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Effect of Temperature on the Short Chain Fatty Acid-induced Inhibition of Lettuce Seed Germination

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ABSTRACT

In lettuce, *Lactuca sativa* short chain fatty acids (C_6 - C_9) vary in their effectiveness as inhibitors of germination according to temperature; the higher the temperature, the greater the inhibition. A linear relationship exists between temperature and the dose causing half-maximal germination. Nonanoic (C_9) acid is the most effective.

When extrapolated to the abscissa, really zero concentration of acid, the regression lines intercept at 36.8 C ± 2.6 C. It may be that at this temperature the whole membrane becomes fluid without acid being present but with acid there was complete fluidizing at lower temperatures the degree of fluidity being determined by the amount and type of acid present. This speculation distinguishes between the fluidizing of the bulk lipids and the character of the lipid layer that may be around certain key membrane proteins.

Lettuce seed germination is dependent on temperature, there being a value above which there is a requirement for light. The need for this light requirement can be overcome if the seed is treated with GA (4) and/or cytokinin (3). On the other hand the temperature at which the seed becomes light-sensitive can be lowered if they are exposed to coumarin (9) and possibly ABA (5).

Short chain length fatty acids (chain length $C_6 - C_{12}$) are known to inhibit seed germination, and in the case of lettuce to impose a light requirement (1).

Inasmuch as these fatty acids might be expected to affect lipid membranes, their action might vary according to the temperature of application. With an increasing level of low mol wt acid incorporated into the lipid membrane it could be predicted that there would be a change in the temperature at which the membrane undergoes a change in its functional characteristics.

It was this hypothesis that we tested.

MATERIALS AND METHODS

A batch of lettuce seed (*Lactuca sativa* L. cv. Grand Rapids) which became light-requiring at high temperatures was obtained from the Page Seed Co., Greene, N.Y. (Batch No. 014576). At 30 C the germination was less than 1%, at 20 C 16%, and when treated with red light, 369 mJ cm^{-2}, the germination rose to 8 and 90%, respectively.

Seeds were counted into batches of 50 prior to dispensing over a 4.25-cm Whatman No. 3 filter paper lining a 4.5-cm Petri dish base. These seed lots were held at −20 C until required. Prior to dispensing the seed the filter paper was loaded with an amount of acid in ether that would give the desired molar concentration when 1.5 ml of H_2O were added after the ether had evaporated. Zero concentration of acid (control) was treated with solvent. A range of concentrations was chosen to encompass the sharp transition from zero effect to maximum effect of each acid and a number of temperatures from 12 to 28 C selected. The temperatures were held to better than ± 1 C.

In this experiment the seeds were irradiated 2 hr after the start with red light (656 nm, band width 10 nm) obtained by filtering the emitted radiation from a 12-v 100-w quartz halogen lamp through 20 cm of H_2O and an interference filter (Barr & Stroud, Anniesland, Glasgow). Levels of radiations were determined by a Molectron Radiometer.

Seeds were scored as germinated if the radicle had emerged at 72 hr from the onset of imbibition.

RESULTS

Lettuce seeds germinate in the presence of these short chain fatty acids in a characteristic way. This is illustrated in Table 1 with respect to pelargonic acid (C_9-nonanoic acid) at 20 C. There is no effect of the acid at concentrations less than 10^{-3} M whereas the acid is absolutely inhibitory at concentrations of 3×10^{-3} M and above. This steep transition is general for all acids at the temperatures tested.

The higher fatty acids decanoic, undecanoic, and dodecanoic, while inhibitory to germination, are much less so than the $C_6 - C_9$ acids. Within the concentration ranges employed it was not possible to treat them in the manner which follows, and it would appear that the alteration in water solubility which occurs at C_9 (6) is responsible for their lessened activity.

We decided that the best indication of the acid/temperature effect would be to determine that concentration which resulted in half-maximum potential germination at the temperature considered, *i.e.* half of the control value. This would give us the concentration that brought about $G_{0.5}$ and could be called $D_{0.5}$. $D_{0.5}$s could be determined for each acid and then plotted against temperature.

The transition from capacity to germinate fully to nongermination is essentially linear. With sufficient values to give a good curve it is possible to obtain the $D_{0.5}$ concentration by inspection but a better estimate of this concentration can be obtained by either obtaining the linear regression for that part of the curve that conforms to a straight line and determining $\bar{x}$ ($D_{0.5}$) for $\bar{y}$ where $\bar{y}$ is mean germination (what $G_{0.5}$ is essentially), or by using the following expression concentration for half-maximum potential germination ($D_{0.5}$ conc.) =

$$\frac{G_{0.5} - \left(G_2 - \frac{G_2 - G_1}{d_2 - d_1} d_2\right)}{\frac{G_2 - G_1}{d_2 - d_1}}$$

where d_1 = lower dose in molarity; d_2 = higher dose in molarity; G_1 = number germinated at dose d_1; G_2 = number germinated at

Table I. The effect of nonanoic acid on the germination of lettuce seed at 20 C irradiated at 2 hr imbibition with 369 mJ cm^{-2} red light

Concentration of nonanoic acid M	No. germinated/fifty at 72 hr	S.E.
0	45.75 ±	1.32
3.16×10^{-4}	45.25 ±	1.03
5.59×10^{-4}	46.00 ±	0.71
10^{-3}	46.00 ±	0.71
1.77×10^{-3}	15.50 ±	2.10
3.16×10^{-3}	1.00 ±	0.58
5.59×10^{-3}	0 -	-
10^{-2}	0 -	-

dose d_2; $G_{0.5}$ = half-maximum potential germination = $\frac{\text{control value}}{2}$. It will always be the case that $G_1 > G_{0.5} > G_2$ and equal numbers of seeds should be treated. If seed numbers vary percentages can be substituted for observed values of G_1 and G_2.

When the values for $D_{0.5}$s of the acids are plotted against temperature the family of curves obtained is illustrated in Figure 1. The curve for each acid is linear and the correlation coefficients, and linear regressions, for each acid against temperature were obtained. The correlation coefficients were all highly significant and the linear regressions were used to calculate where the curves would intercept the abscissa. The intercepts fell between 36 and 38.7 C with a mean value of 36.8 C (with ± 2.6 C for 95% fiducial limits).

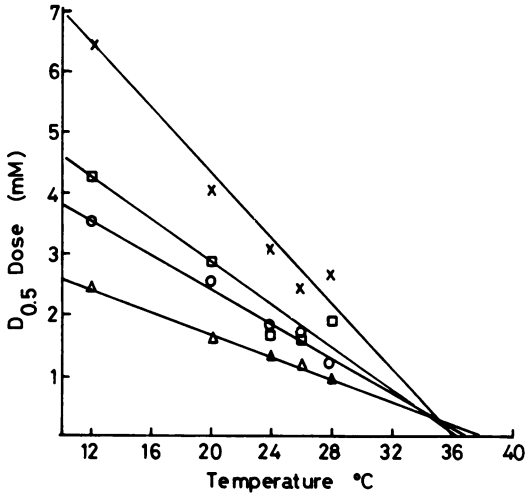

FIG. 1. Dose of short chain fatty acids required to reduce germination to half-maximum value at various temperatures. (x): Hexanoic (C_6) acid, (□): heptanoic (C_7) acid; (○) octanoic (C_8) acid, (△): nonanoic (C_9) acid. Correlation coefficient $C_6 = -0.979$; $C_7 = -0.971$; $C_8 = -0.99$; $C_9 = -0.993$. Regression of y on x. C_6, $y = -0.27x + 9.62$; C_7, $y = -0.18x + 6.56$; C_8, $y = -0.15x + 6.52$; C_9, $y = -0.09x + 3.44$.

DISCUSSION

Nonanoic acid is the most effective of the short chain fatty acids in preventing germination of lettuce. This agrees with previous findings. As the chain length increases or decreases the acid effect becomes less pronounced.

These short chain fatty acids could infiltrate the membrane lipids and change their physical properties. With this change the physiological characteristics of the seed will also change. From this and other work it would seem that as the membrane lipids lose their normal organization the capacity for successful germination is lost (2).

It is necessary to display extreme caution in interpreting these and other bioassay results. Obviously any amphipathic molecule, like these short chain fatty acids, which is present in the medium *ab initio*, could be incorporated into the membrane bilayer in a totally artifactual way. However, this does not invalidate our argument, because such an incorporation appears to increase fluidity with *in vitro* bilayers, and could thus be substituting for the elevated temperature normally required for complete fluidity. The short chain fatty acids could become incorporated between the lipid molecules and would thus reduce the interaction between the neighboring long chain aliphatic "stearate" residues of the normal membrane. The active chain lengths in this study correspond to the number of carbon atoms which do not take part in the "melt" (11). The fluidity due to the short chain fatty acids would thus be manifest as an increase in the mobility of the distal portions of the hydrocarbon chains, increasing the number of gauche configurations in the chains, just as elevated temperature does. This increased fluidity could alter the kinetics of membrane-associated enzymes (*cf.* 10), specific permeability characteristics or some other process required for germination.

Phase transitions in the membranes can be recognized by alterations in permeability (2) or by physical means such as differential scanning calorimetry, fluorescent probes, or spin labels (7). Such observations will apply to the bulk lipids and not to any domain which remains at a different phase when these bulk lipids liquefy. There is evidence for these domains, which could be conceived within the Singer and Nicolson (12) fluid mosaic model for the membrane, from the work of Wunderlich *et al.* (14).

Promoters of germination may stabilize the membrane systems. It has been reported that gibberellins increase membrane fluidity (13); it is likely that, by analogy with cholesterol (8) GA may act as a "fluidity buffer," increasing fluidity below the transition and reducing it above. On the other hand GA may act on specific domains associating with these domains by a binding site which is not part of the lipid bilayer proper. Our fatty acids are likely to be nonspecific with regard to binding sites on the membrane and thus result in substantial alteration phase transition temperature throughtout the membrane bulk lipids and specific domains.

Thus, even if the short chain fatty acids are not involved *in vivo*, their application may mimic the natural control of thermodormancy.

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