Table 1. Percentage (mean ± standard deviation) of slowly dissociating component in anti-BSA sera of various types 6, 8, and 15 days after the last injection. Means were derived from five to eight individual animals. The injection sequence is indicated by italic type; sera indicated were absorbed with HSA.

6 days	8 days	15 days
BSA, unabsorbed		
14.9 ± 4.8	18.4 ± 4.8	20.1 ± 4.0
BSA-BSA, unabsorbed		
55.7 ± 9.0	58.1 ± 9.4	58.8 ± 9.8
*BSA-BSA, absorbed**		
53.9 ± 13.4†	57.0 ± 9.9†	Not done
HSA-BSA, unabsorbed		
44.1 ± 7.5‡	45.6 ± 12.0‡	50.3 ± 9.9†
HSA-BSA, absorbed		
15.7 ± 5.4§	17.3 ± 5.5§	26.3 ± 6.8§

* This control indicates that absorption did not affect the results nonspecifically. Statistical analysis (99 percent confidence interval) was carried out by the method of Duncan (*8*). † Not different from secondary value. ‡ Different from both primary and secondary values. § Not different from primary value.

45 percent of an I^{131}-labeled albumin (*7*) preparation (0.16 μg of nitrogen per milliliter) was mixed with the albumin solution and incubated at 37°C. After the mixture had reached equilibrium (30 minutes was sufficient), a 2000-fold excess of unlabeled albumin was added. The amount of I^{131}-albumin remaining bound to antibody was determined at intervals of 1, 2.5, 5, 10, and 20 minutes by stopping the reaction with ammonium sulfate added to 50-percent saturation. This concentration of ammonium sulfate precipitates albumin-antibody complexes and leaves unbound albumin in the supernatant. The activity of the precipitates was determined in a well-type scintillation counter, and appropriate corrections were made for nonspecific binding to normal serum. The results were plotted on a semilogarithmic scale as the percentage of the bound antigen that had been formed in a sample taken before the addition of excess unlabeled antigen (Fig. 1).

All antisera, regardless of origin, yield dissociation curves (Fig. 1) which can be treated as summations of two independently dissociating components. By applying the methods used in calculating half-lives of mixtures of two radioactive isotopes with different decay constants, mean half-lives of approximately 1 minute for the rapidly dissociating component and 50 minutes for the slowly dissociating component were calculated for all sera tested. Statistical analysis (*8*) revealed no differences in half-lives of either component in any type of antiserum; therefore the only difference among the various types of antisera was change in the ratio of the two components. Thus we were able to express our results as the percentage composition of either component in any given antiserum. All results are percentages of the slowly dissociating component present at zero time. All anti-BSA determinations are given in Table 1; anti-HSA responses were only analyzed graphically because fewer animals were involved. No obvious differences in response pattern were observed between the two antigens.

The data show that antibodies *specific* for the second antigen of a sequence of related antigens are of primary quality and that no maturation of the immune response to the second antigen has occurred. Further, the increased percentage of the slowly dissociating component in the total response was demonstrated, by the absorption technique, to be a result of antibodies of secondary-response quality produced against the cross-reacting determinants of the two antigens. Based on the results with chemically defined cross-reacting antigens (*4*) we expect these antibodies to have their greatest affinity for the first antigen. Little can be said about the reappearance of antibodies *specific* for the first antigen (*3*, *5*) because just before the second antigen injections antibodies of approximately secondary quality were found in the serum (three samples averaged 48 percent slow component).

Considering the overall result, there is an interesting dichotomy in response to cross-reacting and unique determinants of the second antigen; the unique (specific) determinants stimulate primary quality antibody while the cross-reacting determinants stimulate secondary quality antibody (*9*).

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Germination of Melampyrum lineare: Interrelated Effects of Afterripening and Gibberellic Acid

Abstract. *Seed germination of* Melampyrum lineare *proceeds only after activation followed by an extended period of chilling. Up to one-third of seeds can be activated by storage at room temperature, while all seeds are activated if treated with gibberellic acid. Chilling before activation is ineffective.*

Melampyrum lineare Desr. is an annual, chlorophyllous angiosperm which forms root connections with various other vascular plant species in its habitat. Studies of the life cycle and host-parasite relationship have been reported elsewhere (*1*), and indicate that for normal growth *M. lineare* must form connections with other species. In attempting to germinate *Melampyrum* seeds we have encountered a complex germination pattern, involving an apparently unreported relation between gibberellic acid and afterripening.

Various batches of seed were collected from natural populations growing in a jack pine forest near Grayling, Michigan, during the late summer and fall of 1960, 1961, and 1962. After harvesting, the seeds remained viable [as tested with tetrazolium chloride (*2*)] if stored turgid on moist blotters, either at room temperature (19° to 24°C) or in the cold (about 3°C). Seeds were found to require a period of chilling (moist storage at 3° ± 1°C) for germination, and remained dormant if stored at room temperature. This dormancy is complex, involving both a radicle- and a separate epicotyl-dormancy. Longer cold treatment is necessary to overcome epicotyl dormancy than to cause the radicle to emerge. In the present report "germination" is

considered to be emergence of the radicle, and subsequent remarks will refer to this aspect of dormancy.

Cold storage immediately following harvest resulted in widely varying germination percentages in different seed batches, but in all cases less than 35 percent was obtained. Seeds germinated in the cold (3°C) and removal of any batch to room temperature caused germination to cease. In all trials germination started only after a period of 40 to 80 days in the cold, and stopped after 80 to 100 days. The remaining ungerminated seeds, though viable as tested with tetrazolium chloride, have continued dormant in the cold, some for as long as 2 years.

Storage at room temperature, after harvest, resulted in increased germination when seeds were subsequently chilled. Figure 1 (broken-line curves) shows the effect on a batch of seed (which gave 6 percent germination when chilled immediately) of differing lengths of time at room temperature prior to chilling. In general (*3*), longer periods at room temperature up to 28 days produced higher germination percentages. As with cold treatment immediately after harvest, germination started only after an extended period (about 40 days) in the cold and stopped after about 80 days.

At present it appears that no combination of room temperature preconditioning and cold storage will result in germination of more than about 35 percent, at least during the first year after harvest. Seed batches which had more than the above 6 percent germination (Fig. 1) when samples were chilled immediately after harvest, showed less increase in germination when samples were preconditioned prior to chilling. Room-temperature preconditioning after a period of chilling, if also followed by chilling, had the same effect on germination as preconditioning immediately after harvest.

Differences in germination between batches when samples are chilled immediately after harvest are thought to reflect differences in the preharvest history of the seed, perhaps differences in preconditioning on the plant. In the field these differences are presumably made up between seed set and winter. Spring field counts indicate that on the average less than 40 percent of the seeds in the forest litter normally germinate each year.

The high counts of dormant seed in normal field populations confirmed our laboratory data and led us to investigate further the dormancy mechanism. Various unsuccessful manipulations (alternating temperatures, light effects, leaching, and treating with nitrate, thiouria, and host-root extracts) preceded the successful use of gibberellic acid (GA).

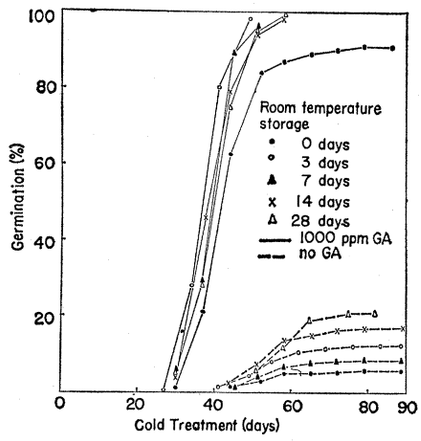

Fig. 1. Germination of *Melampyrum lineare* seed in cold storage, with and without gibberellic acid treatment. Effect of varying periods of room temperature treatment immediately after harvest and before cold storage. Each curve represents three replicates of 100 seeds each. Seed batch collected 13 September 1962.

Gibberellic acid has been widely used during the past decade as a plant-growth promoter which has powerful effects on many stages of plant growth, including seed germination. In most of the reported examples of the action of GA on germination, the effect is to substitute for an apparent requirement for light, for example in lettuce (*4*) and tobacco (*5*), while in other cases GA substitutes for cold treatment [peach (*6*)] and for afterripening at 37°C [sweet cherry (*7*)].

Melampyrum seeds were treated immediately after harvest with GA (*8*) by soaking for 24 hours at room temperature in a solution of 1000 parts of GA per million (1000 ppm). The seeds were then transferred to petri dishes containing blotters moistened with the same solution. Treated seeds maintained at room temperature did not germinate, but those transferred to the cold after the 24-hour treatment germinated completely (greater than 95 percent). Figure 1 (solid-line curves) shows the effect of GA combined with varying periods (up to 28 days) of room temperature storage after treatment and before transfer to the cold. All treatments resulted in germination of greater than 95 percent. However, in the samples with no room temperature treatment (that is, GA applied in the cold) germination was slightly less. The pattern of germination was the same as that observed in germination without GA (Fig. 1, broken-line curves) in that an extended period of chilling was required before germination started. However, GA had the effect of shortening this period of cold-incubation by approximately one quarter (about 30 days in the case of GA-treated seeds compared to about 40 days for those without the promoter).

In another set of experiments the GA treatment was made at room temperature after seeds had been stored 150 days in the cold. Complete germination resulted and again the process began only after a period of about 30 days of additional chilling after treatment with GA.

To observe the effects of GA concentration and length of application time, we used seeds from the ungerminated remainder (about 95 percent) of a batch which had been subjected to 120 days of continuous chilling after harvest. Treatments with solutions of 25, 100, 500, and 1000 ppm of GA were made for 12 or 24 hours at room temperature. In half of the 24-hour treatments GA was left in contact with the seeds, while the remaining 24-hour-treated samples and all of the 12-hour-treated ones were washed with running water for 5 minutes after treatment. Then, replicates of each sample were stored, one in the cold (after a total time of 24 hours at room temperature),

Table 1. Effect of gibberellic acid concentration and duration of application on germination of *Melampyrum lineare* seed. The seed batch was collected 19 September 1961 and then cold-stored (3°C) continuously (120 days) prior to the experiment, which was conducted on the ungerminated remainder (about 95 percent) of the original batch. Figures are final germination percentages for 100-seed samples.

GA (ppm)	Germination (%) when seed were stored under two different conditions after GA application		
	12 hr GA	24 hr GA	Continuous GA
Stored at room temperature			
25	0	0	0
100	0	0	0
500	0	0	0
1000	0	0	0
Stored at 3°C, except for 24 hours at room temperature			
25	5	5	23
100	23	17	46
500	53	44	77
1000	77	65	89

and one at room temperature. Results, shown in Table 1, indicate that cold is required for GA-triggering of germination over the range studied. The time course of germination was in all cases similar to that observed in previous experiments, involving 1000 parts per million of GA in continuous contact with seeds. The percentages of germination were approximately proportional to concentration of GA over the range studied.

The above-described experiment shows that the lag in germination after GA treatment is not due to difficulty in penetration of the seed testa by growth promoter. Seeds which received only a 12- or 24-hour application of GA, followed by thorough washing, started and stopped germination after the same amount of cold storage as those in continuous contact with the promoter. Thus it seems that in *Melampyrum lineare* all germination is associated with a final cold process, whether germination is initiated (i) with GA, or (ii) in its absence (for a percentage of the seeds) by preconditioning on the plant or forest floor, or at room temperature in the laboratory. Chilling before initiation cannot replace this post-activation cold process. A search of the literature indicates that such a germination pattern has not been noted previously.

In the field up to about 40 percent of the seeds present in the forest floor germinate by late spring. The remainder lie viable but dormant and are apparently subject to heavy depradation by the biota of the forest floor. Nevertheless, it seems likely that the field germination counts in late spring include some seeds from more than the preceding year's crop. In the laboratory, dormancy in the ungerminated seeds can be broken by GA, although a natural agency has not been found. Preliminary experiments suggest that a light process is not involved. Possible natural activators include short periods of seed-exposure to desiccation or to elevated temperatures, or a relatively long exposure to a sequence of temperatures corresponding to the seasonal changes of temperature in the field. Also, germination promoters might be present in host-root exudates or their natural decomposition products. These possibilities are being investigated (*9*).

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Temperature Dependence of Wing Abnormality in Tribolium confusum

Abstract. *When* Tribolium confusum *pupae were exposed continuously to temperatures from 10°C to 40°C, the adults which finally emerged exhibited wing abnormalities typical of those obtained with x-irradiation. The data indicate the implication of two processes. Two mathematical models are suggested to account for this phenomenon.*

Pupae of *Tribolium confusum* Duval were harvested within 30 hours of formation and incubated in the dark at temperatures from 10°C to 40°C. The particular wing abnormalities were indistinguishable from kinds seen when pupae were irradiated with a variety of accelerated atomic nuclei (*1*) or x-rays (*2*). These abnormalities are uniform but exhibit varying degrees of intensity; the membranous wing is displaced with irregular warping of the elytra. Continuous incubation at 10°C prevented eclosion for periods up to at least one month, while the higher temperature entirely inactivated the system and resulted in the death of all of the organisms within 7 days. Minimal effects were observed between 30°C and 35°C.

Table 1. Abnormalities (percentage) as a function of temperature.

(deg. C)	X	
	Exptl.	Calc.
20	27.1	26.9
25	5.2	5.88
30	1.6	1.46
35	1.6	1.60
38	5.3	4.41
39	5.8	6.47

At intermediate temperatures adults emerged with varying wing abnormalities. When the percentages of abnormalities were plotted against temperature, the resulting curve (Fig. 1) had two branches. It appears that two processes are implicated. The experimental data appear to be best fit by the function

$$X = 1.19 \cdot 10^{4}\, e^{-0.305t} + 1.32 \cdot 10^{-6}\, e^{+0.394t}$$

as shown in Table 1, where t is the temperature in degrees Celsius. Thus we believe that the wing abnormalities are caused by two additive independent processes with each separate process causing a change in the relative effect proportional to the change in temperature. In differential form, the equation given may be written

$$X = X_1 + X_2$$

$$\frac{dX_1}{dt} = -0.305X_1$$

and

$$\frac{dX_2}{dt} = +0.394X_2$$

where $X_1 = 100$ for $t = 15.16$ and $X_2 = 100$ for $t = 46.1$

In a second, more elaborate, model one may suppose that X is a function of two independent quantities X_1 and X_2, where X_1 is the percentage of abnormalities caused by the first process and X_2 is the percentage of abnormalities caused by the second process. The percentage of abnormalities caused then by the second, but not by the first process, is given by $X_2 \cdot (100 - X_1)/100$. Therefore

$$X = X_1 + X_2 \cdot [(100 - X_1)/100]$$

and

$$X = X_1 + X_2 - (X_1X_2/100)$$

The processes responsible for production of abnormality in the second model may also be considered to occur as follows, where two sets of precursors may be in dynamic equilibrium with two distinct end products:

$$nA + B \underset{}{\overset{k_1}{\leftrightarrows}} A_nB$$

$$k_1 = (A_nB)/(A)^n(B)$$

$$(A_nB) = k_1(A)^n(B)$$

and

$$(A_nB) = (A)^n(B) \cdot ae^{(-b/T)}$$