PHYSIOLOGICAL ECOLOGY

A Series of Monographs, Texts, and Treatises

2nd Edition

RESPONSES OF PLANTS TO ENVIRONMENTAL STRESSES

VOLUME I

Chilling, Freezing, and High Temperature Stresses

ACADEMIC PRESS
A Subsidiary of Harcourt Brace Jovanovich, Publishers
New York London Toronto Sydney San Francisco

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Levitt, Jacob, Date
Responses of plants to environmental stresses.

(Physiological ecology)
Includes bibliographies and index.
CONTENTS: v. 1. Chilling, freezing, and high temperature stresses.
1. Plants, Effect of stress on.
2. Plants--Hardiness. 3. Botany. I. Title.
QK754.L42 1979 581.2'4 79-51680
ISBN 0-12-445501-8 (v. 1)

PRINTED IN THE UNITED STATES OF AMERICA

80 81 82 83 9 8 7 6 5 4 3 2 1

PHYSIOLOGICAL ECOLOGY

A Series of Monographs, Texts, and Treatises

EDITED BY

T. T. KOZLOWSKI

University of Wisconsin
Madison, Wisconsin

T. T. KOZLOWSKI. Growth and Development of Trees, Volumes I and II – 1971

DANIEL HILLEL. Soil and Water: Physical Principles and Processes, 1971

J. LEVITT. Responses of Plants to Environmental Stresses, 1972

V. B. YOUNGNER AND C. M. MCKELL (Eds.). The Biology and Utilization of Grasses, 1972

T. T. KOZLOWSKI (Ed.). Seed Biology, Volumes I, II, and III – 1972

YOAV WAISEL. Biology of Halophytes, 1972

G. C. MARKS AND T. T. KOZLOWSKI (Eds.). Ectomycorrhizae: Their Ecology and Physiology, 1973

T. T. KOZLOWSKI (Ed.). Shedding of Plant Parts, 1973

ELROY L. RICE. Allelopathy, 1974

T. T. KOZLOWSKI AND C. E. AHLGREN (Eds.). Fire and Ecosystems, 1974

J. BRIAN MUDD AND T. T. KOZLOWSKI (Eds.). Responses of Plants to Air Pollution, 1975

REXFORD F. AUBENMIRE. Plant Geography, 1978

JOHN G. SCANDALIOS (Ed.), Physiological Genetics, 1979

BERTRAM G. MURRAY, JR. Population Dynamics: Alternative Models, 1979

J. LEVITT. Responses of Plants to Environmental Stresses, 2nd Edition. Volume I: Chilling, Freezing, and High Temperature Stresses, 1980

In Preparation

J. LEVITT. Responses of Plants to Environmental Stresses, 2nd Edition. Volume II: Water, Radiation, Salt, and Other Stresses, 1980

JAMES A. LARSEN. The Boreal Ecosystem, 1980

Preface

Until recently, a monograph on a specialized subject such as stress remained current and in need of only minor revisions for one or more decades. Now, the explosion of information has ended this relatively long life. Lange (1975), reviewing a mere two years of investigations in only one of the stress areas, covered 2700 publications and admitted that this did not include all the published works. On this basis, it can be estimated that the total number of publications in all areas of environmental stress *since* completion of the first edition of this monograph must be in the tens of thousands and of the same order as the total number published in the whole history of the subject *before* the first edition.

To allow this mass of publications to accumulate without attempting to incorporate them into an overall treatment of the subject would lead to a tragic loss of valuable information contributed by the intense efforts of countless able scientists. It would also leave the subject of environmental stress in an archaic and even chaotic state. Someone had to have either the courage or foolhardiness to attempt an integration of as many as possible of these newer investigations with one another and with the earlier work and to propose general principles based on this integration. Even if most of such proposals should prove to be incorrect, they will contribute to the science by suggesting the most reasonable direction of future meaningful research.

I have, therefore, taken the liberty of hypothesizing liberally throughout this second edition, which is composed of two volumes. The reader must always remember, however, that in our present state of ignorance all such hypotheses must be tested, not accepted. Some of them may even be proved incorrect on the basis of newer information appearing between completion of the manuscript and its publication.

I apologize to those scientists whose valuable work has been overlooked or inadequately treated in this first volume of the second edition.

J. Levitt

Preface to the First Edition

For many years, bits of information have been accumulating on the effects of stresses on plants. I have long felt the need to integrate these in an attempt to discover the basic principles. This need has now become more urgent due to the increasing importance of stress injuries, largely as a result of man's activities. Previously known stresses are becoming more important, and new ones are constantly arising. The practical aim is, therefore, to learn how to control the stresses, or to decrease the injuries they produce.

But the practical goal, though sufficient in itself, is not the sole reason for investigating environmental stresses. It has been said that to understand the normal cell we must study the abnormal cell. To paraphrase this statement, if we wish to understand life we must also study death. The causes of death as a result of exposure to environmental stresses are, therefore, of fundamental importance to all biology, and, for that matter, to all human activities since these are all impossible without life. An understanding of the nature of environmental stresses and of the plant's responses to them may, therefore, help to answer the age-old question: What is life?

It is, therefore, essential that we understand how stresses produce their injurious effects and how living organisms defend themselves against stresses. Why then confine our attention to plants? The simplest answer, of course, is my ignorance. But there is also another reason. The plant has succeeded in developing defenses against stresses that the animal (with few exceptions) has not developed, for instance, against freezing and drought. These also happen to be the stresses that have been most intensively studied. As a result, the research on animals has been mainly confined to responses of quite a different kind. At this stage, therefore, the resistance of plants to environmental stresses is a field in itself. This does not mean that investigations of other organisms can be completely ignored. Some of the most important aids to our understanding of the effects of stresses on plants have come from investigations of animal cells and microorganisms. Such information must, of course, be included.

I have covered four stresses in previous publications: "Frost Killing and Hardiness of Plants" (1941, Burgess, Minneapolis), "The Hardiness of Plants" (1956, Academic Press, New York), Frost, drought, and heat resis-

tance (1958, *Protoplasmatologia* **6**), and Winter hardiness in plants [1966, *in* "Cryobiology" (H. T. Meryman, ed.), Academic Press, New York]. The first two are now out of print, and all are out-of-date. This monograph will include essentially all the environmental stresses which have been intensively investigated (with the exception of mineral deficiencies, which comprise too broad and involved a field to be incorporated with other stresses) and will attempt to bring the information on the above four stresses up-to-date. An attempt will then be made to analyze the possibilities of developing unified concepts of stress injury and resistance. The aim of this synthesis is, therefore, a comprehensive, unified, and molecular point of view. Descriptive aspects of the plant's responses have been largely excluded. For a diagnostic approach to the problem, the reader is referred to Treshow (1970, "Environment and Plant Response," McGraw-Hill, New York).

Only too often in the history of science, parallel investigations by different investigators have led to parallel but different systems of nomenclature. This has occurred in the field of stress research. Any attempt to integrate the results of such parallel investigations requires the adoption of a single, exactly defined terminology. In the case of stresses, this terminology should be applicable to all organisms, plant as well as animal. I have, therefore, attempted to introduce such a uniform terminology in this monograph. The earlier term "frost" has, for instance, been discarded in favor of "freezing," which is now used more generally by cryobiologists. Similarly, the term "tolerance" is adopted in place of the older "hardiness." It is my hope that such adoptions will clarify rather than confuse the concepts.

Unfortunately, the information explosion has prevented an all-inclusive integration. I tender my apologies to all investigators whose important contributions have not been included.

J. Levitt

Contents

Preface ix
Preface to the First Edition xi

I. STRESS CONCEPTS

1. Stress and Strain Terminology
A. Physical Stress and Strain 3
B. Biological Stress and Strain 5

2. The Nature of Stress Injury and Resistance
A. Stress Injury 10
B. Stress Resistance 11
C. Kinds of Stress Tolerance 17

Bibliography (Preface, Chapters 1–2) 19

II. CHILLING TEMPERATURES

3. Chilling Injury and Resistance
A. Chilling Stress 23
B. Chilling Injury 24
C. Chilling Resistance 46

Bibliography (Chapter 3) 56

III. FREEZING TEMPERATURES

4. Limits of Low-Temperature Tolerance
A. Dehydrated Protoplasm 67

B. Hydrated Protoplasm 68
C. Cryoprotectants 73

5. The Freezing Process
A. The Freezing Stress 79
B. Extracellular versus Intracellular Freezing 80
C. Freezing, Undercooling, and Eutectic Points 90
D. Freeze-Dehydration 94
E. Measurement of Ice Formation in Plants 96

6. Freezing Injury
A. Occurrence 99
B. Primary Direct Freezing Injury 99
C. The Time Factor in Relation to Injury 101
D. The Moment of Freezing Injury 105
E. Primary Indirect Freezing Injury 107
F. Secondary Freezing Injury 108

7. Freezing Resistance—Types, Measurement, and Changes
A. Possible Types of Resistance 116
B. Measurement of Freezing Tolerance 129
C. Changes in Freezing Tolerance 137
D. The Nature of Freezing Tolerance 153

8. Factors Related to Freezing Tolerance
A. Morphology, Anatomy, Organelles 163
B. Physiological Factors 166
C. Resistance Induced by Applied Substances 223

9. Theories of Freezing Injury and Resistance
A. Primary, Direct (Intracellular) Freezing Injury 228
B. Secondary, Freeze-Induced Dehydration Injury 230

10. Molecular Basis of Freezing Injury and Tolerance
A. Evidence for a Molecular Basis 248
B. Membrane Damage as the Initial Injurious Strain 254
C. The SH Hypothesis of Freezing Injury 262
D. Mechanism of Freeze-Induced Membrane Damage 274
E. Freezing Resistance and the Hardening Mechanism 276
F. Metabolic Control of the Hardening Process 283

Bibliography (Chapters 4–10) 290

IV. HIGH-TEMPERATURE STRESS

11. High-Temperature or Heat Stress

A. Quantitative Evaluation of Stress 347
B. Limit of High-Temperature Survival 348
C. The Time Factor 351
D. Occurrence of High-Temperature Stress and Injury in Nature 353
E. Nature of the Injury 359
F. Protective Substances 391

12. Heat Resistance

A. Heat Avoidance 394
B. Heat Tolerance 402
C. Molecular Aspects of Thermotolerance 433
D. Relationship between Thermotolerance and Low-Temperature Tolerance 445

Bibliography (Chapters 11 and 12) 448

Index 471

1

STRESS CONCEPTS

1. Stress and Strain Terminology

The responses of plants to the severities of their environment have occupied the attention of man long before the beginnings of the science of biology (Levitt, 1941). To the farmer, plants that survive in these environments are "hardy," those that do not are "tender." The scientist, however, requires a more quantitative terminology. Therefore, in recent years, biologists have adopted the term *stress* for any environmental factor potentially unfavorable to living organisms, and *stress resistance* for the ability of the plant to survive the unfavorable factor and even to grow in its presence. Unfortunately, although stress has been exactly defined in mechanics, no such exact terminology has been developed in biology. Since the lack of exact terminology in science commonly leads to a lack of exact concepts, an attempt will first be made to apply the definitions of mechanics to biology. It must be recognized at the outset, however, that the mechanical and the biological stresses are not completely identical, and that, therefore, the terminology can be transferred only up to a point. Furthermore, the medical concept of stress (Selye,1973) is quite different from both the biological and the physical.

A. PHYSICAL STRESS AND STRAIN

According to Newton's laws of motion, a force is always accompanied by a counter force (Duff, 1937). If a body A exerts a force on body B, then body B must also exert a counter force on A. The two forces are called action and reaction and are parts of an inseparable whole, known as a stress. When subjected to a stress, a body is in a state of strain. The external force produces internal forces between contiguous parts of the body leading to a change in size or shape. The magnitude of the stress is the force per unit area. The magnitude of the strain is the change in dimension (e.g., length or volume) of the body.

Up to a point, which is specific for each body, a strain may be completely reversible. Such reversible strains are said to be *elastic*. Beyond this point, the strain will be only partially reversible, and the irreversible part is called

the *permanent set* (Fig. 1.1). The permanent set is also called a *plastic strain*. The elastic strain produced in a specific body as a result of a specific stress will always be the same, and the strain is proportional to the stress. Therefore,

$$M = \text{stress/strain}$$

The constant M is known as the modulus of elasticity of the body, which differs for different bodies: the greater the modulus, the more elastic the body. The more elastic the body, the greater is its resistance to deformation (i.e., the larger the stress required to produce a unit strain). It should be noted that elasticity is *not* the same as elastic extensibility, which is a measure of the maximum possible elastic (i.e., reversible) strain. Unlike elastic strains,

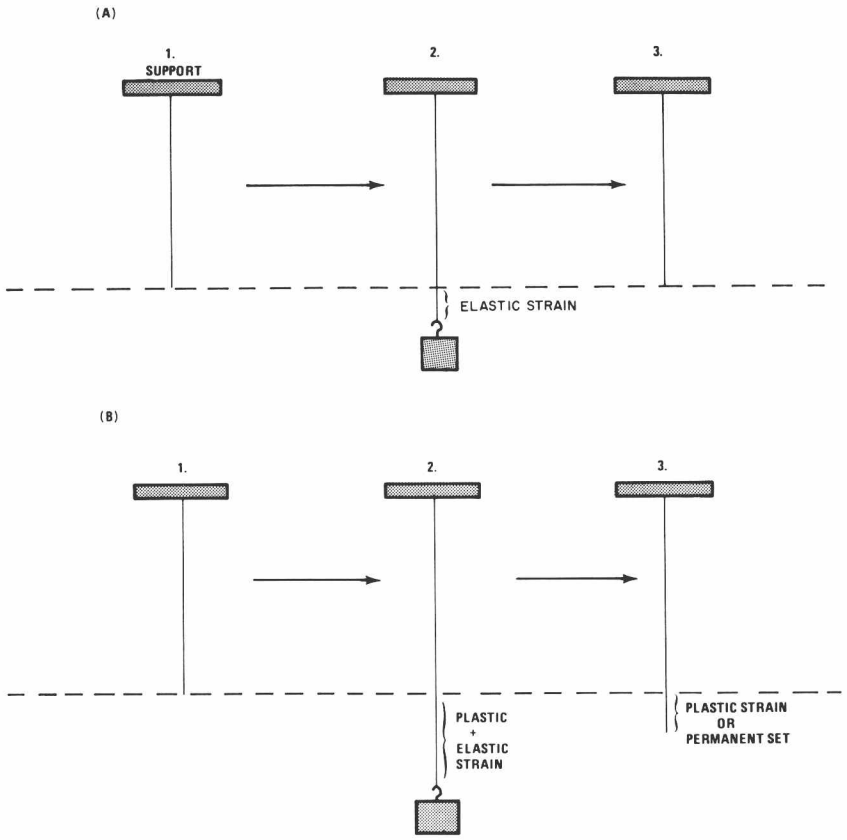

Figure 1.1. Elastic (A) and plastic (B) strains in a simple physical system.

plastic strains are not constant for specific stresses, since they may eventually lead to rupture of the body. There is, therefore, no modulus of plasticity.

B. BIOLOGICAL STRESS AND STRAIN

Biological stresses differ from mechanical stresses in two main ways. First, since the plant is able to erect barriers between its living matter and the environmental stress, the stress must be measured not in units of force but in units of energy. Second, the term stress in biology always has a connotation of possible injury—i.e., of irreversible or plastic strain. A biological stress may, therefore, be defined as any environmental factor capable of inducing a potentially injurious strain in living organisms. Since the biological stress is not necessarily a force, the biological strain is also not necessarily a change in dimension. The living organism may, however, show a physical strain or change (e.g., cessation of cytoplasmic streaming) or a chemical strain (a shift in metabolism). If either strain is sufficiently severe, the organism may suffer a permanent set, i.e., injury or death. Like the physical body, a specific organism will undergo a specific strain when subjected to a specific stress. It will, therefore, have its own modulus of elasticity, or resistance to physical or chemical change. By analogy with disease resistance, the term "elastic resistance" is more in agreement with biological terminology than modulus of elasticity. In biological systems, unlike physical systems, "plastic resistance" is more commonly measured than elastic resistance. Since plastic strains may be dependent on the time exposed to the stress, the time factor must be measured whenever the plastic resistance of biological systems is determined. The above stress terminology for the two systems is compared in Table 1.1.

The stress resistance of biological organisms is, therefore, of two main types. *Elastic resistance* is a measure of the organism's ability to prevent reversible or elastic strains (physical or chemical changes) when exposed to a specific environmental stress. *Plastic resistance* is a measure of its ability to prevent irreversible or plastic strains and, therefore, injurious physical or chemical changes.

One advantage of a precise biological terminology based on an analogy with mechanics now becomes apparent. The term resistance to environmental stresses has been mainly used for plastic resistance. The concept of an elastic resistance has not been as clearly recognized. There is, therefore, a whole field in physiology waiting to be investigated—a determination of the comparative elastic resistances of different organisms and an attempt to discover the mechanisms involved. As an example, when a corn plant is cooled from 30° to 5°C, its growth comes to a complete stop. Wheat, on the

TABLE 1.1

Stress Terminology

Term	Physical sense	Biological sense
Stress	A force acting on a body (F/A = dynes/cm^2 or bars)	An external factor acting on an organism (e.g., bars of water stress)
Strain	A change in dimension produced by a stress	Any physical or chemical change produced by a stress
Elastic strain	A reversible change in dimension	A reversible physical or chemical change
Plastic strain	An irreversible change in dimension	An irreversible physical or chemical change
Modulus of elasticity (or elastic resistance)	Stress/elastic strain	Intensity of external factor/amount of reversible physical or chemical change
Modulus of plasticity (or plastic resistance)	Not measured	Intensity of external factor producing a standard irreversible physical or chemical change[a]

[a]The organism must be exposed to the stress for a standard time.

other hand, continues to grow, though at a slower rate. In both cases, when returned to the normal growing temperature, normal growth is resumed. The strain is, therefore, reversible, i.e., elastic. Why does the corn plant suffer a greater elastic strain than the wheat plant when cooled? Or, using resistance terminology, what is the cause of the greater elastic resistance of wheat than corn when cooled?

Another advantage is that the importance of the time factor becomes obvious in the case of plastic strains. The plastic stretch of a wire may be just as dependent on the time exposed to the stress as on the stress itself. Similarly, injury to an organism is just as dependent on the time exposed to a high-temperature stress as on the high temperature used. On the other hand, this is not completely true of freezing stresses, as will be seen below.

There are two pronounced differences, however, between the responses of a nonliving body and of a living organism to stress.

1. Plastic strains in biological systems may be reparable. As in the case of the physical systems, the plastic strain will increase with the stress, producing more and more injury; the plastic strain is irreversible only in the spontaneous (thermodynamic) sense. The plant may be able to repair the strain by an active expenditure of metabolic energy. As the stress increases, the plastic strain also increases until the "rupture" point, when the strain is irreversible both thermodynamically and by metabolic repair, and the plant is killed. It is obvious, then, that stress resistance has two main components:

(a) The innate internal properties (or "forces") of the plant which oppose (i.e., resist) the production of a strain by a specific stress. (b) The repair system which reverses the strain. Only the first of these is analogous to the modulus of elasticity in physical systems.

2. Living organisms are adaptable. They are, therefore, capable of changing gradually in such a way as to decrease or prevent a strain when subjected to a stress. Both the elastic and plastic resistances of a plant to a specific stress may, therefore, increase (or decrease). This adaptation may be either stable, having arisen by evolution over a large number of generations, or unstable, depending on the developmental stage of the plant and the environmental factors to which it has been exposed. The unstable adaptation must, of course, also have arisen by evolution, but the hereditary potential is wide enough to permit large changes during the growth and development of the organism.

This adaptation is important both in the case of elastic and plastic strains. Plastic strains are by definition injurious. Therefore the adaptation leading to increased plastic resistance will obviously prevent injury by a stress which injures the unadapted organism. This kind of adaptation has been called "resistance adaptation" by Precht *et al.* (1955), since the adaptation implies a resistance to injury. Injury due to elastic strain would seem to be precluded, by analogy with nonliving systems. Although elastic strains are reversible by removal of the stress and therefore, by definition, are noninjurious, it must be realized that if they are maintained for a long enough period, they may lead to injury and even death. This may simply be due to the inability of the organism to compete with others that undergo less elastic strain when subjected to the same stress (e.g., mesophiles versus psychrophiles at low temperatures). The elastic strain may also eventually injure the plant even in the absence of competition, due to a disturbance of the metabolic balance. Thus, a low-temperature stress may simply decrease the rates of all metabolic processes reversibly, but not all may be decreased to the same degree. Therefore, if the stress is maintained for a long enough period, the strain may conceivably lead to an accumulation of toxic intermediates or to a deficiency of essential intermediates. In either case, a long enough exposure to the stress may injure or kill the organism. An adapted organism, on the other hand, may live, grow, complete its life cycle, and regenerate in the presence of the stress. This kind of adaptation has been called "capacity adaptation" by Precht (1967). Resistance adaptation may not permit growth and may merely prevent the plastic strain and therefore the injury until the stress is removed or decreased to the level permitting growth and development.

Nevertheless, both adaptations involve a *resistance* to the effects of a stress; on the one hand, a resistance to elastic strain, and, on the other, a