stratification increased germination of 3-month-old de-apexed seed of P. hispidum and P. pensylvanicum from 23.3 to 56.6% and 13.3 to 66.6% respectively, and of 9-month-old de-apexed seed of these species from 70.8 to 88.3% and 90 to 91.6% respectively (Table V). Stratification appeared to yield a more pronounced effect in stimulating germination of 3-month-old seed than of 9-month-old seed. This was likely due to the relative amounts of germination inhibitors and/or stimulators in these two ages of seed. It might be expected that treatment which destroyed or at least altered the effects of an inhibitor or enhanced stimulatory activity, would have exhibited a

less pronounced effect on seeds already possessing the capacity to germinate.

Gibberellic Acid

Gibberellic acid effectively increased germination of de-apexed seed of P. biocrne and P. scandens, but not of de-apexed seed of P. pensylvanicum (Table VI). Interpretation of the data of Table VI is complicated by the use of two seed lots of both P. biocrne and P. pensylvanicum. In both instances, the control and GA concentrations of 750 and 1000 ppm were performed with one seed lot of each species, but GA concentrations of 125, 250, and 500 ppm were used on another seed lot of these species. This change in procedure necessitated separate analysis of the GA effects because different seed lots are known to exhibit variance in germination ability.

TABLE VI. Effect of gibberellic acid on germination of de-apexed Polygonum seed.

GA (ppm)	Mean Germination ± SE and Per Cent Germination* 1966 Harvest P. biocrne	P. scandens	1967 Harvest P. pensylvanicum
0	18.5 ± 2.3 (62)	1.5 ± 0.5 (5)	28.3 ± 0.5 (94)
125	23.0 ± 2.8 (77)	7.8 ± 0.9 (26)	23.0 ± 0.6 (77)
250	25.8 ± 1.6 (86)	3.0 ± 2.0 (10)	21.2 ± 1.8 (71)
500	27.2 ± 0.8 (91)	5.2 ± 0.8 (18)	23.0 ± 1.9 (77)
750	21.2 ± 2.8 (71)	5.5 ± 1.3 (18)	26.2 ± 0.8 (88)
1000	24.0 ± 1.1 (80)	8.0 ± 0.7 (27)	27.2 ± 0.6 (91)

* Per cent germination shown in parentheses.

Gibberellic acid at concentrations of 750 and 1000 ppm increased germination of P. bicorne seed over that of the control (62%) to 71 and 80% respectively. Gibberellic acid concentrations of 125, 250, and 500 ppm induced seed germination of 77, 86, and 91% in P. bicorne. Seeds of P. pensylvanicum germinated 90% in the control plates and 88 and 91% in GA concentrations of 750 and 1000 ppm respectively. Gibberellic acid concentrations of 125, 250 and 500 ppm resulted in P. pensylvanicum seed germination of 77, 71, and 77% respectively. P. scandens seed germinated 5, 26, 10, 18, 18, and 27% as a result of GA treatments of 0, 125, 250, 500, 750, and 1000 ppm concentrations respectively. It was instructive to note the harvest year of each of these species' seeds and recall that seeds harvested in 1966 were kept dry at 4 C, whereas those harvested in 1967 were kept dry at room temperature. Furthermore, seeds kept at room temperature lost their dormancy whereas those stored at 4 C did not seem to lose dormancy. With these facts in mind, re-examination of Table VI related the fact that only seed of the 1966 harvest (P. bicorne and P. scandens) exhibited stimulated germination due to GA treatment. Seed of the 1967 harvest (P. pensylvanicum), which was relatively non-dormant, was not significantly effected by GA. Gibberellic acid was then stimulatory to dormant Polygonum seed but did not enhance germination of seed which had already lost its dormancy. Gibberellin was thought to

function in stimulation of dormant lettuce seed germination by promoting amylase activity which supplies the monosaccharides for embryo respiration (Ikuma and Thimann, 1963). This, or similar action, also seemed to be a possible function of GA in breaking dormancy of Polygonum seed.

Nitrogenous Bases

Nitrogenous bases of adenine, cytosine, and thymine, stimulated seed germination significantly only in P. pensylvanicum (Table VII), but showed essentially no stimulation in either P. biserne or P. scandens.

TABLE VII. Effect of nitrogenous bases on germination of de-apexed Polygonum seed of the 1966 harvest.

Base	ppm	Mean Germination ± SE and Per Cent Germination*		
		P. biserne	P. pensylvanicum	P. scandens
Adenine	0	22.2 ± 0.5 (74)	10.8 ± 2.1 (36)	0.0
	10	22.8 ± 2.7 (76)	20.2 ± 1.0 (68)	
	100	24.2 ± 1.0 (81)	25.0 ± 1.9 (83)	
	1000	0.0	0.0	0.0
Cytosine	0	10.2 ± 1.0 (34)	10.8 ± 2.1 (36)	0.0
	10	14.0 ± 1.4 (47)	18.0 ± 0.8 (60)	
	100	13.8 ± 3.5 (46)	16.5 ± 0.9 (55)	
	1000	10.2 ± 1.7 (34)	19.0 ± 0.7 (63)	0.8 ± 0.8
Thymine	0	10.2 ± 1.0 (34)	10.8 ± 2.1 (36)	0.0
	10	9.0 ± 1.0 (30)	15.8 ± 1.0 (52)	
	100	9.0 ± 1.4 (30)	14.8 ± 2.1 (49)	
	1000	11.5 ± 1.8 (38)	18.0 ± 1.5 (60)	0.0

* Per cent germination shown in parentheses.

Adenine at concentrations of 10 and 100 ppm effected seed germination in P. bicorne of 76 and 81% respectively. Although these results were slightly higher than the control (74%), the difference in mean germination was not significant at the 5% confidence level. P. scandens seed was tested only at concentrations of 0 and 1000 ppm adenine and there was no germination in either instance. P. pensylvanicum, on the other hand, exhibited significant increases in mean germination at both 10 and 100 ppm adenine (68 and 83% respectively) over germination of the control (36%). Adenine at 1000 ppm stimulated initial germination in both P. bicorne and P. pensylvanicum, but inhibited lateral root growth and subsequent seedling development. Since this concentration was toxic to growth, this result was interpreted as no germination. This procedure did not relate the germination stimulating activity of this adenine concentration however, and further, it was possible that normal development would have occurred if the seeds had been removed from this concentration.

Cytosine at concentrations of 10 and 100 ppm stimulated seed germination in P. bicorne of 47 and 46% respectively, whereas cytosine at 1000 ppm and the control both resulted in seed germination in this species of 34%. P. pensylvanicum seed germinated 36% in the control whereas concentrations of 10, 100, and 1000 ppm cytosine

stimulated seed germination in this species of 60, [illegible] 63% respectively. P. scandens seed was only slightly stimulated (2%) at 1000 ppm cytosine concentration, [illegible] was not significant at the 5% level.

Thymine at concentrations of 10, 100, and 1000 ppm stimulated seed germination in P. bicarne of 30, [illegible] 38% respectively as compared to 3% in the control. P. pensylvanicum seed germination varied from 36% in the control to 52, 49, and 60% in concentrations of 10, 100, and 1000 ppm thymine respectively. Although all concentrations of thymine tested increased germination percentages in P. pensylvanicum over that of the control, only concentrations of 10 and 1000 ppm increased mean germination significantly at the 5% level. Thymine did not stimulate seed germination in P. scandens.

A possible explanation for the stimulatory action of nitrogenous bases was based upon the theory of the necessity for a series of hydrolytic enzymes to become activated or synthesized. Such hydrolysis of carbohydrates, proteins, and lipids may have provided specific monomers which were required before synthetic processes could take place resulting in germination (Amen, 1968). These exogenous nitrogen bases might serve to initiate or by-pass the hydrolytic enzyme systems which naturally form these compounds for utilization in subsequent synthetic processes.

SUMMARY

Seed of Polygonum bicorne, P. pensylvanicum, P. punctatum, and P. scandens possessed dormancy, whereas that of P. lapathifolium germinated easily in all tests. Seed dormancy in P. punctatum was apparently due to the presence of a hard pericarp which mechanically restricted growth of the embryonic tissues. It was theorised that dormancy in P. bicorne and P. pensylvanicum was due to inhibitors which repressed hydrolytic enzyme action within the seed and/or the necessity for seed germinating stimulatory compounds as well as a piercing restriction.

De-apexing was found to effectively remove the mechanical restriction imposed by the pericarp and several methods were found which acted to overcome the dormancy thought to be caused by relative concentrations of inhibitors and/or stimulatory compounds. Seed germination of these dormant Polygonum species increased with the length of dry storage at room temperature over a 9 month period. Seed metabolism at this temperature was considered to alter the inhibitory nature of compounds which promoted dormancy and/or to initiate production of stimulatory compounds. Stratification at 4 C also functioned to alleviate the dormant state in these seeds. Treatment of de-apexed dormant Polygonum seed with solutions of gibberellic acid or the nitrogenous bases

adenine, cytosine, or thymine likewise resulted in increased germination. Gibberellic acid was considered to function in stimulation of hydrolytic enzyme systems within the seed which provided specific monomers required for embryo respiration and growth. Supplying the dormant seed with nitrogenous bases was suspected to have stimulated hydrolytic enzyme action, or alternatively, to have by-passed the need for enzymatic hydrolysis which supplied these compounds to the embryo.

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