Reports

Effect of Electrostatic Field on Freezing of Supercooled Water and Insects

Abstract. Supercooled water and two species of insects froze at higher temperatures than normally when placed in an electrostatic field. The effect became more certain and occurred earlier as the amount of supercooling was increased. The amount of supercooling in the presence of the electrostatic field was not related to the amount in its absence.

Larvae of the wheat stem sawfly, *Cephus cinctus* Nort., when supercooled to −20°C, freeze at once on exposure to the discharge of a spark coil. Under certain conditions they freeze in the presence of the electrostatic field with no discharge. Water drops and larvae of the goldenrod gallfly, *Eurosta solidaginis* (Fitch), react similarly.

The two species of insects have widely different supercooling abilities: *C. cinctus* supercools about 25°C, *E. solidaginis* about 10°. Water was tested either as 20-mm³ hemispheroidal drops placed on 1-in. squares of aluminum foil, or as 50-mm³ drops injected into drops of fresh rubber cement, also on squares of foil. Experimental samples were placed at various constant temperatures on a horizontal metal plate in the vertical temperature gradient of a deep-freeze. The electrostatic field was generated by a Ford Model T spark coil energized by a 4 volt, 60 cy/sec source. One terminal led to the cold plate, the other to a 15-in. wire probe sheathed with glass tubing except for the terminal inch. The probe was held vertically ½ in. above each sample for 5 seconds, under which conditions no sparking occurred and a peak potential difference of about 15,000 volts was obtained.

Preliminary testing revealed that: (i) A spark or discharge is not necessary, though it produces a more certain effect, probably because the probe must be closer in order to discharge. (ii) The sample to be frozen must be on or close to one electrode. With the horizontal metal plate electrode, the samples could be separated from it by one or two thicknesses of paper, an oil film, or up to 0.5 mm of silicone grease. Separation by 2 mm or more of air suppressed the effect completely. Parallel-plate electrodes may be substituted for the point-and-plate pair to give a larger field area. (iii) The effect is directly proportional to the duration of the stimulus; hence probability is a determining factor, as it is in ordinary nucleation.

The effect of temperature on the percentages of larvae and water samples freezing in response to the electrostatic field is shown in Fig. 1. The response in all tests increased with increased supercooling. In addition, freezing was observed to occur earlier in the 5-second exposure period as the temperature was lowered.

Cephus cinctus exhibited a complete response to the stimulus at −10° to −12°C (Fig. 1*A*), with more than 10° of supercooling ability still remaining. Since *Eurosta solidaginis* supercools only to −10° to −12°C, most specimens were frozen at these temperatures before the electrostatic field was turned on, and they are not included in the calculations. That only about half of the remainder were nucleated by the field at these temperatures indicates a much less pronounced response by *E. solidaginis* (Fig. 1*B*) than by *C. cinctus*.

Water samples also showed a variable response to the electrostatic field, depending somewhat on their source but more on their configuration, size, and containing surfaces. The 20-mm³ drops froze in the presence of the field only at temperatures below −6°C (Fig. 1*C*), whereas all the drops encased in rubber cement froze at temperatures above −5°C (Fig. 1*D*). Both sets supercooled at least 10° when cooled in the absence of an electrostatic field.

Variability of response is thus shown both by insects and by water. Whereas *C. cinctus* larvae show a ready response to the stimulus of the electrostatic field, *E. solidaginis* larvae appear to have some resistance to its action, or perhaps they require a stronger field. The temperature ranges within which the two species responded to the stimulus were not determined by their usual supercooling abilities, though these of course impose a lower limit. Similarly, the water samples encased in rubber cement froze much more readily under the influence of the electrostatic field than did the bare droplets, even though both supercooled similar amounts in the absence of the field. The rubber cement therefore aided the action of the field in some unknown way, but did not affect ordinary supercooling.

The nature of the action of the field in stimulating nucleation can only be surmised at this time. The molecules of supercooled water, or the nucleating agents, or both, may be oriented by the field in a manner favorable to nucleus formation (*1*). Since the field oscillates, the molecules are not held stationary as in a crystal, but their polarity makes them subject to a certain amount of orientation, and the oscillation is a superimposed action. A d-c potential difference of equivalent magnitude may

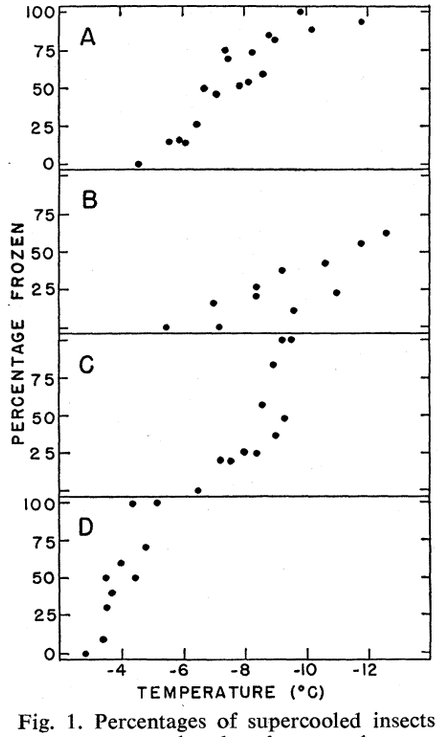

Fig. 1. Percentages of supercooled insects and water samples that froze at the constant temperatures indicated when exposed for 5 sec to an electrostatic field. *A*, Larvae of *Cephus cinctus*; *B*, larvae of *Eurosta solidaginis*; *C*, 20-mm³ water drops; *D*, water encased in rubber cement.

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

prove effective, but 1000 volts d-c gave negative results with *C. cinctus* larvae at −12°C when a ⅜-in. air gap and exposures up to 1 minute were used.

The necessity of having the sample close to an electrode or even forming an extension of it suggests that the conditions used in these experiments were near-minimal. The incomplete response by *E. solidaginis* larvae also supports such a view. However, if the field is strengthened by decreasing the gap, discharges will occur. Although nucleation was induced under these conditions, the side effects of the sparks, such as possible heating, were not investigated.

These results represent a preliminary excursion into a field that is new, biologically at least, and it is quite likely that some aspects that will later assume importance have been neglected or overlooked at this time. It has been demonstrated, however, that supercooled water and certain insects can be induced to freeze at higher temperatures than otherwise when placed in an electrostatic field. The effect becomes more certain and occurs earlier as the amount of supercooling is increased.

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Reference

R. W. Salt, *J. Insect Physiol.* **2**, 178 (1959).

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Lipids of Ankistrodesmus braunii

Abstract. Ankistrodesmus braunii was grown to stationary phase on a chemically defined medium and its cellular lipids were analyzed. The lipid content was found to vary from 18 to 73 percent of dry weight for cultures of different age and method of analysis. The pigments of the nonsaponifiable fraction were separated by adsorption chromatography and counter-current extraction and tentatively identified. The fatty acid fraction was converted to the corresponding methyl esters and analyzed by gas chromatography. The principal fatty acids present were: palmitic, oleic, and linolenic acids. Traces were detected of caprylic, capric, lauric, and palmitoleic acids.

Previous microscopic studies of *Ankistrodesmus braunii* during stationary phase indicated that the quantity of lipids synthesized by this green alga might be equal to or greater than that made by *Chlorella* spp. and related organisms (*1*). Potential use of algal metabolic products both as human nutritional supplements and in the closed ecosystems of space travel invited further investigation of the lipids of *A. braunii*.

Cultures of *A. braunii* strain 750 (Indiana University algal culture collection = McMillan clone 245-St 1.1) were grown in large erlenmeyer flasks to stationary phase in GFS medium previously described (*1*). The cells were maintained at 25° to 27°C with continuous daylight fluorescent illumination of 400 to 500 ft-ca for 4 to 8 weeks.

Cells were harvested by centrifugation and washed once with water. They were then suspended in 95 percent ethanol and placed in a boiling-water bath for 15 minutes to inactivate enzymes. The suspension was cooled, an equal volume of hexane was added, and the system was treated for 30 minutes in a 9 kcy/sec Raytheon sonic oscillator at maximum frequency. The suspension was transferred to a flask, most of the hexane was removed under vacuum, and the volume was made up with alcohol. A large aliquot was transferred to a boiling flask, potassium hydroxide was added, and the solution was saponified under reflux for 2 hours. The alkaline saponification mixture was extracted with hexane, then it was acidified and extracted with ether by the usual technique. All fractions were made up to volume and aliquots were removed for compositional analysis.

Pigments were separated on columns of powdered sugar and of anhydrous calcium carbonate by the procedure of Cowgill and Pardee (*2*). The hexane-soluble pigments were also subjected to countercurrent extraction on a 100-plate Craig apparatus in a solvent system consisting of 1.8 parts of petroleum ether and 1.0 part of 99 percent methanol.

Methyl esters of the algal fatty acids were prepared by the method of Clinton and Laskowski (*3*). Gas chromatographic separations were achieved on the Beckman GC-2 gas chromatograph with an 18-foot column of Resoflex R-446. Identification was based on standard samples.

Varying results were obtained in the determination of lipid content of *A. braunii* in stationary phase, depending on the age of the culture and method of analysis. Total lipids ranged from 18.6 to 33.7 percent for cultures from 4 to 7 weeks of age respectively. This is comparable to the findings of Collyer and Fogg (*4*) for a number of Chlorophyceae. Of the total lipids, 85.5 percent were recovered in the following distribution: nonsaponifiable, 17.1 percent; saponifiable, 61.5 percent; and glycerol, 6.8 percent. In one experiment where lipids were extracted from vacuum-dried cells, a lipid content of 72.8 percent was obtained, a value which would appear quite unreasonable were it not for similar observations of extremely high lipid content in cells subjected to vacuum drying while still living (*5*).

Adsorption chromatography and countercurrent distribution led to the separation of four principal pigments which were detectable by both techniques. Identification was based on known carotenoid spectra and is tentative: beta carotene, astaxanthin, lutein, and possibly a derivative of neoxanthin. The correct spectra and solubility were obtained for the first three. All of these pigments have previously been detected in algae (*6*).

The characteristic fatty acid composition of *A. braunii* in stationary phase is shown in Fig. 1. The principal fatty acids were found to be palmitic, oleic, and linolenic acids with traces of caprylic, capric, lauric, and palmitoleic acids. One sample showed a trace of linoleic acid. By the peak area method, the relative amounts of the three principal acids were linolenic acid, 13 percent; oleic acid, 54 percent; and palmitic acid, 33 percent. This algal fatty acid fraction is generally similar in composition to other vegetable oils, although

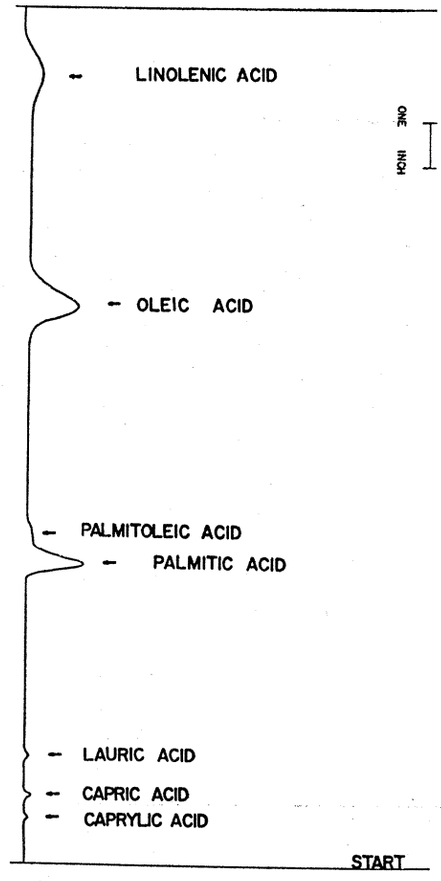

Fig. 1. Gas chromatogram of methyl esters of fatty acids isolated from *A. braunii*. Column: 18-foot Resoflex R-446; helium flow rate, 140 cm³/min; filament current, 320 ma; temperature, 240°C; chart speed, 0.4 in./min.