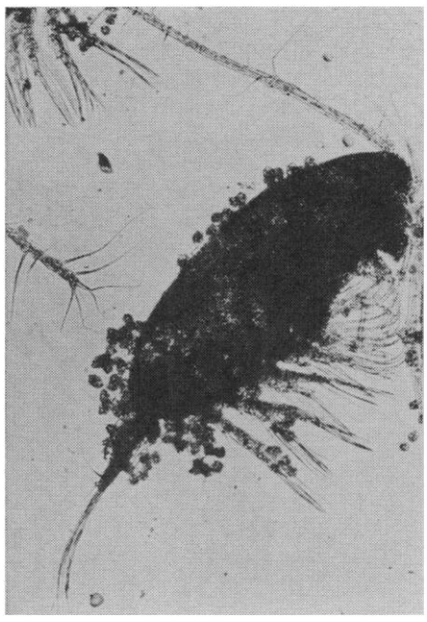

Fig. 1. Infestation of copepods with peritrichous ciliate, *Zoothamnium* sp.

2 net. The copepod *Acartia tonsa* is by far the most numerous zooplankter. Other workers have shown that this copepod is the most abundant species in Chesapeake Bay (*1*), and members of this genus are considered to be the most abundant inshore copepods along the east coast of the United States.

The seasonal cycle of *Acartia* was described by Conover for Long Island Sound (*2*) where *A. tonsa* was dominant from the summer through late winter and was replaced in dominance during early spring by *A. clausi*.

In the Patuxent River in 1963–64, *A. tonsa* dominated the zooplankton population from May 1963 through March 1964 and was replaced by *A. clausi* in late March and April 1964.

During early March 1964, all specimens of *A. tonsa* along the entire river became infested with a peritrichous ciliate identified as a member of the genus *Zoothamnium* (*3*). Each copepod had from 25 to 200 of these stalked protozoans present mainly around the appendages (Fig. 1). *Acartia clausi*, which was present in smaller numbers, was not infested nor were other zooplankters. By late March *A. clausi* and *A. tonsa* were equally abundant; and all *A. tonsa* were infested while only a few *A. clausi* had attached ciliates. Mid-April brought further changes; *A. clausi* became the dominant copepod and only a few *A. tonsa* were present. Each *A. tonsa* present was still infested while no *A. clausi* were affected. Table 1 indicates the relative abundance of the two species of copepods and the temperature and salinity ranges during the infestation period.

Partial examination of zooplankton samples from collections made during the spring of 1963 also showed that *Zoothamnium* sp. were present on some *A. tonsa* during March and April (*4*).

It would appear that *Zoothamnium* sp. was highly specific for *A. tonsa*. While there are numerous reports of individual organisms infested with large numbers of different epizoic ciliates, there have been few reports of entire populations being affected in this manner. Conover (*2*) noted that during winter many older stages of *A. tonsa* were host to a stalked protozoan and attributed this association to a steady-state condition of the copepod population due to wintering over.

Without experimenting with live organisms it is not possible to assess the ciliates' effect on the copepods or to account for the high specificity to *A. tonsa*. It is possible to envision the mechanical effect of numerous ciliates on the copepods. Sinking rates of preserved material were 19.2 cm/sec with infested *A. tonsa* and 13.8 cm/sec for noninfested organisms. It is possible that those copepods laden with ciliates were at a disadvantage in the natural environment.

Although other workers (*2*, *5*) have suggested temperature or salinity, or both, as being directly or indirectly responsible for the change of copepod species, it would now appear that other factors, such as infestation with protozoans, may be involved with changeover in dominance from *A. tonsa* to *A. clausi*.

SIDNEY S. HERMAN
Department of Biology and Marine Science Center, Lehigh University, Bethlehem, Pennsylvania
JOSEPH A. MIHURSKY
University of Maryland, Natural Resources Institute, Chesapeake Biological Laboratory, Solomons, Maryland

References and Notes

1. C. B. Wilson, *Proc. U.S. Natl. Museum* **80**, 1 (1932); T. E. Bowman, *Chesapeake Sci.* **2**, 206 (1961).
2. R. J. Conover, *Bull. Bingham Oceanogr. Coll.* **15**, 156 (1956).
3. J. O. Corliss, personal communication.
4. Material was supplied through the courtesy of W. R. Dovel, Chesapeake Biological Laboratory, Solomons, Md.
5. G. A. Deevey, *Bull. Bingham Oceanog. Coll.* **17**, 1 (1960); H. P. Jeffries, *Limnol. Oceanog.* **7**, 3 (1962).
6. We thank A. J. McErlean, Chesapeake Biological Laboratory, for his field assistance. Photographic work was done by L. Land and T. Loder, Lehigh University. The project was supported by funds from NSF, GP883, and Maryland Dept. of Water Resources, grant No. 1. Contribution 64-1, Marine Science Center, Lehigh University; contribution 267, Chesapeake Biological Laboratory.

17 July 1964

Inducing Resistance to Freezing and Desiccation in Plants by Decenylsuccinic Acid

Abstract. *Decenylsuccinic acid induces resistance to desiccation, cold, and frost in young bean plants. When decenylsuccinic acid is sprayed on flowering peach, apple, and pear trees, most of the flowers are resistant to a frost of −6°C.*

Resistance to drought and resistance to freezing in plants generally occur together (*1*). Water permeability of roots should affect survival of plants during desiccation. Also, frost resistant cells show more rapid plasmolysis (*1*), and hence evidently have more permeable membranes. Therefore, since compounds are available that will change permeability, their effect upon resistance to desiccation and freezing was measured.

When decenylsuccinic acid [CH_3—$(CH_2)_6$—CH=CH—CH_2—CH(COOH)—CH_2—COOH] penetrates into the lipid layer of the membrane of bean root cells it increases water permeability eightfold at 30°C. Of most significance here, is the fact that permeability becomes only slightly temperature dependent (*2*). These observations of both water permeability and its response to temperature plus the general correlation of drought and frost resistance suggested that decenylsuccinic acid might render plants resistant to both dry and cold weather.

First, the induction of drought hardiness was demonstrated. The roots of bean (*Phaseolus vulgaris*, var. "Bountiful," 14 days old) were placed in $10^{-3}M$ decenylsuccinic acid (*3*) for 2 hours. The roots were subsequently placed in a 10 percent solution of polyethylene glycol (osmotic pressure 1.3 atm) (*4*). This large molecule (molecular weight 20,000) is commonly used as an os-

motic agent to create a constant water stress. The leaves were illuminated by incandescent lamps (5×10^6 erg sec^{-1} cm^{-2}). The treated plants remained turgid, while the untreated plants wilted severely.

Next, the effect of treatment on plants exposed to a persistent drought was observed. Bean seedlings, 12 days old, were grown for 14 days in vermiculite to which solutions of various concentrations of polyethylene glycol and of decenylsuccinic acid were added (Table 1). In water, decenylsuccinic acid decreased leaf growth; it made the leaves darker green and increased their dry weight per square centimeter. The relative turgidity and osmotic pressure (*4*) were unaffected. When the roots were in an environment of 0.6, 1.3, or 2.9 atm water tension, however, treatment with decenylsuccinic acid increased leaf growth. Dry weight of leaves and of roots followed the same pattern as leaf area. Further, when water stress was severe, survival was increased markedly by the treatment.

When the roots are cold and the shoot is in warm air and bright light, a bean plant normally wilts. Since water uptake of treated roots is only slightly dependent on temperature, decenylsuccinic acid might prevent this type of wilting. Again, roots of beans were exposed to decenylsuccinic acid for 2 hours. The roots were then placed in ice water, and the leaves were illuminated by incandescent lamps (5×10^6 erg sec^{-1} cm^{-2}). A $5 \times 10^{-4}M$ solution prevented wilting of leaves, while lower concentrations were ineffective.

Table 1. Expansion of the first pair of leaves of bean plants (cm^2) and survival as affected by decenylsuccinic acid when the roots are subjected to varying water potentials. Potentials were created by 0, 5, 10, and 15 percent polyethylene glycol. Initial leaf area was 14.8 cm^2, and results are presented as mean increase in leaf area for 20 replicates (standard error 1.55 cm^2).

Potential (atm)	Conc. of decenylsuccinic acid (M)			
	0	10^{-5}	10^{-4}	10^{-3}
	Leaf growth			
0	51	44	41	40
0.6	21	25	28	26
1.3	13	15	21	20
2.9	0	4	4	8
	Survival (%)			
2.9	35	90	100	100

Finally, it remained to learn whether this unsaturated fatty acid would render tissues resistant to actual freezing. The observation of Lyons *et al.* (*5*) that mitochondrial membranes of plant species resistant to chilling contain more unsaturated fatty acids than do those of sensitive species is encouraging. Also, I observed that epidermal cells treated with decenylsuccinic acid plasmolyzed and deplasmolyzed more rapidly than untreated ones, and rapid dehydration of cytoplasm during freezing might increase survival of freezing. Young bean plants grown in vermiculite were given $10^{-3}M$ decenylsuccinic acid for 1, 3, and 5 days before exposure for 1 hour to −5°, −10°, or −15°C. One day of treatment permitted no plants to survive any frost. Three days of treatment permitted 8 plants out of 20 to survive a frost of −5°C, while 2 plants even survived a frost of −10°C. Five days of treatment made all plants resistant to a frost of −5°C, 9 at −10°C, and 1 at −15°C. Clearly the application of the chemical to the roots rendered the whole plant resistant to injury by freezing.

In the final experiments the chemical was applied directly to the organ to be protected, the organ being the extremely frost-sensitive flower. Cut branches of peach in full blossom were sprayed or dipped in $10^{-3}M$ decenylsuccinic acid and kept at 4°C. Two hours later they were exposed to a frost of −7°C for 2 hours and were then kept at room temperature. If the stamens and styles were turgid 24 hours later, the flowers were classified as survivors. No control flowers survived the treatment, while 65 of 100 sprayed flowers and 92 of 100 dipped flowers survived the frost. Most petals of the treated flowers kept their pink color. Evidently, decenylsuccinic acid had penetrated nearly all cell tissue and made it resistant.

Several branches of apple (Baldwin, Golden Delicious) and pear (Bosc) trees in an orchard were sprayed with $10^{-3}M$ decenylsuccinic acid and kept wet for 2 hours with polyethylene bags. The twigs within their bags were then placed in an insulated styrofoam box. The branch was surrounded by a mixture of ice and salt at −6°C. After 2 hours the branches were removed from box and bag. The flowers generally showed some brown spots on the petals, probably due to the mechanical pressure of the ice. Most flowers survived (Fig. 1, Table 2). All flowers were later pollinated, and many set fruits. Several leaves were visibly injured by frost, perhaps because the chemical had not penetrated the thick leaves as completely as it had the thin petals. Untreated flowers and leaves exposed to the frost were completely dead. So far no inhibitory effect of decenylsuccinic acid on fertilization and fruit growth has been observed.

Fig. 1. Effect of decenylsuccinic acid on frost resistance in apple blossoms. Top: control background, frozen at −4.5°C for 2 hours. Bottom: treated branch, frozen at −6°C for 2 hours; the photograph was made the next day.

Alkenylsuccinic acids are useful for the reduction of transpiration by closure of the stomata (*6*). These compounds

Table 2. Survival of frozen apple and pear flowers. The treated flowers were sprayed with $10^{-3}M$ decenylsuccinic acid during the period 11–14 May 1964. Controls were not sprayed.

Treatment	Frost temperature (°C)	No. of flowers		No. of fruits set on 26 May 1964
		Alive	Dead	
	Pear (Bosc)			
Control	−5	0	40	0
Sprayed	−6	20	10	7
Sprayed	−6	30	10	12
	Apple (Baldwin)			
Control	−4.5	0	85	0
Sprayed	−6	12	3	9
Sprayed	−4	55	3	48
Sprayed	−6	12	7	0
	Apple (Golden Delicious)			
Sprayed	−6	14	5	10

and perhaps other fatty acids also promise to become powerful agents to prevent the loss of crops by drought, cold, and frost.

PIETER J. C. KUIPER
Department of Soils and Climatology, Connecticut Agriculture Experiment Station, New Haven

References and Notes

1. J. Levitt, *The Hardiness of Plants* (Academic Press, New York, 1956), p. 100.
2. P. J. C. Kuiper, *Science* **143**, 690 (1964).
3. Samples were provided by B. J. Humphrey, Humphrey-Wilkinson, Inc., North Haven, Conn. The chemical was dissolved in a few drops of hot ethanol; then it was added to distilled water and the slightly cloudy liquid was used.
4. C. R. Frink showed that Al, Fe, and heavy metals were virtually absent in our sample of polyethylene glycol. S. Rawlins determined the osmotic potentials of the bean leaves and of the solutions of polyethylene glycol.
5. J. M. Lyons, T. A. Wheaton, H. K. Pratt, *Plant Physiol.* **39**, 262 (1964).
6. P. E. Waggoner, J. L. Monteith, G. Szeicz, *Nature* **201**, 97 (1964); I. Zelitch, *Science* **143**, 692 (1964).
7. I gratefully acknowledge the receipt of an O.E.C.D. postgraduate travel grant from the Netherlands Organization for the Advancement of Pure Research and I thank all my colleagues at the Station who helped me to get things done in time.

28 July 1964

Potassium and Sodium Content of Tissues of Hamsters and Ground Squirrels during Hibernation

Abstract. *Samples of kidney cortex, diaphragm, and atrial and ventricular muscle were taken from hamsters and ground squirrels to determine whether the low body temperature of hibernation caused loss of potassium and gain of sodium. Gradients between tissues and plasma are maintained or even increased during hibernation.*

In most mammalian tissues inhibition of metabolism by low temperature causes a decrease in ionic gradients across cell membranes. The loss of potassium and gain of sodium caused by cold in such tissues as mammalian erythrocytes and parenchymatous tissues (*1*) constitutes a failure of volume regulation and results in swelling (*1, 2*). In excitable tissues such as cardiac muscle the depression of ionic pumping by cold also leads to a lowering of the membrane potential and failure of conduction (*3*). In tissues of mammalian hibernators, however, excitability (*4*) and regulation of water content (*5*) persist at low temperature. Since maintenance of ionic gradients is essential for both of these cellular activities, it is surprising that little attention has been paid to the ionic content of tissues during hibernation. Eliassen and Leivestad have reported (*6*) that in muscles of two species of hibernators, hedgehog (*Erinaceus europaeus*) and bat (*Myotis daubentonii*), potassium concentration in tissue water increased somewhat during the winter months, while sodium concentration remained constant during the year but was greater than in nonhibernating species. They did not specify whether all "winter" bats and hedgehogs were actually hibernating when the tissues were collected. The results described in this report show that the total sodium and potassium content of tissues of hamsters (*Mesocricetus auratus*) and ground squirrels (*Citellus tridecemlineatus*) either remains unchanged during hibernation or changes in a way that would not be consistent with diminished cation transport.

Awake animals were kept in an animal room maintained at about 22°C, whereas hibernating hamsters and ground squirrels were housed in a cold room at 5°C. These animals were placed in the cold on 11 November. The ground squirrels began hibernating on 14 November and were killed between 11 and 24 February. The hamsters, as is typical of the species, delayed hibernation for 4 to 6 weeks (until 6 to 23 December), and 3 to 5 weeks of regular hibernation ensued before these animals were killed (between 5 and 22 January). Records were kept of the length of hibernating periods and frequency of periodic arousal. Of the six hamsters used, three were killed on the 3rd consecutive day of a hibernating period, one on the 2nd day and two on the 1st day. Of four ground squirrels, one was sacrificed on the 6th day of a period, one on the 5th day, and two on the 2nd or 3rd day. Cheek-pouch temperatures were measured prior to killing and were in all cases below 7°C.

After the animal was killed by a blow on the head, the kidneys, diaphragms, atria, and ventricles were removed through a midline incision. The atria and small sections (about 150 mg) of diaphragm and ventricle were rinsed briefly in an ice-cold Krebs bicarbonate-buffered medium to wash out the blood. These tissues were then blotted, placed in a tared tissue beaker, and dried overnight at 105°C for determination of dry weight. The capsule was removed from the kidney, and slices of the cortex were made with a dry razor blade. The outermost slice was rejected because in guinea pigs this has a higher potassium and lower sodium concentration than the rest of the tissue (*7*). The next slice was placed directly into a tared beaker to be desiccated.

Sodium and potassium values of dilutions of nitric acid digest of dried tissue were made by internal standard flame photometry on a Baird flame photometer.

The results of these measurements (Table 1) clearly show that in no tissue was there a significant decrease in potassium concentration or an increase in sodium concentration. Hence the low body temperature of hibernation does not cause the reduction in ionic gradients observed in most tissues of nonhibernating mammals at comparable temperatures.

In kidney cortex of both species, in hamster ventricles, and in ground

Table 1. Cation concentration of tissues of hamsters and ground squirrels. The value given (microequivalents per gram dry weight) in each case is the mean and standard error. Figures in parentheses indicate the number of cases in each column.

Tissue	Concentration			
	Potassium		Sodium	
	Hibernating	Awake	Hibernating	Awake
		Hamster		
	(6)	(8–10)	(6)	(8–10)
Kidney cortex	443 ± 16*	339 ± 6*	239 ± 18	242 ± 17
Diaphragm	332 ± 9	350 ± 6	218 ± 22	177 ± 17
Atria	367 ± 4	377 ± 10	288 ± 18*	393 ± 20*
Ventricles	383 ± 13*	336 ± 3*	144 ±8*	184 ± 5*
		Ground squirrel		
	(3–4)	(6)	(3–4)	(6)
Kidney cortex	469 ± 1*	331 ± 11*	208 ± 32	249 ± 16
Diaphragm	331 ± 12	335 ± 11	185 ± 12	163 ± 12
Atria	314 ± 7*	223 ± 15*	329 ± 40	271 ± 14
Ventricles	350 ± 11	344 ± 11	157 ± 9*	185 ± 6*

* Denotes pairs of values between which the differences between columns are statistically significant ($p < 0.01$).