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Changes in the germination capacity of three Polygonum species following low temperature moist storage

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SUMMARY

Changes in germination capacity of Polygonum aviculare L., P. convolvulus L. and P. persicaria L. were studied following wet storage at 4°C for one to five months, and after subsequent periods of dry storage for up to three months at two temperatures (4° and 25°C). Germination capacity of P. aviculare steadily increased during five months' moist storage. However, the germination capacity of P. convolvulus declined after two or more months' storage, while P. persicaria showed little change. Subsequent dry storage of the seeds caused a decline in germination in all species which was most severe at the higher temperature. On the basis of limited tests on seeds of P. persicaria, the decline in germination was thought to be largely due to a loss of viability, but with some seeds acquiring a secondary dormancy. The essential conditions for "after-ripening" of seed of P. persicaria were confirmed as being low temperature (4°C constant) in the presence of moisture. Only fifteen days of such treatment was needed to after-ripen P. persicaria.

INTRODUCTION

Four annual Polygonum species are of special importance in the U.K. as arable weeds (Anon., 1962). These are P. aviculare L. (knotgrass), P. convolvulus L. (black bindweed), P. lapathifolium L. (pale persicaria) and P. persicaria L. (redshank). All of these species produce seeds (dispersed as nuts) which are dormant initially and which generally require a period of after-ripening before they will germinate (Justice, 1941). The after-ripening requirement can be satisfied by a period of low-temperature moist storage (stratification) which is provided naturally in the soil by winter conditions. The requirement for after-ripening varies not only between species, but also intraspecifically (Hammerton, 1964, 1967a, 1967b). Of the four species, P. lapathifolium probably shows the greatest variation in this respect for some samples completely lack a chilling requirement (Timson, 1965; Hammerton, 1967a). P. lapathifolium therefore does not present a problem to the herbicide agronomist, who wishes to cultivate the weed for experimental purposes, for completely non-dormant strains are available.

The remaining three species all require some degree of moist chilling, although germination of P. convolvulus in particular can be induced by filing the edge of the pericarp or by treatment with conc. H_2SO_4 (Justice, 1941). Acid treatment has also been effective on P. persicaria (Justice, 1941; Steinbauer and Grigsby, 1959; Timson 1965) and on P. aviculare (Justice, 1941; Courtney, 1968), but this treatment involves the risk of damaging the embryo.

In addition to innate dormancy, an acquired or induced dormancy may also prevent germination. Courtney (1968) found that high temperatures in combination with high levels of CO_2 induce dormancy in P. aviculare. The effect of low temperature and moist conditions in breaking dormancy,

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and high temperature in re-imposing it, is consistent with the cyclic pattern of germination found to occur in the field.

Stratification would seem to be the most effective and useful means of inducing germination of these species in the glasshouse, but because of the variation in response previously mentioned, it appears necessary to treat each strain of seed as having an individual requirement. The object of the work described here was: (1) to determine the stratification requirements of seed batches that were to be used to raise seedlings for experiments with herbicides and (2) to determine the tolerance of after-ripened seed to subsequent dry storage at several temperatures, and hence to define the conditions under which treated seed could be stored without detriment to germination capacity.

MATERIALS AND METHODS

Nuts (hereafter called seeds) of P. aviculare and P. persicaria were collected in summer 1967, the former from a field at Wytham near Oxford, and the latter from pot-grown plants raised in the glasshouse at the Weed Research Organization (WRO). The P. convolvulus seed used was collected in 1964 from plants in pots at WRO. Before use all seeds were cleaned and stored in paper bags and submerged in distilled water at a temperature of 4°C commencing in mid-December 1968. At intervals one bag of each species was withdrawn and the seeds dried on filter paper. Sub-samples of the seeds were then:

a) tested immediately for germination at 25°C (after drying for 1-2 h at room temp.);

b) placed dry in the refrigerator at 4°C;

c) placed dry in an incubator at 25°C.

Seed b) and c) was tested for germination at 25°C after one, four and twelve weeks of dry storage.

For germination, seeds were placed on a single filter-paper in 9.0 cm petri dishes, 25 per dish and moistened with distilled water as required. The seeds were kept for 20 days and assessed regularly every 2-3 days. They were counted as germinated once the radicle had emerged. Germination data are presented as the percentage recorded within 10 days (little, if any germination occurred after this period), and each figure is the mean of four replicate dishes.

The viability of seeds of P. convolvulus (as determined by germination following part removal of the pericarp) was found to be approximately 54%. Pericarp injury and use of acid and alternating temperatures failed to increase germination beyond 52% for P. aviculare and 90% for P. persicaria. Attempts to devise a satisfactory technique for estimating seed viability using the Redox indicator TTC (trimethyl tetrazolium chloride) were unsuccessful.

RESULTS

1. Response to moist low-temperature storage and to subsequent dry storage at two temperatures

The effect on germination of storage under water at 4°C, and the effect of subsequent drying and storage of the seed at 4°C and 25°C is shown, for the three species, in Table I. P. persicaria was the most responsive, its chilling requirement being satisfied by one month's wet cold storage. It was also the species least harmed by storage conditions; it had as high a

germination capacity after five months as after one months storage under water. High germination was maintained after drying provided storage continued to be at 4°C.

P. aviculare had a more prolonged stratification requirement and its germination continued to increase steadily through the wet storage period. The best germination of P. convolvulus occurred after one month's cold wet storage and germination decreased with longer storage periods. Dry storage at 25°C decreased germination of all species and germination of P. aviculare and P. convolvulus was also reduced by dry storage at 4°C.

2. Rate of decrease in germination capacity

Following the main experiment reported above, further studies were undertaken to determine more precisely the rate at which germination capacity declines following drying and the termination of cold wet storage. The results for P. aviculare and P. convolvulus (Table II) show that P. convolvulus suffers a rapid loss in capacity to germinate when dried.

The decline in germination of P. persicaria was studied for samples stored for both one and five months under water at 4°C and then subsequently stored dry at 4° and 25°C. The rate of decline was most rapid at 25°C (Table III).

In a subsequent experiment with dry storage at 45°C the germination capacity of chilled seed of P. persicaria decreased rapidly, falling from 52% to 16% in twelve days. Older seed (harvested 1966) with a lower initial viability and giving approximately 40% germination after one month's wet chilling gave only 1% germination after 24 hours at 45°C.

3. Nature of the reduction in germination capacity

The decrease in germination capacity, most marked at high temperatures, could have been due to either an adverse effect on viability or to a re-imposition of dormancy of the seed. If a secondary or acquired dormancy was operative then germination might be obtained by subjecting seed to a further period of low temperature moist storage. Justice (1941) found the seeds of a large number of Polygonum spp., including P. aviculare, to revert to secondary dormancy if stored dry at room temperature. Dormancy was establised as the cause, for germination was obtained when further cold wet storage was given. With P. persicaria, however, Justice found little reduction in germination even after 50 days' dry storage at room temperature (from 90% to 71%), a much less marked decline than that observed in the present study.

Therefore germination tests were made on seed of P. persicaria after second wet chilling periods, utilising some of the treatments in the main experiment. While a proportion of the seed acquired a secondary dormancy and so germinated in response to further cold wet treatment, the rest (ranging from 27% to 77%) was unable to germinate and may be considered largely non-viable (Table IV). Seed stored dry at 4°C was capable of giving a high % germination without further treatment and so little extra germination occurred following a second wet chill. These results are too fragmentary to give a complete picture but they do indicate that both processes of dormancy acquisition and of viability loss occur. In other experiments on P. persicaria where seed was stored dry for up to six months after wet chilling and then given a second chilling period of four weeks, no germination occurred for seeds stored dry at 25°C, while a mean germination of 77% was recorded for seeds which had been maintained at 4°C.

Table I

The percentage germination of *P. aviculare*, *P. convolvulus* and *P. persicaria* after storage under water at 4°C and after subsequent dry storage at 4° and 25°C

Species	Temperature of dry storage	Number of weeks of dry storage	Number of months stored under water at 4°C				
			1	2	3	4	5
P. aviculare	-	0	12	23	34	42	52
	4°C	1	7	5	10	39	35
		4	3	7	14	38	27
		12	7	8	10	15	16
	25°C	1	2	2	2	14	15
		4	3	3	2	6	2
		12	0	0	0	0	0
P. convolvulus	-	0	35	14	3	0	3
	4°C	1	0	1	1	0	0
		4	1	2	0	0	0
		12	2	2	0	1	0
	25°C	1	1	0	0	0	0
		4	0	0	0	0	0
		12	0	0	0	0	0
P. persicaria	-	0	81	83	71	77	78
	4°C	1	71	90	79	64	66
		4	55	74	79	74	68
		12	66	78	70	52	*
	25°C	1	50	63	47	17	38
		4	28	16	14	8	0
		12	0	1	0	0	*

N.B. Germination of untreated seeds (stored dry at room temperature throughout): *P. aviculare*: 0%; *P. convolvulus*: 2%; *P. persicaria*: 0%.

* Seed unavailable for testing

Table II

The decline in germination capacity of P. aviculare and P. convolvulus stored for one month under water at 4°C and subsequently dried

(% germination in 10 days)

Number of days' dry storage	P. aviculare		P. convolvulus	
	Temperature of dry storage			
	4°C	25°C	4°C	25°C
1	23	12	30	3
2	15	7	3	1
3	13	6	0	0
5	12	10	0	0
7	7	1	0	0
11	8	11	0	0
15	10	1	-	-

Table III

The decline in germination capacity of P. persicaria stored for one and five months under water at 4°C and subsequently dried

(% germination in 10 days)

a) One month's wet storage

Number of days dry storage		2	3	4	7	14	28	56
Temperature of dry storage	4°C	-	62	-	45	60	49	44
	25°C	51	-	42	33	35	20	0

b) Five months' wet storage

Number of days dry storage		7	14	21	28	35	42
Temperature of dry storage	4°C	66	66	70	68	60	45
	25°C	38	11	8	0	-	-

Similar tests on P. aviculare and P. convolvulus failed to induce more than a few seeds to germinate. Puncturing of the pericarp of all three species also proved ineffective in stimulating germination of chilled and dried seeds although control seeds of P. convolvulus germinated after such treatment.

Table IV

The percentage germination of P. persicaria following a second period of low temperature wet storage for one month

Number of months of initial wet storage	% germination after first wet storage	Number of weeks of dry storage prior to second wet storage period	% germination after dry storage		% increase in germination after second wet storage period		Total % germination	
			Temperature during dry storage (°C)					
			4°	25°	4°	25°	4°	25°
3	71	12	70	0	4	11	74	11
4	77	1	64	8	0	0	64	8
		12	52	0	26*	0	78	0
5	78	1	66	38	2	13	68	51
		2	66	11	1	11	67	22

* 16% germination occurred while in cold store

4. Conditions required for "after-ripening"

Timson (1965a) has confirmed that for breaking dormancy by low temperature storage the seeds of Polygonum spp. must be moist. Justice (1941) found water alone to be more effective with P. persicaria than moist cotton, paper towels or peat. The results of an experiment comparing seeds stored dry, submerged in water and stored between moist filter papers (Table V) show little difference between the two wet storage methods.

Table V

The percentage germination of P. persicaria after storage for three methods at 4°C

	Number of weeks storage	
	4	5
seeds dry	0	1
seeds between filter paper	64	67
seeds under water	59	63

5. Minimum length of wet low temperature storage required

In further experiments using shorter wet cold storage periods P. persicaria gave a high germination after fifteen days while P. convolvulus continued to give an increase in germination throughout the thirty day period (Table VI). As already seen P. aviculare continues to respond to up to five months' wet storage.

Table VI

The increase in germination capacity during thirty days storage under water at 4°C of P. convolvulus and P. persicaria

Number of days' storage	% germination	
	P. convolvulus	P. persicaria
0	0	0
5	0	13
10	0	36
15	4	64
20	4	57
25	7	68
30	16	70

The low germination obtained for P. convolvulus in this experiment is probably due to the decline in viability which had occurred during the period in which the work was undertaken. Such a decline has also been recorded by Chancellor (1968).

6. Changes in germination capacity immediately following low temperature moist storage

When testing seed of P. persicaria to ascertain the rate at which germination capacity is lost during dry storage, fluctuations in percentage germination are noticeable shortly after ending the cold wet treatment and drying the seed as appropriate. (All tests in the experiment described above (page 2) were commenced after the seed had been allowed to dry at room temperatures for approximately two hours). When seed was tested without drying, or as soon as it became dry on exposure on filter paper at room temperature, germination was lower than when samples were taken after 2 - 3 days' drying at room temperature. (Table VII). The differences were in the order of 10% and were not statistically significant, and may have arisen due to sampling error. Alternatively such fluctuations might be biologically significant and if so are of particular importance, since they indicate one way in which data of the effects of cold wet storage on seeds might be dependent on small details of the technique used.

Table VII

Short-term changes in percentage germination of P. persicaria following low-temperature moist storage for one month

% germination		before drying = 42%; immediately on drying = 41%				
Storage temperature of seeds after drying at room temperature	4°C	Storage time (days)	3	7	14	28
		% germination	62	45	60	49
	25°C	Storage time (days)	2	4	7	14
		% germination	51	42	33	35
	45°C	Storage time (days)	0.5	1	2	3
		% germination	52	44	43	47

DISCUSSION AND CONCLUSIONS

The main aim of the investigation has been to gather information of practical use for other types of experimental work at WRO. The main conclusions to be drawn from the results are:

1. The length of cold wet storage necessary to break dormancy of seeds of the three species studied appears to be at least five months for P. aviculare, one month for P. convolvulus and fifteen days for P. persicaria;

2. P. aviculare and P. persicaria seeds can both be stored under water at 4°C without detriment for as long as five months;

3. Subsequent drying of the seed and storage, even at the same temperature as that at which the seed was chilled wet, results in a decrease in germination capacity most marked with P. convolvulus and least marked with P. persicaria. At 25°C the decrease in germination capacity is accelerated.

As mentioned previously, seeds of Polygonum species are known to vary with regard to the length of wet chilling required to break dormancy. Thus the results obtained here differ from previous findings (Table VIII), due to differences in origin, environmental factors during growth and seed maturation, harvesting etc. A further factor in the case of P. convolvulus is that seed used in this study was collected in 1964 and therefore had declined in viability and possibly in sensitivity to wet - cold induced changes. The P. aviculare seed used was also of rather low viability.

The concept of optimal length of stratification time cannot be applied with P. persicaria which shows no decline in germination capacity with prolonged storage, unlike P. convolvulus which Timson (1966) found to have quite a distinct optimum of 17 weeks. Conversely P. aviculare retains its peak germination capacity for a period of at least 9 - 12 weeks (Hammerton, 1964).

The determination of the main effect of drying the seed, viz: viability loss or dormancy re-imposition is made difficult by the lack of a reliable technique for estimating seed viability in these species. Seeds not responding to a second period of low temperature wet storage have here been categorised as non-viable, but it is conceivable that they may have entered a state of dormancy unaffected by stratification. That the rate of reduction of germination on drying is increased with temperature does, however, suggest that viability loss is the main process. Effects of low temperature pre-treatment are not always reversed by subsequent drying.

Table VIII

The percentage germination of the three Polygonum spp. obtained by various authorities after stated periods of cold wet storage at 2 - 4°C

Authority	Species studied	Number of weeks' wet storage	Germination	Notes
Courtney, 1968	P. aviculare	4 8 14 18 20	approx. 18 approx. 58 approx. 79 approx. 78 approx. 78*	*% germination at 4°; i.e. during the period of cold treatment
Hammerton, 1964 Hammerton, 1966	P. aviculare P. persicaria	3 9 3 6	approx. 80 approx. 95 approx. 70 approx. 80	Data for seed originating from Oxford populations
Justice, 1941	P. aviculare P. convolvulus P. persicaria	15 8 12	93 75* approx. 80	* seed stored moist between cotton
Steinbauer and Grigsby, 1959	P. persicaria	1	37	Seed germinated using alternating temperature and 0.2% KNO_3
Timson, 1965a Timson, 1966	P. persicaria P. convolvulus	11 17	>65 >55	Figures are derived from cumulative data
Present Study	P. aviculare P. convolvulus P. persicaria	22 4 4	52 35 81	-

Borthwick and Robbins (1928a) found the stimulation due to such treatment to be retained through long periods of dry storage of lettuce. The effects of drying in relation to such differences are worthy of further study.

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