THESIS

This is to certify that the

thesis entitled

SIMULTANEOUS INHIBITION OF TRANSLOCATION OF PHOTOSYNTHATE AND OF THE FLORAL STIMULUS BY LOCALIZED LOW-TEMPERATURE TREATMENT IN THE SHORT-DAY PLANT PHARBITIS NIL

presented by

David Leon Kavon

has been accepted towards fulfillment
of the requirements for

M.S. degree in Botany

Major professor

Date August 8, 1979

O-7639

SIMULTANEOUS INHIBITION OF TRANSLOCATION OF PHOTOSYNTHATE AND OF THE FLORAL STIMULUS BY LOCALIZED LOW-TEMPERATURE TREATMENT IN THE SHORT-DAY PLANT *PHARBITIS NIL*

By

David Leon Kavon

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1979

ABSTRACT

SIMULTANEOUS INHIBITION OF TRANSLOCATION OF PHOTOSYNTHATE AND OF THE FLORAL STIMULUS BY LOCALIZED LOW-TEMPERATURE TREATMENT IN THE SHORT-DAY PLANT PHARBITIS NIL

By

David Leon Kavon

There is considerable evidence to indicate that the floral stimulus moves along with photosynthate in the phloem. In examining this phenomenon, the effect of cooling a localized region of the stem of the short-day plant Pharbitis nil Chois. was investigated. The movement of both ^{14}C-labeled assimilates and the floral stimulus was followed.

Three methods were devised to apply low temperature to the translocation path. The initial method, using an ice-filled Plexiglas trough involved a gentle bending of the stem. It was shown that the bending itself contributed to an inhibitory effect on translocation of assimilates as well as movement of the floral stimulus. An erect version of this cold block, eliminating the bend, was shown to apply mechanical pressure on the stem which also appeared to contribute to an inhibitory effect. The final apparatus, based exclusively on the circulation of low-temperature air around the stem zones, eliminated all mechanical contact with

the translocation path. The effective low-temperature treatment simultaneously inhibited translocation of photosynthate and of the floral stimulus, thus further supporting the idea that the floral stimulus is transported concurrently with assimilates in the phloem.

ACKNOWLEDGMENTS

I am pleased to thank Jan Zeevaart, chairman of my guidance committee, for the time and effort he invested in this project. I sincerely appreciate the encouragement he offered on a continual basis during the course of my research. I would also like to thank Debbie Delmer, Andrew Hanson, and Hans Kende for serving on the guidance committee.

Foremost, I feel immeasureable gratitude to my wife, Susie, whose patience was exceeded only by her wisdom and foresight. Finally, I think my daughter, Yedida, whose first year made this final year a veritable joy.

TABLE OF CONTENTS

	Page
LIST OF TABLES	v
LIST OF FIGURES	vi
INTRODUCTION	1
Assimilate Translocation	1
Tissue of Assimilate Translocation	1
Substances Transported in the Sieve Tubes	3
Mechanism of Translocation	11
Early Observations	11
Solute Translocation Independent of Solvent Water	13
Mass Flow	15
Resistance to Flow	19
Other Theories	23
Role of Metabolism	23
Anoxia Effects	24
Inhibitor Studies	25
Low-Temperature Inhibition	28
Transient inhibition and recovery	32
Floral Stimulus Translocation	36
Recognition of a Flowering Signal and a Transmissible Floral Stimulus	36
Floral Stimulus Movement	38
Tissue of Floral Stimulus Transport	38
Inhibition of Flowering by Noninduced Leaves	43
Other Factors Affecting Floral Stimulus Production and Movement	47
Velocity of Floral Stimulus Movement	49
Attempts to Divert or Impede the Movement of the Floral Stimulus	51
Experimental Objectives	53
MATERIALS AND METHODS	55
Growing Conditions	55
Low-Temperature Treatment	57
^{14}C-Labeling of Assimilates	57
Movement of the Floral Stimulus	61

Page

RESULTS AND DISCUSSION 62

Labeling Method 62
^{14}C-Assimilate Detection by Rapid-Scan Method . . . 62
Movement of Floral Stimulus Out of Cotyledons of *Pharbitis* Seedlings 65
Effect of Leaf Size on Perception of the Inductive Conditions 65
Effect of a Localized Cold Block on ^{14}C-Photosynthate Translocation 71
Effect of a Localized Cold Block on ^{14}C-Photosynthate Translocation and on Floral Stimulus Transport . . 71
Effect of an Erect, Localized Cold Block or Simulated-Ice Block on the Transport of the Floral Stimulus . 82
Effect of a Localized Cold Block, Based on Low-Temperature Air, on Translocation of ^{14}C-Photosynthate and on Transport of the Floral Stimulus 85

SUMMARY AND CONCLUSIONS 95

LITERATURE CITED 97

LIST OF TABLES

TABLE		Page
1.	Effect of Leaf Size on the Perception of the Inductive Conditions	72
2.	Effect of Cold Block, Bending and Excision at Various Heights Above the Cold Block on Movement of the Floral Stimulus	81
3.	Effect of an Erect, Localized Cold Block or Simulated-Ice Block on the Transport of the Floral Stimulus	83
4.	Effect of Localized Chilling of the Stem on Translocation of the Floral Stimulus in *Pharbitis nil* . .	91

LIST OF FIGURES

Figure		Page
1.	Devices used for localized chilling of *Pharbitis* stems	58
2.	Distribution of ^{14}C-activity along *Pharbitis* stems after labeling of the source leaves by two different methods	63
3.	Scan of ^{14}C-activity along *Pharbitis* stems	66
4.	Movement of floral stimulus out of cotyledons of *Pharbitis* seedlings	69
5.	Distribution of ^{14}C-activity along *Pharbitis* stems in initial cold block after labeling of the source leaves	73
6.	Distribution of ^{14}C-activity along *Pharabitis* stems in initial cold block and in bent control after labeling of the source leaves	78
7.	Distribution of ^{14}C-activity along *Pharbitis* stems in cold block based on low-temperature air 7 h after labeling of the source leaves	86
8.	Profile of distribution of ^{14}C-activity along the stem of *Pharbitis* 12 h after labeling of the source leaf	89

INTRODUCTION

Assimilate Translocation

Tissue of Assimilate Translocation

In the course of scientific inquiry, it is not uncommon to note observations whose significance becomes apparent only years thereafter. Such is the case with some early studies on translocation in plants. Stephen Hales (65) conducted girdling or "ringing" experiments on shoots of a dwarf pear tree. In 1727 he reported that where a ring of bark was removed both above and below a segment containing a thriving, leaf-bearing bud, by the end of the growing season this remaining ringlet swelled greatly at the bottom, yet not at the top. No swelling was observed if the segment contained no bud. He further correlated the extent of the bark swell at the bottom of the ringlet with the extent of leaf tissue produced by the adjoining, developing bud. While he neglected to interpret his results in terms of interruption of assimilate flow, his experiment clearly demonstrated that such interruptions of the continuity of a transport apparatus results in the accumulation of the transported substances just above the region of interruption. Such experimental results later generated speculation that the site of the tissue responsible for the translocation of photosynthate is in the bark and that removal of this tissue by girdling will result in the accumulation of photosynthate which causes the swelling.

In the nineteenth century Hartig (73) described the sieve tube of the phloem and proposed that it is through this tissue that the basipetal transport of organic assimilates from the leaves occurs. Schneider-Orelli (137) observed that the mobilization of the starch accumulated in apple leaves during the day is blocked in areas where the leaf-mining larvae stage of the moth Lyonetia clercella L. sever the phloem channels. This premise was strongly supported in 1928 by convincing girdling experiments performed by Mason and Maskell (109). They were able to demonstrate that the diurnal fluctuations of leaf sugar concentrations could be observed several hours later in the inner bark further down the stem, but not in the wood. They further showed that when the bark was removed, sugar translocation halted, whereas it continued when the bark and wood were merely separated. They also reported that translocation continued even into loosened flaps of bark. Fluorescent dyes were later used to visually ascertain that the sieve tubes of the phloem were, in fact, the channels of translocation. Schumacher (138) applied a fluorescent dye to a Pelargonium leaf and was able to follow its transport down the petiole. Furthermore, by inducing callose-plug formation at the sieve plates, he was able to block further transport.

The strongest evidence for movement of photosynthate in the phloem was presented once radioisotopes were available for biological research. Colwell (31) was the first to employ autoradiography to study the movement of assimilates through the phloem. Bieleski (6) reported that following labeling with $^{14}CO_2$ the cells which accumulated the most label were in the secondary phloem. The movement of

^{14}C-assimilates in the sieve tubes of Cucurbita was shown by Webb and Gorham (182). Cucurbita is an ideal plant to study translocation since in addition to the usual vascular bundles it contains isolated strands of sieve tubes and companion cells in the vascular region and cortex. While they showed that the sieve tubes do indeed carry translocate, it remained for Trip and Gorham (163) and Schmitz and Willenbrink (136) to show that cells other than the sieve tubes do not carry photosynthate.

Another approach, which has become very useful for translocation studies, is the aphid-feeding technique developed by Mittler (114,115). Aphids feed on phloem sap by puncturing a single sieve tube with their stylets. Eventually they will exude a droplet of honeydew which is quite similar in chemical composition to the phloem sap. The honeydew can be analyzed or the aphid can be severed from the stylet and the sap collected directly. Aphids which are feeding on a plant recently photosynthesizing in the presence of $^{14}CO_2$ will release honeydew containing radioactive sugars (14), further confirming the sieve tubes as the translocation channels.

Substances Transported in the Sieve Tubes

There exist several methods by which sieve-tube contents can be studied. Tapping sieve-tube sap from bark does not work with many species, although the method reported by King and Zeevaart (96) using chelating agents to enhance phloem exudation from petioles makes it possible to collect the contents of phloem from more herbaceous species. There are other drawbacks to the tapping method, including

delivery of only part of the sieve-tube contents, dilution or contamination by cut cells or metabolic changes by the sap enzymes. The aphid-stylet technique avoids most of these problems, but it produces very small quantities for analysis. Analysis of the entire conducting bundle makes it difficult to differentiate between the content of the sieve tubes and the accompanying parenchyma and companion cells. Microautoradiography can be a helpful procedure and indirect evidence for transport is avilable if importing tissues can be shown to require specific compounds which they cannot synthesize from simpler, transported substances. While no single method is adequate for positive confirmation, a combination of methods may be accepted as proof. If a compound can be isolated from sieve-tube sap, will accumulate above a girdle and is part of a moving front of radioactive assimilates, then one may conclude that it travels in the phloem.

Water is the most abundant and important substance translocated in sieve tubes. Second only to water, carbohydrates--sugars and sugar alcohols--form the bulk of the transport substances in the phloem. An exception to this rule occurs in certain species of the Cucurbitaceae where nitrogenous compounds form the bulk of the transport substances, whereas carbohydrates are only 1% of the fresh weight (199). Zimmermann and Brown (208) classified three types of plants according to the form of carbohydrate translocated in the phloem: (a) species with sucrose as the major sugar, (b) species which contain large quantities of oligosaccharides of the raffinose family in addition to sucrose, and (c) species with considerable

amounts of sugar alcohols such as mannitol, sorbitol, and dulcitol. Free hexoses are rarely found. All transport sugars are non reducing as noted by Trip et al. (165). Reducing sugars appear to be excluded at the phloem loading step. Sucrose and sugar alcohols predominate probably because of their high solubility in water, their ease of synthesis from first stable products of photosynthesis, their ease in undergoing further metabolism by receiving cells, their protection from degradation in the sieve tubes, and their ability to be actively transported across membranes (199).

Except for species of the Cucurbitaceae, nitrogenous substances are less abundant in sieve-tube sap than carbohydrates. In Salix Mittler (115) found the nitrogen content to range between 0.3-2.0 mg ml^{-1}, varying with season. The protein of the phloem sap consists mainly of "P-protein," a class of proteins with a filamentous and tubular structure. Although there had been speculation that it may be a contractile protein responsible somehow for phloem transport, all evidence now points against this (187). The amino acids asparagine, aspartate, glutamine, and glutamate are predominant in sieve-tube sap (66). Serine, which is also common, is readily formed from 3-phosphoglycerate, an early photosynthetic product. Proline may be the most abundant amino acid transported in sieve tubes of some species at the end of the growing season following leaf-protein mobilization. Putrescine, canavanine, allantoin, allantoic acid and citrulline have all been detected in some species, but are clearly less common.

There are scattered reports of ether-soluble compounds in phloem exudate (199), but no systematic study of lipids in sieve-tube sap has been reported as yet.

Citric, oxalic, malic, tartaric, and other metabolically important organic acids have been identified by Peel and Weatherly (127), but are minor components of the sieve-tube sap. Sugars, rather then any transported organic acids, undoubtedly serve as the basic skeletons for sink-tissue building materials.

Mature sieve elements are cells which have lost their nuclei, yet there are some indications that nucleic acids or their components are transported in the sieve tubes. Both DNA and RNA levels have been measured in sieve-tube sap. AMP and ADP have also been detected but ATP concentrations are noted to be particularly high with concentrations ranging from 34.9-976 $\mu g\ ml^{-1}$ (199). While free adenine has been detected, this may be the result of an enzymatic degradation of ATP to adenine. The ATP concentration in sieve tubes remains relatively constant and yet turns over rapidly. The companion cells are probably responsible for maintaining the ATP level. Uracil derivatives, which may function in transport-sugar biosynthesis, and cytosine derivatives have also been found in phloem exudate.

Correlative control of developmental growth processes by growth regulators requires their transmission via a long-distance transport system. It is, therefore, not surprising that the known growth regulators are easily found in phloem exudate. IAA appears to follow the direction of assimilates in bulk flow. Bioassay

activity of IAA-like substances in sieve-tube sap was first detected by Huber et al. (82). Some indole derivatives apparently cannot enter the sieve tubes. Hall and Baker (66) estimated the IAA concentration in sieve tubes to be 0.6×10^{-7} M. There are several possible roles for auxin transported in the phloem. Auxin may affect activation of sieve tubes, initiate cambial activity in overwintered trees and influence sugar translocation in sieve tubes both laterally from storage tissue to sieve tube and longitudinally within the sieve tubes.

Using the dwarf corn (d_5) bioassy, Kluge et al. (97) found gibberellin-like activity in the phloem exudate of several species. The concentration was about 5×10^{-3} µg ml^{-1}. Similarly, gibberellin-like activity has been detected in the honeydew of aphids feeding on several plant species (80). In Salix it was shown that the concentration of gibberellin-like substances in sieve-tube sap was related to the day-length in which the plants were growing (80).

Phillips and Cleland (129) obtained evidence, using the soybean-callus bioassay, of cytokinin activity in honeydew of aphids feeding on Xanthium. The absence of such activity in honeydew of aphids feeding on a totally defined diet clearly showed that the plant is the source of the cytokinin-like substances. Three cytokinin-like substances from Ricinus phloem exudate were separated by Hall and Baker (66).

Abscisic acid is known to markedly affect the transpirational status of plant tissues. Hoad (78) detected ABA in honeydew of

aphids feeding on Salix viminalis. Its concentration was reported to be inversely related to plant daylength (9). Zeevaart (194) further demonstrated that not only ABA, but also its two metabolites, phaseic acid and dihydrophaseic acid are translocated in the phloem to the sink tissue, the shoot tips. Hoad (79) reported that under conditions of water stress the ABA level in lupin sieve-tube sap was very high when compared to the ABA levels in leaf, seed and pod tissues. The author suggested the possibility of active secretion of the hormone into the phloem from the leaf based on these results.

Eschrich (42) reviewed the unidentified hormonal factors reported to be translocated in the phloem. Included in these factors are a cold-hardiness factor, a leaf factor necessary for successful graft unions, a factor for root assimilation of certain substances, and the floral stimulus.

Many herbicides and synthetic growth regulators are phloem mobile. This mobility in the phloem is neither correlated with the water solubility of the herbicides, nor with the rate of their metabolic degradation, nor with their degree of chlorine substitution. However, the presence of carboxyl groups on the molecules appears to be closely related to their phloem mobility. There are several exceptions whereby compounds are mobile but lack a carboyxl function. In such cases their products of degradation may be responsible for their mobility. Alar (B-995) and morphactins such as chlorfurenol contain carboxyl groups and are mobile in the phloem. AMO-1618 and CCC are not carboxylated, but their phloem mobility has not been investigated (199).

Nonautotrophic plant tissues, such as roots, require the supply of certain vitamins from leaf tissue. Based on tissue culture and girdling experiments Ziegler and Ziegler (201) showed that thiamine, niacin, pantothenic acid, B6-complex vitamins including pyridoxine and its derivatives are transported in the phloem. Riboflavin, biotin, folic acid, vitamin B_{12} and "Crithidiafactor" may occur in phloem exudate, but only in very small amounts. Inositol, which functions in biosynthesis of galactose-containing oligosaccharides and as a pectin precursor can be found in phloem exudate in high concentration. Ascorbic acid, dehydroascorbic acid and diketogulonic acid have also been detected in considerable concentration (201).

Numerous organic-phosphate compounds and phenolic compounds have been detected in small quantities in sieve tubes and phloem exudate. The fact that they also accumulate above a girdle adds credence to the claim that they are phloem mobile and not simply contaminants from adjacent, damaged cells.

Inorganic substances are also present in the phloem. Bukovac and Wittwer (13) studied the mobility of mineral elements in the phloem and categorized them according to mobility. Potassium is the predominant cation in the phloem and other alkali metals such as sodium, rubidium and cesium are likewise phloem mobile with the exception of lithium. Magnesium is readily mobile in the phloem, while calcium, strontium and barium are not.

Phosphate appears to be the predominant anion although chloride is present in comparable concentration in Ricinus (66). The

abundance of phosphate determines the low concentration of cations which form phosphate salts of low solubility. The abundance of potassium and phosphate appears to largely account for the generally alkaline pH of sieve-tube sap. Most recent studies indicate an absence of nitrate in phloem exudate.

Heavy metal nutrients including manganese, iron, zinc, copper, molybdenum, cobalt, aluminum and titanium have all been detected in phloem exudates of some species and are generally classed as relatively mobile elements.

Calcium, boron, and lead are particularly immobile elements. Calcium is an element important to plant growth and the Ca/K ratio in an organ is indicative of the balance between phloem supply and xylem supply to the importing tissue. Boron is likewise an essential nutrient which must be taken up in the xylem owing to its phloem immobility. Under very humid conditions, newly-developing leaves may become deprived of adequate boron. Lead is a significant air pollutant resulting from gasoline combustion, and as in the case of calcium, the phosphate salt is very insoluble in water and cannot enter the sieve tubes.

While viruses are not normal constituents of phloem sap, certain virus particles, owing to their size, can occur in the sieve tubes, while others move from cell to cell via the plasmodesmata.

Upon reviewing the many different substances of varying molecular weights, chemical substituents, charges and shapes which move

along with water in the phloem, it becomes evident that the phloem transport mechanism is based on mass flow driven by an osmotic gradient.

Mechanism of Translocation

Early Observations

Both the tissue of translocation and the mechanism of translocation have been studied concurrently. De Vries (173) in 1855 felt that diffusion down a concentration gradient was much too slow a process to account for the quantity of material translocated per unit time and transverse sectional area. Having observed protoplasmic streaming in many species, he suggested that perhaps protoplasmic streaming might supply a driving force for translocation. Support for this view came from the work of Curtis (36) in 1935. While he strongly advocated the protoplasmic streaming theory, the role, if any, of this phenomenon in phloem transport has yet to be demonstrated. More recently, Thaine (154) has reported observing transcellular streaming in sieve tubes and Canny (15) calculated that the energy required to drive protoplasmic streaming at phloem transport rates compares well with the experimental values of sugar transported per unit time. Nevertheless, the general objections are strong. The transcellular streaming observation has not been clearly confirmed. Esau et al. (39) argued that such transcellular streaming is highly improbable. Yet, like Thaine, Parker (125) also observed some type of transcellular strands. Transcellular fibrillar or tubular material was observed by Evert and Murmanis (50), but these fibrils may

be very different from what Thaine observed (208). The velocity generally recorded for protoplasmic streaming is only 10-20% of the velocity of a radioactive front moving through the phloem. Lastly, applied substances should be distributed evenly throughout the plant if protoplasmic streaming is the motive force, yet observation simply does not bear this out.

There were others who, unable to reconcile specific mass transfer with diffusion in the phloem, advocated the xylem as the tissue of transport. Birch-Hirschfeld (7) calculated that diffusion was too slow to account for transport and insofar as protoplasmic streaming did not accelerate transport in her experiments, she suggested the xylem as an alternative route. Dixon and Ball (37) also offered the xylem tracheae as the transport conduit since they assumed that the flow of a 10% sugar solution at 40 cm h^{-1} could not be accomplished in the phloem. Similar calculations made by Mason and Maskell (110) showed that the observed specific mass transfer could not possibly be accounted for by diffusion, which encouraged speculation as to alternate mechanisms.

In 1927, Münch (121) described a physical system which, when applied to the plant, would seemingly account for many of the observations in the translocation literature. He envisioned two osmotic cells connected via a channel through which a solute concentration gradient would be maintained. If one cell were at a higher concentration than the other, a mass flow would result provided water exchange and return could occur through the semipermeable cells. The

products of photosynthesis would maintain the high concentration in cells which would connect via the sieve tubes to meristematic or storage cells, with water returning through the xylem. In short, a gradient of decreasing turgor pressure would exist between a source region and a sink region. His proposal generated much interest, and its virtues are even currently the subject of debate in the literature (144).

Solute Translocation Independent of Solvent Water

While any mass-flow theory requires the movement of solute molecules along with the solvent water, other theories espouse the transport of solutes, independent of the solvent water, by some active process. This differentiation can be useful in examining the experimental evidence.

Protoplasmic streaming is one theory according to which the solutes move independently of the solvent water. The bases of and objections to this theory have already been reviewed (see pp. 11-12).

Van den Honert (170) proposed that phloem transport may be explained in terms of rapid movement at interfaces along a concentration gradient. His proposal was based on the observation that oleate spreads rapidly at the interface between water and ether. There is no evidence to indicate that one may generalize from this case to the many different molecules translocated in the phloem.

The theory put forward by Canny (16) was based on Thaine's transcellular strand observation. He postulated that half of the

strands move in each direction and carry dissolved substances in equilibrium with the vacuolar solutes. The transport follows a concentration gradient. It is essentially a diffusion theory accelerated by protoplasmic streaming or contractile proteins. The same objections to the protoplasmic streaming theory also hold in this case in addition to questions regarding the nature of the proteins in the sieve tubes.

Two important questions have been asked by researchers in an attempt to establish if solutes flow with the solvent water or independently and thus approach the mechanism of translocation. First, does simultaneous bidirectional transport occur within a single sieve tube? If it did occur, then obviously the solutes are moving independently of the solvent. Second, are there solutes which move in the same sieve tube at different velocities? If the answer is in the affirmative, then once again it would appear that solute and solvent must move independently.

Biddulph and Cory (5) found that both ^{3}H-water and ^{14}C-sugars move together in a single conducting bundle. Trip and Gorham (164) followed simultaneous movement of ^{14}C-sugars and ^{3}H-water in the phloem of squash plants and both were blocked by steam girdling. These and similar evidence would argue against bidirectional transport. However, Gage and Aronoff (54) were unable to detect ^{3}H-water movement in the phloem. Furthermore, bidirectional movement in phloem has been reported by Ho and Peel (76), Peel et al. (178), Canny and Askham (17), and Eschrich (41). In interpreting the above

results, it is imperative to ascertain if: (a) the observed transport is occurring in the very same sieve tube; (b) the observed transport is not movement occurring in the same sieve tube, but from opposite directions to a common sink such as an aphid stylet; and (c) the ^{3}H-water often used in such experiments exchanges through the sieve tubes resulting in dilution and, therefore, poor detection. These reservations render the interpretations inconclusive. The entire question of bidirectional transport was addressed by MacRobbie (107) and reviewed most recently by Eschrich (43).

There exists much evidence for the nonspecificity of transport in the phloem. Virus particles, various ions, herbicides, and hormones all seem to be carried together. The results of differential translocation experiments, however, as was the case with bidirectional transport experiments, are subject to alternate explanations. When conducted over long periods of time, one may actually be dealing with both phloem and xylem circulation. Altered sink activities in inhibitor studies may account for apparent differential translocation. Cataldo et al. (19) have demonstrated that ^{3}H-water is very mobile and is subject to rapid local diffusional exchange and adsorption during translocation through the sieve tubes. This, too, can account for the appearance of differential tracer translocation.

Mass Flow

While the results of the simultaneous and bidirectional transport experiments are equivocal, most other evidence points to a mass-flow system. In Heracleum montegazzianum, the vascular strands lie

near the surface of the inner cavity of the petioles such that the phloem can be removed from the xylem and ground tissue. Ziegler (198) surgically separated xylem from phloem, but removal from its osmotic environment complicates the picture. Respiration of the isolated bundles increased dramatically. Ziegler and Vieweg (200) isolated such a phloem strand and applied a heat pulse whose movement was detected by a thermocouple located some distance from the source. It seemed that the heat pulse was carried by mass flow. But Hoddinott and Gorham (81) noted that even slight disturbances of the vascular strands of H. montegazzianum lead to a loss of translocation.

Kursanov (99), studying the movement of radioactive tracer fronts as a measure of translocation velocity, recorded velocities of up to 200 cm h^{-1} which are clearly too rapid for diffusion and probably indicate some form of mass flow. Zimmermann (207) studied translocation velocity by following the progress of oligosaccharide ratios in phloem exudate along the trunk of actively photosynthesizing trees. He, too, found that high velocities can be achieved, pointing again to a mass flow.

In a very creative study, Walding (174) demonstrated mass flow using a willow stem without leaves or roots. While the ends were submerged in water, ^{32}P was applied to a region of abraded bark near the upper end, and radioactivity was monitored some distance from the source. Little movement was noted over one week. Following the establishment of a feeding aphid further down the stem, in a few hours the honeydew from the aphid already contained radioactivity.

Apparently the turgor pressure had dropped as a result of the feeding and a mass flow resulted.

Assuming that phloem transport is a solution flow, water would return to the leaves via the xylem when sugars are removed at a sink such as storage tissue in a tree. By enclosing a downward-pointing bark flap in plastic, it is possible to collect water provided that transport continued. Continuation of phloem transport was confirmed by formation of wood on the cambium side (12).

That phloem transport occurs via a solution flow can be best concluded on the basis of the observed aphid stylet exudation rate and a few simple calculations. Typical values of exudation rate from the stylet of Longistigma caryoe Harris inserted in a single sieve element are 2-5 $mm^3 h^{-1}$ or about 0.001 $mm^3 sec^{-1}$. A typical sieve element has a diameter of 0.025 mm and a length of 0.35 mm--a volume of about 0.0002 mm^3. Clearly, then, it must deliver five times its own volume per second. The only way to account for refilling five times per second is by a flow mechanism (207). On this point most are agreed in contrast to the controversy surrounding the mechanism providing the force for this flow.

Zimmermann (203), in an attempt to order the evidence for a pressure-driven, mass-flow mechanism, established three criteria: (a) the sieve tube system must be semipermeable with respect to the apoplast. That is, the longitudinal walls must be lined with a semipermeable membrane to prevent leakage of osmotically active molecules. Both entry and removal of these solutes must be the result of active

loading and unloading. (b) the sieve plates between elements must be permeable to the transported solutes. (c) the turgor must be positive in the direction of flow. In other words, a turgor gradient must exist from source to sink.

The first criterion has been met numerous times. Currier et al. (34) demonstrated plasmolysis in sieve elements which indicates the presence of a semipermeable membrane. Weatherly et al. (178) perfused xylem tissue with increasing concentrations of a mannitol solution. As the osmotic concentration was raised, the concentration in the adjacent sieve tubes also increased and the rate of exudation decreased reflecting the drop in turgor pressure. Clearly the walls are bound by some semipermeable membrane and the sieve elements act as osmotic cells.

Weatherly et al. (178) further performed razor-incision experiments on a stem containing an exuding aphid stylet. Longitudinal cuts made a distance of two cm away from the stylet had very little effect when compared with transverse cuts made ten cm above the stylet which resulted in the immediate drop in exudation rate. These latter results provide support for meeting the second criterion. In other incision experiments Zimmermann (205) studied the concentration of sugar in sieve tubes and observed dilution of the sugars with time, apparently the result of dilution by water from the surrounding apoplast. In a later study (206) he was able to show, using double incision experiments, that exudate flows axially toward the incision from above and below. Thus both the criteria of lateral semipermeability and longitudinal permeability have been met.

There have been several attempts to answer the question of turgor gradients from source to sink. Huber et al. (82) obtained sieve-tube exudate by incision and measured in Quercus rubra L. a concentration gradient of 0.01 mole m^{-1} or about 0.2 atm m^{-1}. By defoliation treatments, Zimmermann (204) was able to manipulate the concentration gradient in sieve-tube exudate. Defoliation resulted in removal of the sugar source and lowering of the xylem tension. Both of these processes caused a decrease in the exudate concentration in the tree. The total molar concentration gradient, which was positive in the direction of flow prior to defoliation, disappeared when the leaves were removed, presumably when transport ceased. This would seem to indicate that turgor pressure is the driving force for translocation in the sieve tubes. Hammel (67) attempted to directly measure sieve-tube turgor pressure in red oak using a syringe-type manometer. He found a pressure gradient on the order of 0.2-0.4 atm m^{-1} which seems to match well the theoretical estimates for turgor-pressure gradients (208). Further confirmation of such values is needed before there is general acceptance of these data as definitely meeting the third criterion.

Resistance to Flow

If the driving force behind the solution flow of translocation is turgor pressure, as posited by the pressure-flow hypothesis, then the resistance to flow in the sieve tubes must be of a magnitude which can be overcome by the turgor-pressure gradient. Furthermore, it must be small enough to account for the observed flow rates.

It is not possible to accurately calculate the resistance to flow in sieve tubes. At best, it can only be approached by the Hagen-Poiseuille equation which describes the theoretical steady-state laminar flow through ideal capillaries. It states the relation connecting the rate of change of pressure (dp) with distance (dx) along a long, circular, horizontal tube of radius (r) containing a fluid of viscosity (η) flowing at a volumetric rate (Q) per unit time.

$$\frac{dp}{dx} = -8\eta Q/\pi r^4$$

Accordingly, a sap solution of 20% sucrose, i.e., about 2 centipoise viscosity at room temperature can be driven by a pressure gradient of 0.1 atm m^{-1} up to 1000 cm h^{-1} through a 30μ diameter capillary. In considering the parameters of sieve tubes, however, they are neither long, nor consistantly circular or horizontal tubes whose "radius," viscosity and flow rate are continually changing. Since the radius term is raised to the fourth power, the equation becomes very sensitive to changes in the circular cross section. In short, they are nonideal capillaries. The above equation is based on a paraboloid flow with the peak velocity on the axis of the tube and an hypothetical zero velocity at the tube wall. However, such a paraboloid flow can result only when the tube length approaches 50 times its diameter (208).

There are, however, modifications of the basic equation such that it may apply to nonideal capillaries (123). They include a

coefficient for cross-section shape other than circular (for particular shapes only), nonhorizontal tubes, the internal surface of the tube, and a calculation of the Reynolds Number, a dimensionless value which defines the flow as either turbulent or laminar. There are also modifications which take into account tubes having a variable cross section and even variable flow and viscosity under some conditions.

The greatest obstacle in applying such equations to flow in sieve tubes is the presence of the sieve plates. The extent of resistance introduced by such structures is a matter of current controversy. In another case of resistance to flow, Münch (122) found that in tracheids of Abies the hydraulic conductivity was 1/3 that of ideal capillaries of the same diameter. This fact would require the tripling of the calculated pressure gradient in the Hagen-Poiseuille equation, if, in fact, the situations are analogous, i.e., bordered pits as compared with sieve pores. Since the question is one of structure particularly with respect to the sieve pores and their in vivo condition, anatomical evidence is often cited in the support of a particular position. Insofar as artifacts are not uncommon in the anatomical literature, care must be exercised in the assessment of anatomical evidence.

Exudation from phloem, which, in some cases, can be maintained for many hours, even in water-stressed tissue, is a clear indication that the sieve-tube contents are under pressure. Sudden release of this pressure causes a surging in the sieve tubes which is likely to

result in displacement of structural material and give an altered picture of the cell anatomy. Therefore, the success of an anatomical investigation, particularly of sieve pores, often rests with the fixation technique. The original chemical fixative for E.M. work, $KMnO_4$, has generally been replaced with buffered glutaraldehyde followed by OsO_4 post fixation. Evert (49) outlined several criteria for acceptable E.M. work in structure-function studies. Thaine (157) claims that even the currently standard fixation procedure fails to preserve cytoplasmic strands in phloem exudate. Another criticism of fixation technique was posed by Kidwai and Robards (92). They noted that while most phloem exudate has been measured at about pH 8.0, the fixation buffer is often 1.0-1.5 pH units lower, which may be critical in terms of surface charge and membrane separation.

Much of the work related to the question of the degree of occlusion of the sieve tubes in vivo was reviewed and critically assessed by Spanner (144). It is largely on this point that the various theories hinge. If the sieve pores are open, then pressure flow is clearly a viable mechanism. If they are mostly occluded, the resistance to flow would be too large for the pressure generated. If, however, there is just some structural material, such as filaments, traversing the pores, the feasibility of a pressure-flow mechanism would depend on filament spacing (177). Because the appearance of the pores varies according to species (33), environmental condition of the tissue (61), and fixation techniques (49), it is difficult to make any universal statement with our present

knowledge. This remains a major limitation to progress on conclusively establishing a mechanism of phloem translocation.

Other Theories

The hypotheses of Canny--accelerated diffusion, and Thaine-transcellular strands--neither based on mass flow, have already been addressed. Other models, while based on a flow of solution propose some source, other than pressure, as the driving force of translocation.

Spanner (143) proposed a theory based on electroosmotic flow. He envisions an electrically-polarized sieve plate maintained by a metabolically-driven ion circulation (of K^+ ions, for example) with ions being recirculated via the companion cells. Bowling (10), using microelectrodes, measured a potential across the sieve plates of Vitis vinifera. This finding lends some support to such an electroosmotic theory. Fensom (51) independently proposed the same type of electroosmotic theory. Since then he has withdrawn his electroosmotic theory (52).

Role of Metabolism

While there is general agreement that translocation is dependent, at some point, upon metabolic energy, the pressure-flow theory, unlike others, proposes no metabolically generated force along the path. Rather, translocation is driven by activities at source and sink, i.e., vein loading and unloading, which are clearly metabolic (2). A recent report of Chamberlain and Spanner (29)

claimed apparent unloading despite chilling of the sink to 0°C. The authors followed ^{14}C-transport from a mature Saxifraga sarmentosa via a stolon to a developing plantlet. Unfortunately, the radioactivity was expressed on a per segment basis, rather than on a weight-of-tissue basis. This could easily account for the apparent accumulation of counts in the relatively heavy plantlets. Loading and unloading are undoubtedly metabolically driven.

There exists, then, a means of testing the validity of the assertion that path metabolism is not directly responsible for translocation. There are several methods used to interrupt path metabolism. Anoxia, metabolic inhibitors and low-temperature treatments have all been used as probes. There are problems with these techniques which render their effects somewhat nonspecific or nonlocalized and the results of such studies equivocal.

Anoxia Effects

Treatment with nitrogen removes oxygen as the terminal electron acceptor for respiration, inhibiting oxidative phosphorylation. While the objective is to decrease ATP levels, anoxia also may affect membrane permeability and electrical conductivity. In addition, some products of anaerobic metabolism, which are toxic, will eventually accumulate. There are other factors which may influence the effects of anoxia which include extent of O_2 depletion, presence or absence of light, stomatal aperture, duration of treatment and capacity to remove toxic byproducts.

Mason and Phillis (111) found that O_2-deprivation had no effect on phloem translocation until drastic conditions were imposed. Vernon and Aronoff (172) were also unable to detect inhibition with anoxia. Ullrich (168) confirmed these earlier results. Qureshi and Spanner (130), however, flushed Saxifraga stolons with nitrogen over 20-30 cm lingths and obtained a reversible reduction in ^{137}Cs and ^{14}C-translocation. Callose formation did not appear to be involved. A transient inhibition of assimilate transport was reported by Sij and Swanson (141). They suggested that perhaps the energy generation blocked by anoxia may be required only for sieve-tube maintenance as suggested earlier by Garner and Peel (56). Full recovery was obtained within one hour, while 24 h of O_2-deprivation resulted in further inhibition of translocation, probably a consequence of tissue damage. The available evidence generally seems to favor no direct role for path metabolism in phloem translocation.

Inhibitor Studies

Respiratory inhibitors and other metabolic poisons have also been used to study the role of path metabolism in phloem translocation. Here the difficulties were twofold--localization and non-specificity. Substances such as 2,4-dinitrophenol, azide and cyanide are mobile in phloem tissue and may not remain confined to the localized zone of application. If they affect either source or sink, translocation will surely be perturbed. Therefore, an apparent inhibition of translocation may not reflect any role of path metabolism. In addition, agents which alter the energy state of tissues

may also have effects which are nonspecific. For example, 2,4-dinitrophenol (DNP), is generally used because it uncouples phosphorylation from terminal electron transport, yet if it affects membrane permeability, leakage to the xylem may occur and the inhibitor may reach the leaf blade. In short, if an effect is observed, it still does not prove translocation to be dependent on path metabolic energy.

Willenbrink (185) isolated the central vascular bundle of Pelargonium petioles and treated them with respiratory enzyme inhibitors. The observed inhibitory effect of cyanide was reversible. Here the objections of nonlocalization and nonsepcificity may hold. Duloy et al. (38) cited inhibitor results as consistently below 50% and probably due to either effects on source and/or sink or inhibition of companion cell function. Ullrich (169) found that treatment with cyanide blocked translocation and that this treatment correlated with an increase in callose, although removal of the cyanide restored translocation despite the remaining high callose levels. Callose is a β-1-3 glucan, a structural carbohydrate, which is often associated with sieve pores, particularly when the phloem is cut. This response has suggested callose as a plugging material for damaged sieve tubes. It was reported by McNairn and Currier (112) that warming the hypocotyls of cotton to 40°-50°C over a 4 cm zone for 15 min resulted in callose formation and a halt in translocation. After several hours the callose diminished and translocation was restored. Canny (16) was unable to reproduce these results. Willenbrink (186) reported

blockage of phloem transport by cyanide treatment and suggested that either the path is actively transporting, or some cyanide-sensitive system is crucial to the maintenance of open phloem tissue for normal translocation. Harel and Reinhold (72) re-examined the effect of DNP on phloem translocation. They found no effect when care was taken that the inhibition did not interfere with vein loading. Ho and Mortimer (77) re-examined cyanide inhibition of translocation. The inhibitor, when applied to the path tissue as pretreatment, ultimately reached the xylem and depending on the direction of water flow, reached the source or sink inhibiting photosynthesis in addition to translocation. Without pretreatment photosynthesis was not affected, presumably the inhibitor was diluted, yet translocation was blocked. The effects of inhibitors on rates of sugar exudation via aphid stylets, and ATP levels in willow were studied by Gardner and Peel (56). Oligomycin and DNP both caused a rapid drop in exudation rate with no prior effect on ATP levels. Using sodium fluoride, a glycolysis inhibitor, in only two out of nine replicates did they note a drop in ATP concentration prior to an effect on exudation. They concluded that the energy state of the tissue may only indirectly influence translocation. Qureshi and Spanner (131), who take issue with the methodology of Harel and Reinhold (72), treated Saxifraga stolons with DNP and noted a blockage of transport, although they found no callose accumulation, which is a finding difficult to reconcile with pressure flow. As with anoxia, the results with chemical inhibitors are varied, but in view of the difficulties of the techniques, it appears that

most of the evidence speaks in favor of perhaps some indirect role for path metabolism in translocation, e.g., cellular or organelle maintenance.

Low-Temperature Inhibition

Low temperature is perhaps the most desirable of the metabolic probes. Since most metabolic processes cease at low temperatures, it can be a reversible, nondestructive technique if the temperature is maintained above freezing. It is not without some difficulties, however. While penetration of the low temperature is assured, cooling the phloem will inevitably chill the xylem water as well. This cooled water may reach the source leaf and affect photosynthetic carbon fixation or other source processes such as phloem loading. Both Webb (181) and Wardlaw (175) noted a small delay in vein loading resulting from path cooling although the CO_2 fixation rate remained constant. Webb and Gorham (183) monitored photosynthesis during chilling of a squash node and found no effect of the treatment on photosynthesis. These processes, which may be affected by chilling, are easily monitored. Nonmetabolic effects can also result. Low temperature, besides reducing the turnover rate of ATP or other energy intermediates, may alter membrane fluidity, increase solution viscosity, affect water relations or produce cellular structural changes.

If either sieve pores are densely plugged, or the Münch pressure-flow hypothesis rejected for another reason, one would need to postulate some metabolic pumping system along the path rather than a passive, pressure flow. If the energy for such a process were respiratory in origin, it would be sensitive to low temperature.

Probably the earliest report of localized, low-temperature experiments was that of Child and Bellamy (30) who noted an inhibitory effect on translocation by chilling a few centimeters of the translocation path in Bryophyllum. Curtis (35) cooled the stem of Phaseolus vulgaris and reported reduced transport at 2-5°C but not above 6-8°C. He concluded the existence of a definite threshold for low-temperature inhibition. A further interesting note was that inhibition tended to lessen with the duration of treatment, which may have been the first observation of transient inhibition and subsequent recovery. This topic will be examined in the following section. Swanson and Böhning (148) similarly noted that at 10°C the translocation rate, initially inhibited, gradually increased with time. The same translocation behavior following path cooling was reported by Swanson and Whitney (149) for ^{32}P, ^{42}K, ^{45}Ca and ^{137}Cs. Mortimer (116) completely blocked translocation in soybean by cooling petioles to temperatures slightly above 0°C. Thrower (159) also using soybean, stopped transport at 0-3°C; transport recovered upon return to higher temperatures, although the recovery time varied. Webb and Gorham (183) inhibited translocation by chilling a node of a squash plant to 0°C. Owing to the lag time of recovery, they concluded that the inhibition could not be due to increased viscosity only. An increase in callose plugs in sieve pores in response to low night temperatures was reported by Majumder and Leopold (108). While the frequency was proportional to the length of treatment in bean, no

effect was observed in tomato. This hinted at structural changes rather than metabolic ones in inducing translocation blockage.

Webb (180) found that all parts of the translocation path--stem, nodes, hypocotyl and petiole--all show the same response to low temperature. He further studied the effects of a broad range of temperature on translocation in Cucurbita melopepo and determined an optimal translocation temperature. While all parts of the translocation path may respond similarly, organs of different ages may not. Ford and Peel (53) cooled willow stems, labelled with ^{14}C--assimilates, to 5°C. Young shoots, 3-5 weeks old, showed an 85-98% reduction in translocation over controls, while mature stems, 2 to 3 years old, showed no reduction, but rather up to a 5 fold increase in translocation over controls!

There also exist reports of little or no effect of low-temperature on translocation. Tammes et al. (153) studied the effect of cooling on phloem exudation in Yucca. There was little effect on longitudinal transport as evidenced by exudation from the inflorescence stalk which continued for 24 h at 0°C. The low temperature did stop radial movement and lateral transfer in the stalk. Because lateral loss from the assimilate stream was blocked, the solute content of the exudate rose as a result. Weatherly and Watson (179) lowered the temperature of 10 cm sections of willow stem to 4°C and found that this did not significantly affect translocation, although respiration was inhibited 95%. They even precooled the stem for 18 h in an attempt to exhaust the residual ATP

supply. Using Lolium, Wardlaw (175) obtained results similar to those of Tammes et al. (153). He subjected a leaf zone to 0°C and noted very little effect on translocation, although it reduced the lateral transfer to 1.5-2.0% of the controls. Like Webb (181), he found that the low temperature delayed vein loading slightly, yet not assimilation in the leaf.

In order to reconcile the ineffectiveness of low temperature observed in some cases with the proposition that metabolic pumping along the path may be required, an explanation offered is that while pumping in the small, cooled region is incapacitated, the pumping mechanism above and below may compensate and prove adequate for continued translocation. Lang (103) attempted to test this contention by cooling a long (30 cm) section of a petiole of Nymphoides peltata. He found that cooling temporarily blocked translocation until a new steady state was reached. He calculated a Q_{10} of 1.2 for the difference between the two steady states. A change in viscosity alone would predict a Q_{10} of 1.35, but insofar as only 50% of the petiole was treated, a Q_{10} of 1.17 was derived which closely approaches 1.2. In a similar study Watson (176) cooled 65 cm of a willow stem (most of the path) to 0°C and found that there was neither an effect on distance travelled by a radioactive tracer, nor on the total quantity of label transported.

It is clear that thus far localized low-temperature experiments have yet to provide an unequivocal answer to the original question posed regarding the role of path metabolism in translocation.

The variation in response to low-temperature treatment as well as the documentation of a transient inhibition with resultant recovery may provide some insight into the remaining question.

Transient inhibition and recovery.--Swanson and Geiger (150) lowered the temperature of 2 cm of a sugar beet petiole to 1°C and prepared a time course of inhibition of sucrose translocation. An inhibition of 50% occurred within the first 15 min. However, within an hour of the continued low temperature, translocation returned to pre-cooling levels. Re-warming had no further effect. This type of study is generally conducted by following radioactively labelled sugars. Bowling (11) followed the uptake of K^+ by the roots as a measure of the sugar translocated there. When a 12-cm zone was chilled, a rapid decline in K^+ uptake resulted. But, he also noted that often the initial inhibition was followed by a recovery over several hours. There are, perhaps, several possible explanations for this phenomenon. One could speculate that changes are invoked at the source and/or sink which result in a steeper gradient and, hence the recovery. Perhaps there is an increase in viscosity followed by a subsequent reversal. A temporary stomatal closure and the consequent drop in photosynthesis could produce a similar effect. In line with the theories espousing a role of metabolism along the path, such results could be explained as some process of cold adaptation which restores the requisite energy supply. Perhaps there is a disruption of membrane structure and rearrangement with time. Lastly,

some physical blockage may result from the chilling, which over time is removed.

These possible explanations have been investigated. Coulson et al. (32) maintained sugar-beet petioles at temperatures between 0.7 and 2.5°C. After a transient inhibition translocation returned to normal levels in 2-3 hours. Neither respiration nor ATP levels recovered along with translocation. The authors concluded that energy may be required for structural maintenance, but not for translocation. They further demonstrated that the low-temperature treatments did not cause stomates to close and decrease photosynthesis. Using pulse-labeling techniques with sugar beet Geiger and Sovonick (60) established that the recovery of translocation was not the result of an increase in rate of source loading, but rather the result of the restoration of velocity. Similarly, Gardner and Peel (56) found that the recovery in willow is not due to an increase in sucrose or solute concentration. The data of Swanson and Geiger (150) and Coulson et al. (32), cited earlier, indicate a greater effect than can be accounted for by viscosity changes alone. But in the system used by Lang (103) the change in viscosity alone could account for the Q_{10} calculated from transport rates prior to and following cooling. The notion of membrane disruption and recovery to account for transient inhibition remains to be substantiated particularly with respect to the differential responses to be discussed next.

Geiger (58) had taken particular note of the differential species response to low-temperature treatments. Translocation in

chilling - insensitive species such as willow or sugar beet are unaffected by low temperature or transiently affected, respectively. In chilling-sensitive species such as bean, low temperature will interrupt translocation usually without recovery. Webb (181) studied the effect of long-term cooling on translocation in Cucurbita melo-pepo, a chilling-sensitive species. After 5 h at 15°C translocation had recovered to 12% of controls; by 19 h an 85% recovery was observed. Apparently, even in chill-sensitive plants, recovery may occur, but over a longer priod of adaptation. Geiger (58) has even cited ecotypic differential responses to chilling. Cirsium arvense from Montana responds to low temperature as does sugar beet, while a southern ecotype from California behaves as does bean.

Q_{10} analyses can be informative with regard to the type of processes involved. A Q_{10} value between 1.2 and 1.5 generally implicates some physical process responsible for the change, such as viscosity or diffusion. A higher Q_{10} value of 2.0 to 4.0 generally reflects a thermochemical basis for the change, e.g., enzymatic. A Q_{10} value above 4.0 usually indicates disruption of the system, e.g., protein denaturation or cellular damage (61). Giaquinta and Geiger (62) noted a threshold temperature, 10-12°C for chilling-sensitive and 0°C for chilling-insensitive plants, above which the effect of temperature on translocation, i.e., mass transfer and velocity of ^{14}C-assimilates, can be understood as viscosity changes in light of a Q_{10} of 1.3. Below 10°C in bean a Q_{10} value of 6.0 was noted. This supports their contention that a physical plugging of

the sieve pores occurs which blocks translocation. There is a delay of about half an hour prior to the resumption of translocation upon rewarming. This further supports the idea of a physical blocking and unblocking. Further support derives from their experiments of following protein body displacement resulting from pressure release. At 25°C protein body displacement toward the incision was observed several cm on each side of the incision, whereas in chilled segments, displacement only occurred at a few mm on either side. In an attempt to visually substantiate this view of physical blockage, they presented electron micrographs of sieve plates of bean petioles maintained at various temperatures. They were prepared by rapid freezing and freeze substitution. Tissue at 0°C appeared to have occluded sieve pores, whereas the sieve pores of both the control tissue and tissue cooled one hour and rewarmed for 1.6 h appeared unoccluded. If, in fact, such a blcokage can explain the translocation results, it is unfortunate that the authors did not show micrographs of similar tissue in sugar beet. Are the sieve pores occluded during the initial inhibitory period? Do the occlusions disappear during recovery? The results of such controls would have been very useful in fully assessing their theroy. Of all the possible explanations for the observed transients, a temporary physical blockage in response to cold appears most consistent with physiological observations.

Floral Stimulus Translocation

Recognition of a Flowering Signal and a Transmissible Floral Stimulus

The marked transition from vegetative to reproductive growth in response to some flowering signal was intimated by Tournois (162) early in this century. He noted, after studying seasonal and light effects on the flowering behavior of houblon japonais (160, 161), the flowering response to short periods of daily illumination and that the seasonal flowering response is due to lengthening of nights, rather than shortening of days (162). The actual hypothesis of photoperiodism in flowering was stated and tested by Garner and Allard (57). Subsequent research dealt with the nature of the photoperiodic requirements which proved to vary with genus, species, and even intraspecifically. Of interest were the site of photoperiodic perception, the mechanism of translation of photoperception to developmental change and the translocation of the apparent message over distance within the plant.

Knott (93) reported that in spinach, leaves are the site of photoperiodic perception. He postulated the existence of a leaf-generated stimulus which is translocated to the apical tip, causing flowering. Chailakhyan (20) presented evidence for a transmissible stimulus by localized induction. He also established (21), using Chrysanthemum, that the leaf rather than the apex is the site of photoperiodic perception. Clearly, a transmissable signal is implicated if the sites of photoperiodic perception and floral evocation

are spatially separated or if the stimulus can be transferred from in induced donor to a noninduced receptor via a graft union.

It was established that the signal to flower constituted short days (more specifically long nights) in some plants and long days in others. Even long-short day and short-long day requirements were discovered, which required both treatments in a specified sequence to promote flowering. Within each individual class of photoperiodic plants there is great variation as to the number of photoperiodic cycles to achieve the maximal flowering response. Those plants which had an apparent insensitivity to all tested photoperiodic cycles, and yet flowered upon attaining a particular developmental stage were termed day-neutral plants. There have been many attempts to study the similarity of the floral stimulus in the various response types and perhaps establish the universality of the floral stimulus. This has been done by grafting induced and noninduced partners of different response types. The reviews by Lang (100) and Zeevaart (193) thoroughly covered the documented cases of successful transfer of the floral stimulus via grafts between different photoperiodic response types. Often the barriers to such experiments are either the incompatibility of the graft partners or the technical difficulty in maintaining the plants under the different photoperiods. It has been shown possible to effectively transfer the stimulus even between species of different families. There are also reports of noninterchangeability within the same species with graft compatibility (63). The floral stimuli from long-day and short-day plants have also

proven functionally interchangeable. In the case of plants which require exposure to more than one type of photoperiod in order to flower, the question has been raised whether different transmissible stimuli are generated following each type of treatment or whether a single floral stimulus results only following both types of photoperiodic treatments. The latter was shown to be the case in Cestrum nocturnum, a long-short-day plant, by Sachs (134) who found that the product, if any, of the long day was not translocated.

Floral Stimulus Movement

Tissue of Floral Stimulus Transport

The movement of the floral stimulus has been intriguing, particularly since it is so easily demonstrated, yet chemical isolation has proven so enigmatic. There is much convincing circumstantial evidence that the floral stimulus travels in the phloem tissue. In 1934 Bennett (3) was able to show that the sugar-beet curly-top virus, known to be localized in the phloem of infected tissue, was able to pass from the scion of a tobacco graft to the uninfected stock only after 5-6 days--which is the length of time necessary for the differentiation of continuous phloem. Bennett's data on virus transmission was plotted by Zeevaart (182) together with his own data on floral stimulus transmission from Perilla leaf grafts. This showed the striking similarity of these two phenomena, implying transmission of both via the phloem. Similarly, Moshkov (119) reported that the floral stimulus did not traverse a graft union in

Perilla until tissue union was established. Bennett (4) expanded his earlier study and showed that a parenchyma-localized virus could cross the graft union after merely 2 days, i.e., prior to establishment of phloem continuity, while the phloem-localized virus required 5 days to pass the graft union. Heinze et al. (75) found that when soybean was splice-grafted, a graft which maximizes the contact of vascular bundles, 50% of the plants flowered when the donor leaves were removed after 4 days. Although Phaseolus/soybean produced a successful structural graft in that same study, no floral stimulus was transferred, probably because no photosynthate was translocated.

One isolated report by Wellensiek (184) claimed the successful transfer of the floral stimulus from Xanthium to Silene without phloem union and concluded that the stimulus may also move by cell-cell transport. His grafts produced 1/3 of all plants flowering in 4 days and he relied on de Stigter (146), who showed that 7 days or more are required for phloem connection. It would have been even more convincing had he followed ^{14}C-photosynthate in those very same plants, although such graft partners are incompatible and incompatibility is often accompanied by a failure of phloem connection (145). However, Silene whose roots have been removed also flowers in short days (171) and insofar as these Silene plants served as scions, the root removal may account for their flowering, rather than a novel transport mode for the floral stimulus.

Kalanchoë blossfeldiana, owing to its vasculature, has proven to be a useful plant in studies of floral stimulus translocation

through stem tissue. Harder et al. (70) demonstrated that if a single leaf of a decussate leaf pair is photoperiodically induced, an assymetrical inflorescence results at the apex. This seemed to indicate that Kalanchoë contains few lateral vascular connections. Harder (69) followed up this point by introducing a fluorescent dye, berberin sulfate, into a leaf and found that it travelled primarily in the vascular tissue of the stem region adjacent to the leaf. Lona (105) was also able to show that the floral stimulus travels mainly in the region of the stem adjacent to the leaf. Eschrich (40) found a different translocation pattern in Impatiens holsti. He followed the path of fluorescein dye in the vascular system of Impatiens after removing half of the stem bark. The dye moved laterally from the severed vascular bundles via the parenchyma to intact bundles until new sieve tubes were regenerated. Earlier, Chailakhyan (22) had noted that the floral stimulus moves laterally as well as longitudinally. He also presented evidence for the movement of the floral stimulus in Perilla in either the mesophyll tissue or the epidermis (23). He severed the veins at the base of an induced leaf and this treatment did not diminish the flowering response. In experiments with half-girdled stems of Perilla plants, Zeevaart (189) reported that floral buds appeared earlier on the side of the Perilla stem under the intact bark. Floral buds did develop later beneath the girdled side further showing the capacity for lateral transport.

The floral stimulus appears normally to travel over long distances in the phloem with the photosynthate. Chailakhyan and

Butenko (28) followed the fate of a ^{14}C label from induced and non-induced Perilla leaves. The authors showed a clear correlation between a high-flowering response and high radioactivity from the induced leaf and between a low-flowering response and high radioactivity from the noninduced leaf. This seems to be very clear evidence of simultaneous floral stimulus and photosynthate movement. Zeevaart (189) found that even small strands of phloem are adequate for translocation of the floral stimulus. In the same study he showed, using ^{14}C-sucrose, that the first movement of the floral stimulus and the first transport of label across the graft union occurred simultaneously. De Stigter (146) found that in the case of the donor as scion and receptor as stock, the flowering response was much weaker than if the partners were reversed. He found the identical pattern in terms of ^{14}C-translocation. However, unlike Perilla, in Silene armeria he noted that while no functional vessel union existed after 5 d, a small amount of downward translocation resulted after 7 d and bidirectional transport after 9 d, yet a flowering response required three weeks. In the obscure case of Piquera trinervia, Zimmerman and Kjennerud (202) found that the floral stimulus can move only through a few internodes, which may be more attributable to the floral stimulus itself, rather than any limit in the vasculature.

If the floral stimulus travels in the phloem along with the photosynthate, then perturbation of the latter's transport should similarly affect the former. Early studies which pointed to this

phenomenon related to floral stimulus movement in plants exposed to light or dark just following induction. Kudhairi and Hamner (91) found that if the induced Xanthium leaf is removed immediately following the long day, no flowering will result. If, however, the leaf remained attached for 4 h after a 15 h induction or for 2 h after a 20 h induction, flowering resulted. Stout (147) manipulated the translocation of carbohydrate in sugar beets and found that the reproductive stimulus followed the carbohydrate translocation pattern. Skok and Skully (142) noted that floral stimulus export from Xanthium occurred more rapidly in high-intensity light than in light of low intensity. Single-leaved Xanthium plants, when transferred to light following a 14 h induction, flowered even when the induced leaf was removed after 4-6 h, whereas in the dark the same flowering response required 10-20 h prior to defoliation. Transport did, however, occur even in the dark. Their findings confirmed those of Kudhairi and Hamner (91). Salisbury and Bonner (135) reported similar findings for Xanthium. Carr (18) also reported that the flowering response increased with increasing light intensity following induction. Furthermore, he was able to completely replace the light effect by applying sucrose to the induced leaf. He postulated that the stimulus moved in a mass-flow system and that intense light is needed for photosynthesis which provides assimilates. Searle (139) found no added effect of light following induction. Perhaps this was the result of some noninduced leaves which remained on the plants and which may have been able to provide the assimilate for transport.

Tsybul'ko and Yastrebov (165) reported that brief light interruptions may reduce assimilate export from leaves of short-day plants. The authors suggest that this might account, in part, for some of the observed phytochrome-shift effects on floral induction.

Inhibition of Flowering by Noninduced Leaves

It has been widely noted that noninduced leaves in either short-day or long-day plants diminish the flowering response to induction, so much so that standard procedure in such experiments involves removal of the noninduced leaves to maximize the flowering response (190). There are a couple of approaches to explain this phenomenon. The noninduced leaves may be the source of an inhibitory substance, whose effect counters the stimulus of the induced leaf. On the other hand, the noninduced leaves may interact with the overall source-sink apparatus of the plant such that their presence interferes with the optimal translocation from the induced source leaf to the sink receptor bud. Most of the evidence appears to support the latter contention. A study by Moshkov (120) indicated that this inhibitory effect is only exerted by leaves which are mature. Mature leaves are known to export assimilates in contrast to young, developing leaves which are primarily importers of assimilates. Mature, noninduced leaves could be the source of a competitive assimilate stream which would reduce the import from the induced source. In Kalanchoë, for example, the competition of the induced lower leaf is less effective than the noninduced upper leaf since the upper leaf

pair is the main supplier of photosynthate to the apex (192). Chailakhyan (26) showed that this inhibitory effect is limited to those noninduced leaves located between the stimulus source and the receptor bud in Perilla. Harder et al. (71) examined this question in Kalanchoë, whose vasculature is such that the stimulus moves primarily in the vascular tissue adjacent to the source leaf, and found that only noninductive leaves in the same orthostichy will be inhibitory. Such an inhibitory effect can be artificially created by applying a sugar solution in place of the noninduced leaves (27). The same study showed that if these noninduced leaves are present in a critical location then exposure of these noninduced leaves to low-intensity light exclusively will minimize their inhibitory effect. King and Zeevaart (95) showed that the inhibition of noninduced leaves is one of competition of assimilate streams in Perilla and concluded that the floral stimulus moves in parallel with the photosynthate in the phloem. These several cases are consistent with translocation interference.

Not all the evidence can be explained simply on the basis of translocation patterns. Lincoln et al. (104) contended that factors influencing translocation of carbohydrate in Xanthium, such as a carbohydrate-deficient condition, will influence the translocation of the floral stimulus. However, they also suggested that perhaps there is also a specific inhibitor and not only a translocation effect. Zeevaart et al. (197) also using Xanthium established a correlation between transmission of the floral stimulus and

translocation of photosynthate such that much of the long-day inhibition in Xanthium can be explained by translocation effects. However, they could not entirely rule out the possibility of an inhibitor produced by long-day leaves. Evans (45), studying flowering in the grass Rottboellia exaltata, proposed that while the short-day leaves produced a transmissible stimulus, the long-day leaves may either accelerate or inhibit inflorescence development depending on the progress toward complete induction. Evans and Wardlaw (47) studied assimilate transport patterns with respect to flowering in Lolium and found that noninduced leaves did not interfere with assimilate transport. On the assumption that they may be major sinks, thereby diverting the floral stimulus, they found the leaf was only a 1% sink. On the assumption that they may compete successfully with assimilate from the induced leaf, they found no reduction in transport from induced leaf to apex. Ballard and Grant Lipp (1) found that the leaves of Anagallis arvensis were optimally photoperiodic at 2-3 mm^2 in area, only 1/20 of their full size, yet at that stage they are primarily importers of carbohydrate. Their status as importers was inferred and not confirmed by ^{14}C-labeling. Similarly, Evans and Wardlaw (48) found that young leaves of Lolium temulentum, only 14-20% of their full area, were able to induce flowering although they hardly exported assimilate. Evans (46) has long been an advocate of different mechanisms for assimilate and floral stimulus transport. He based this largely on his observation that in Lolium the rate of floral stimulus transport is far slower than is the rate

of assimilate transport. In Pharbitis he acknowledges that the rates of each are similar and concludes that the actual stimuli differ in Lolium and Pharbitis. He further points to the observation that leaves too small to export carbohydrate in Lolium still export the floral stimulus, while in Pharbitis it is primarily the mature leaves which are effective in exporting the floral stimulus.

There exist some convincing reports of flowering inhibitors, although details of these fall outside the scope of this section. Lang and Melchers (101) found that a defoliated Hyoscyamus plant flowered in any daylength, yet if a single leaf was grafted back it then flowered under long days. The authors concluded the existence of a leaf-localized inhibitor resulting from short days. After Hartmann (74) published his paper on floral stimulus movement in strawberry from an induced mother plant to a noninduced plantlet via the stolon, Guttridge (64) and Thompson and Guttridge (158) presented evidence for an active inhibitory principle from the non-induced plantlet which depressed flowering in the induced mother plant. Paton and Barber (136) claimed evidence for a flowering inhibitor of cotyledonary origin in peas. Evans (44) published evidence for a transmissible inhibitory product in Lolium in addition to the promotive floral stimulus. Most recently, Lang et al. (102) demonstrated the existence of a graft-transmissible flower-inhibitory stimulus in Nicotiana.

While there is some evidence for inhibitors in certain cases, it does not change the inescapable conclusion that in most cases

studied the floral stimulus appears to be translocated along with the photosynthate in the phloem.

Other Factors Affecting Floral Stimulus Production and Movement

Thus far photoperiod has been the major factor presented with regard to floral stimulus production and subsequent light intensity, and position of the noninduced leaves with regard to floral stimulus movement. There are other minor factors which also affect either the production or distribution of the floral stimulus.

Temperature is actually more than a minor factor in determining flowering response to photoperiod, not to mention those plants which require vernalization. Roberts and Struckmeyer (133) noted that photoperiodic requirements are not absolute, but can be modified by temperature and other environmental factors. Zeevaart and Lang (195) reported that the night temperature will markedly influence the flowering response in Bryophyllum daigremontianum under an otherwise inductive photoperiod. Quantitation of this observation in Bryophyllum appeared in a study by Van de Pol (171). He determined a numerical value based on the sum of the products of the hours of light and dark and their respective temperatures in °C. Maximum flowering occurred when the sum of the products was 472 or less as in 17°/8 h light and 21°/16 h dark. A sum of around 504 yielded moderate flowering while greater than 536 produced no flowering response.

Pharbitis nil is another good example of an effect of temperature on photoperiodic induction. Pharbitis is a short-day plant,

which is already sensitive in the cotyledonary stage. Ogawa (124) found that under low temperature Pharbitis behaves as a day-neutral plant. Takimoto et al. (152) cultured Pharbitis plants and found them to be day-neutral at 10°C when supplied with sucrose.

King and Evans (93) have pointed out that the floral stimulus in Pharbitis may be thermolabile and will revert to vegetative growth in either high temperature or following 5-fluorouracil treatment. More likely these treatments are affecting the processes of floral expression in the apex, rather than any direct effect on the stimulus itself. Husain (83) found the floral stimulus in Lolium more stable, at least under drought stress. Translocation of the floral stimulus can be halted by drought stress, but if relieved within a few days the translocation of the floral stimulus resumes. Since drought stress could have many effects, this, too, is not evidence for a direct effect on the floral stimulus. Given our present knowledge, it is not possible to determine the amount of stimulus required to produce a flowering response. However, one can study the attenuation of the response or dilution of the stimulus as upon crossing greater lengths of tissue. Imamura and Takimoto (88) found that the floral stimulus was attenuated greatly on passing through the stem from one branch to another as compared with travel of the floral stimulus to the adjacent axillary bud. Related experiments are reported later in this thesis.

Velocity of Floral Stimulus Movement

Having established that a transmissible floral stimulus indeed exists, the rationale for calculating the velocity at which such a stimulus travels was the possibility to better understand the nature of the stimulus, the method by which it is translocated, and even about the flowering process in general.

The early studies on floral stimulus translocation indicated a velocity far slower than the general velocity of assimilate translocation. Assuming a mass-flow system of phloem translocation where there is no preferential translocation, the early data spoke against the floral stimulus travelling along with the photosynthate in the phloem. Chailakhyan (23) induced a Perilla leaf and observed the length of time required for the adjacent axillary bud to produce flowers. He simultaneously observed the time required for the same result, but with the induced leaf some known distance from the receptor bud. The difference in distance in the two cases divided by the difference in time to flower was taken as the estimated rate of translocation. His calculation showed a velocity of 0.8 mm h^{-1} or 1.9 - 2.0 cm d^{-1} and 0.4 - 0.5 cm d^{-1} across the stem and root, respectively.

Imamura and Takimoto (86) devised a more refined method and arrived at a velocity of 2.6 - 3.8 mm h^{-1} in Pharbitis, higher than Chailakhyan's value, but not yet anywhere near the 20 - 70 cm h^{-1} at which assimilates are translocated. That a graft union presents no obstruction to the translocation of the floral stimulus, i.e., it

can cross the graft union at the same rate as in the intact was shown in a subsequent paper by Imamura and Takimoto (87). Based on the four hours required for the floral stimulus to travel from the cotyledon to the bud in Pharbitis, Zeevaart (190) calculated a velocity of 6.2-9.1 cm d^{-1} or about 3.0 mm h^{-1}, again far too slow to argue in favor of mass flow simultaneous distribution of photosynthate and floral stimulus. All of the above estimates were based on the transport of a threshold quantity of floral stimulus for induction, between source leaf and receptor bud. This underestimated the actual velocity.

Evans and Wardlaw (47, 48) introduced methods which circumvented this problem of underestimation. By removal of the stimulus source at different intervals and locations along the translocation pathway and noting the time at which adequate stimulus for a standard flowering response passed points separated by a known distance, they could calculate a more accurate translocation rate. In Lolium they calculated a velocity of 1.0-2.4 cm h^{-1} while the assimilates moved at 77.0-105.0 cm h^{-1}. Based solely on this result, the authors proposed that in Lolium there is an independent translocation of photosynthate and floral stimulus. Takeba and Takimoto (151) used this method in studying the floral stimulus translocation velocity in long, single-stem adult Pharbitis plants. After a 14 h induction, leaf removal resulted in no flowering in the adjacent axillary bud, but after 16 h all buds flowered even if the stem distance between leaf and bud was 102 cm, a velocity of 51 cm h^{-1}. This rate falls in

line with assimilate-transport velocities. King et al. (94) were able to confirm a high floral-stimulus-translocation velocity in Pharbitis. In three experiments the rates determined were 24, 37, and 29 cm h^{-1} while the concurrent ^{14}C-assimilate translocation velocities were 33, 37, and 37 cm h^{-1}, respectively. This clearly supports the idea of concurrent translocation. Interestingly, they found that in darkness, induced leaves would export the floral stimulus nearly as fast as induced leaves exposed to light. Yet, virtually no movement of ^{14}C-photosynthate accompanied the floral stimulus in the former case. In discussing the rate discrepancy between Lolium and Pharbitis, they (94) suggested that the floral stimulus either moves differently, or may actually differ in these plants.

Attempts to Divert or Impede the Movement of the Floral Stimulus

There have been a number of attempts at imposing treatments on induced plants in order to observe the effect on the movement of the floral stimulus. Of necessity some have been noted in previous sections, particularly with reference to the tissue of floral stimulus translocation. These treatments, which are often localized to a specific region of the translocation system, were usually designed either to learn more of the nature of the stimulus itself, or to study its transport in relation to assimilate transport. The treatments used are similar to those used in the straight phloem-transport studies.

Chailakhyan (21), in 1937, girdled the stem of an induced Chrysanthemum plant and thereby blocked floral-stimulus transport

implicating a requirement for continuous phloem. Moshkov (118), in the same year, reported transport of the floral stimulus across an incomplete graft union of Chrysanthemum, separated by a water gap. This observation could not be repeated by Galston (55) in soybean. The floral stimulus would not move across a steam-girdled stem. Lubimenko and Bouslova (106) severed the midrib of an induced leaf at the leaf-blade base and found that it hindered the floral-stimulus movement out of the leaf. Chailakhyan (24) could not repeat Lubimenko's findings. Perhaps, while ultimately the treatment plants may flower as do the controls, such a treatment is not unlikely to slow the translocation rate. Imamura and Takimoto (88) found that the floral stimulus did not cross a stem which was killed by electrically heating a wire loop around the stem. It traversed a stem subjected to a series of incisions, although more slowly than through control stems. Undoubtedly lateral transfer is a slower process than direct phloem transport. Withrow and Withrow (188) were also unable to repeat the findings of Moshkov (118). They found that floral-stimulus transport across a graft did not occur in Xanthium until tissue union occurred. While they noted that the floral stimulus had crossed lens paper, as Hamner and Bonner (68) had reported, tissue union had occurred in their experiments. The floral stimulus did not cross a steam-girdled stem. It did not travel in the xylem, which was still functional. A later paper by Moshkov (119) reported results which were quite different from his earlier Chrysanthemum work. He found that floral-stimulus transport across a graft did not take place in Perilla until there was tissue union. Selim (140)

claimed that girdling did not interfere with floral stimulus movement. However, since he also reported extensive callus formation at the wound surface, it is likely that there was a functional vasculature and his report should be viewed skeptically.

Chailakhyan (25) attempted other localized treatments to influence floral stimulus transport. In Perilla, ether, chloroform, and low temperature seemed to block the transmission of the floral stimulus. Borthwick et al. (8) attached a cooling jacket directly to the stem or petiole of soybean and reported a decreasing flowering response with decreasing temperature. Lang (100) cited unpublished data that localized cold and heat treatments of Hyoscyamus niger petioles blocked floral stimulus translocation. In that same reference he also compiled a table of treatments which blocked floral-stimulus translocation in various species.

Experimental Objectives

The objective of the experimental work described herein was to study various aspects of the floral stimulus, particularly its transport, in Pharbitis. In light of the considerable evidence indicating that the floral stimulus moves along with photosynthate in the phloem, experiments were designed to further test this hypothesis in adult Pharbitis plants. Of primary interest was the effect of localized low temperature on transport of the floral stimulus and on translocation of ^{14}C-labeled assimilates.

If the floral stimulus moves in the phloem along with the photosynthate, it should be possible to block the translocation of

both photosynthate and floral stimulus simultaneously, by applying localized low temperature to the stems. Previous attempts to inhibit floral stimulus transport with low temperature (8, 25) usually involved either bending of, or mechanical attachment to, the petiole or stem which may have contributed to any low-temperature inhibition of translocation.

Three methods were devised which ultimately enabled the separation of mechanical from temperature effects. The response of Pharbitis to long-term, localized low-temperature treatment, the accumulation of the floral stimulus in transport-blocked stems and the effect of leaf size on perception of the induction treatment were all examined.

MATERIALS AND METHODS

Growing Conditions

Seeds of Pharbitis nil Chois., cv. "Violet," were germinated as described by Zeevaart (191). Germinating seeds were planted, radicle down, in a mixture of gravel:vermiculite:soil (1:2:3, v/v) in 340-ml plastic containers. The seedlings were maintained in a growth chamber at 30°C for the first 2 d, and thereafter at 23°C, under continuous light from Sylvania Gro-Lux-WS fluorescent lamps (Danvers, Mass., USA) at a fluence rate of 4.0 mW cm^{-2} until the plants had reached a height of 30 cm. The plants were then transferred to a larger growth chamber and maintained at 23°C and 20-h photoperiod; the light source was as described by Zeevaart et al. (197). Mature plants were watered daily with 1/2-strength Hoagland's nutrient solution. Each plant was allowed to wind around a glass support rod with a diameter of 3 mm. In the first experiment five-day-old seedlings, in the cotyledonary stage, were given a 14 h inductive cycle with hourly cotyledon excision treatments at 14-20 h following the start of induction.

In older plants the effect of leaf size on the perception of the inductive conditions was tested. Plants were selected with a uniform distance between the induced leaf and the receptor bud; the width of the donor leaves ranged from 1-5 cm. All plants were

trimmed as described in the following paragraph. They were induced by one 16-h dark period at 28°C and the subsequent flowering was recorded.

When the plants had reached a height of 130 cm, they were selected each for an expanding leaf between 2.5 and 3.5 cm at the widest point of the apical half. The stem above this leaf, including the axillary bud, was removed; so were all the other leaves and axillary buds, except one "receptor" bud 80 cm below the remaining "donor" leaf. The trimmed plants were returned to the growth chamber for 3 d to allow the source leaf to expand and to free the receptor bud from apical dominance.

Just prior to induction the cold-block chamber was built around the stems of eight plants. Three of the plants were designated for labeling with $^{14}CO_2$; the remaining five plants were used to measure transport of the floral stimulus. This group of plants was subjected to low-temperature and is referred to as "cold block" (CB). Eight additional plants were grown and trimmed in the same way, except that they remained outside the Plexiglas chamber. This second group is referred to as "CB control." The same experiment was repeated the following day with a new group of plants enclosed in the chamber, but exposed to warm ari ("warm block" or WB) and again a control group (WB control) for floral induction and possible physical effects of the chamber was included. All source leaves were maintained at the same height with respect to the light source. The plants were induced by one 16-h dark period at 28°C.

Low-Temperature Treatment

In initial experiments either an ice-bath in a Plexiglas trough (10 x 10 x 51 cm^3) (Figure 1A), or an ice-filled or simulated ice-filled plastic cup surrounding the stem was employed. The results obtained with these cold blocks indicated that both transport of photosynthate and of the floral stimulus were affected by bending the stems and by mechanical contact, respectively. Therefore, a new system eliminating these problems was developed in which low-temperature air was forced into an insulated Plexiglas cold-block chamber (20 x 28 x 28 cm^3) surrounding 20 cm of the plant stems (Figure 1B). With this set-up, mechanical disturbance of the stem was avoided and there was no direct contact between the chamber and the plants. Air was dried through anhydrous $CaSO_4$ and fed into a copper coil immersed in a 6% (w/v) LiCl-ice bath (-7°C). At the same time air was bubbled through the bath to enhance mixing and thus maintain a uniform temperature. The cold-air outlet was attached directly to the cold-block chamber. ^{14}C-labeling was not begun until the temperature in the cold-block chamber had dropped below 5°C. The final temperature was maintained at 1-2°C by adjusting the air flow. Warm-block experiments were always run with a room-temperature (25°C) water bath coupled to the Plexiglas chamber.

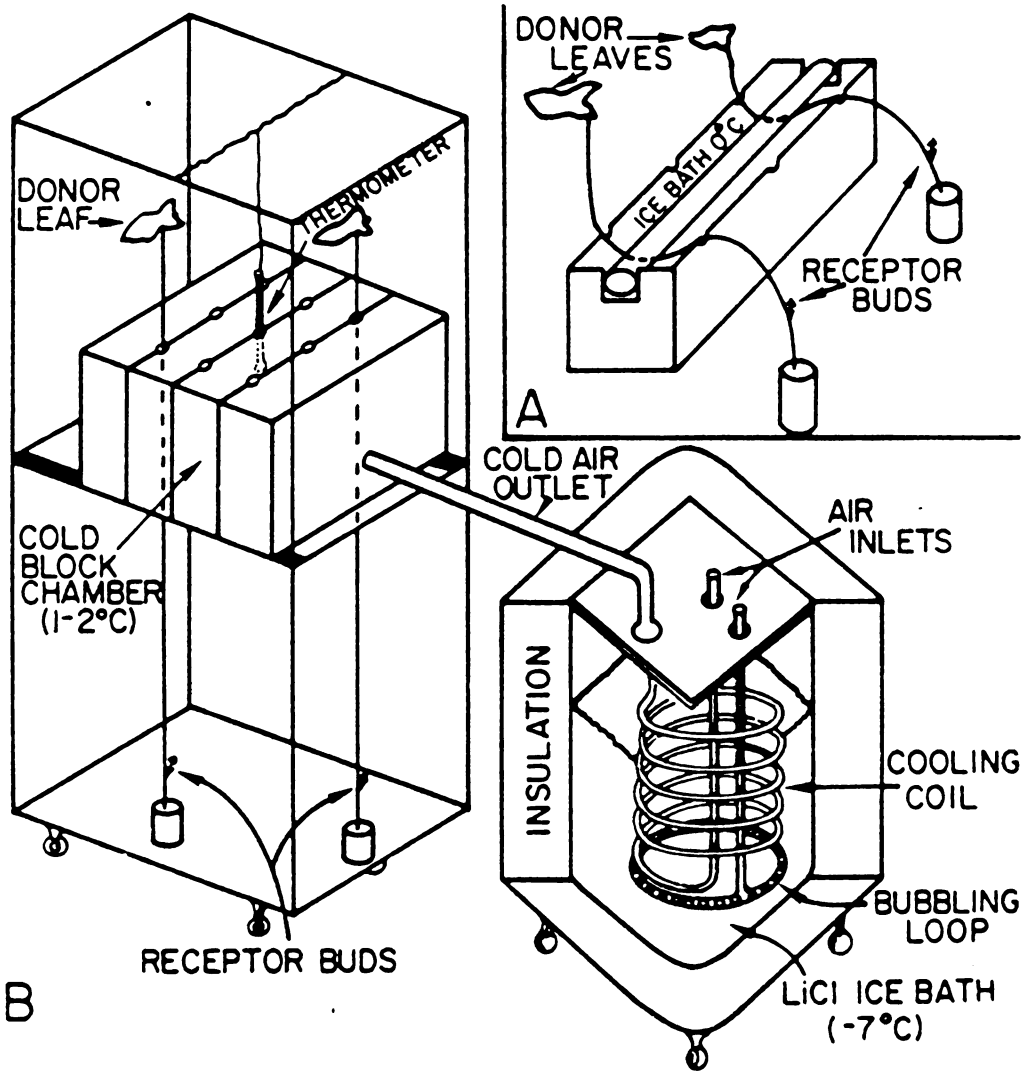
^{14}C-Labeling of Assimilates

Initially, two methods for labeling the assimilates with $^{14}CO_2$ were compared. In later experiments a Plexiglas labeling chamber was used routinely. Following photoperiodic induction and

Figure 1A and B.--Devices used for localized chilling of *Pharbitis* stems.

A. Cold block used in initial experiments. *Pharbitis* stems were gently bent under an aluminum bar through an ice-bath contained in a Plexiglas trough.

B. Apparatus used in later experiments. Low-temperature air, generated through the cooling coil in the LiCl-ice bath was fed into an insulated Plexiglas cold-block chamber built around the *Pharbitis* stems.


DONOR LEAVES
ICE BATH 0°C
RECEPTOR BUDS
A
DONOR LEAF
THERMOMETER
COLD BLOCK CHAMBER (1-2°C)
COLD AIR OUTLET
AIR INLETS
INSULATION
COOLING COIL
BUBBLING LOOP
LiCl ICE BATH (-7°C)
RECEPTOR BUDS
B

cooling of the cold-block chamber to 5°C, 6 leaves, three CB and three CB control, were enclosed in a Plexiglas labeling chamber (17.5 x 31 x 2 cm^3) through which $^{14}CO_2$ was circulated. $^{14}CO_2$ was generated and the leaves were fed $^{14}CO_2$ for 8 min as previously described by Zeevaart et al. (197). After 7 h of transport or 12 h in the case of the last experiment, the labeled plants were harvested cutting either the entire 130-cm stem into 2-cm segments or excising 2-cm stem segments every 10 cm. The segments were immediately frozen in liquid N_2, lyophilized, and their dry weight was determined. Because combustion and subsequent counting of 65 2-cm stem segments per plant were lengthy procedures, a nondestructive scanning method was devised to quickly determine if ^{14}C-translocation was affected by whichever localized low-temperature treatment was being tested. Every fifth 2-cm stem segment of the 65 was affixed in series to a strip of chromatography paper and run through a Packard Radiochromatogram Scanner model 7200 (Packard Instrument Co., Downers Grove, Ill., USA). If the method appeared effective, then the radioactivity of each of the 65 stem segments was determined. The radioactivity was determined as reported by Zeevaart et al. (197). The radioactivity was counted for 5 min in a Packard Tri-Carb liquid scintillation spectrometer model 3255 (Packard Instrument Co., Downers Grove, Ill., USA). The counting efficiency was determined and all data except those of figure 2 were converted to dpm mg^{-1} dry weight. Background subtractions were made. On the following day the same procedure was repeated except that plants were treated as WB and WB control.

Movement of the Floral Stimulus

The induced leaves of the nonlabeled plants were allowed to export floral stimulus for 7 h in the light while treated either as CB, CB control, WB or WB control. Following this 7-h period, the stems were cut off at the top of the cold-block chamber or in the case of the controls without a block, at the same height. The receptor buds grew out over the next several weeks while the plants were kept in the growth chamber under the previously described non-inductive conditions. The axillary buds along the new stem were scored either as vegetative or floral. The total number of flower buds produced was taken as a measure of the floral stimulus that had been translocated from the donor leaf.

RESULTS AND DISCUSSION

Labeling Method

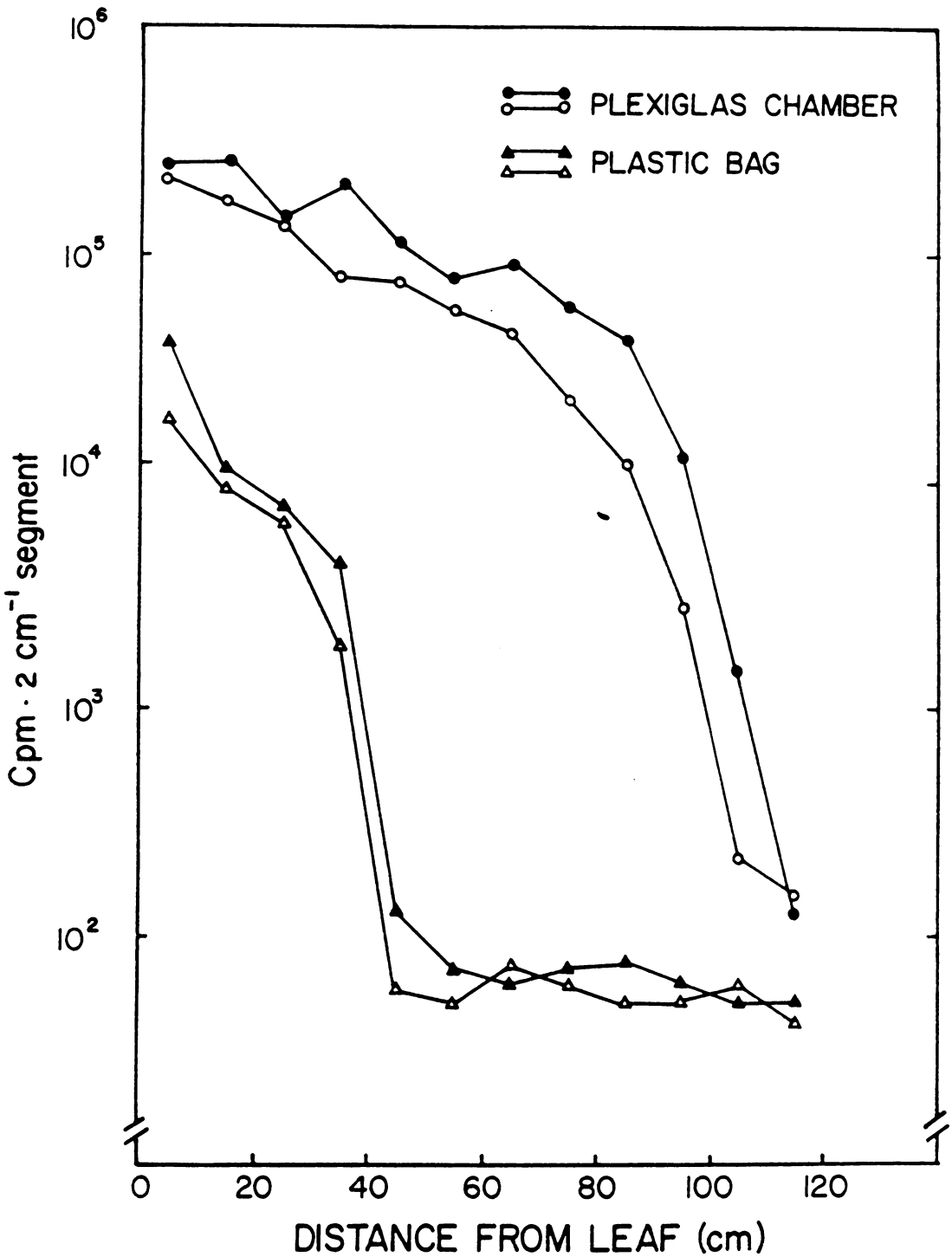
Two methods for labeling the leaf assimilates with $^{14}CO_2$ were compared (Figure 2). In the plastic-bag method only an aliquot of the $^{14}CO_2$ generated was injected into a small plastic bag enclosing each leaf. Consequently, it fixed far less total radioactivity than did the plants enclosed in the Plexiglas labeling chamber. The upper 30 cm of the stems of the plants labeled by the latter method contained an order of magnitude more counts per minute than the stems of the plants labeled by the former method. As a result the plants labeled in the chamber were able to transport a significant amount of radioactivity even beyond 100 cm from the leaf. In contrast, the plants labeled by the bag method were able to transport significant radioactivity only up to 40 cm from the leaf. The Plexiglas-chamber method, which was proven clearly superior, became, therefore, the method routinely employed in the studies that follow.

^{14}C-Assimilate Detection by Rapid-Scan Method

Using the rapid-scan method it was possible to quickly determine if a labeled plant was translocating normally. If a control plant translocated poorly as indicated by the scan, or if a low-temperature treatment showed no effect whatsoever, a great deal of time and effort could be spared by not combusting and counting the stem segments. The experiment could then be repeated or modified

Figure 2.--Distribution of ^{14}C-activity along Pharbitis stems after labeling of the source leaves by two different methods. Two representative samples of each method are shown.

The Plexiglas labeling chamber method is described in Materials and Methods. A 2-cm stem segment cut each 10 cm was combusted to determine radioactivity per segment. The plastic bag method required enclosing each individual source leaf in a small plastic bag. $^{14}CO_2$ was injected by syringe into the plastic bag, which was removed following 1/2 h assimilation time in the illuminated growth chamber.


PLEXIGLAS CHAMBER
PLASTIC BAG
Cpm · 2 cm⁻¹ segment
10⁶
10⁵
10⁴
10³
10²
0
20
40
60
80
100
120
DISTANCE FROM LEAF (cm)

accordingly. Typical scans of an effective cold block and the complimentary control are shown in Figures 3A and B respectively.

Movement of Floral Stimulus Out of Cotyledons of Pharbitis Seedlings

The induced cotyledons of 4-day old plants were excised 14-20 h following the start of the 14 h dark, inductive period. While 14 h was too short for flowering, the flowering response increased with time until at least 20 h (Figure 4). The intact controls, which retained a continuous source of floral stimulus, produced an average of 10.8 flowers per plant. There was a minimum time for floral stimulus production and transport over such a distance to a receptor bud which appears to exceed 14 h. As the cotyledons remain attached for longer periods of time beyond the critical photoperiod the flowering response increases until some undetermined, maximum level. This was already demonstrated by Zeevaart and Marushige (196). While 14 h may exceed the critical photoperiod, it appears in this case not to be the optimal photoperiod since the intact controls formed no terminal flower buds.

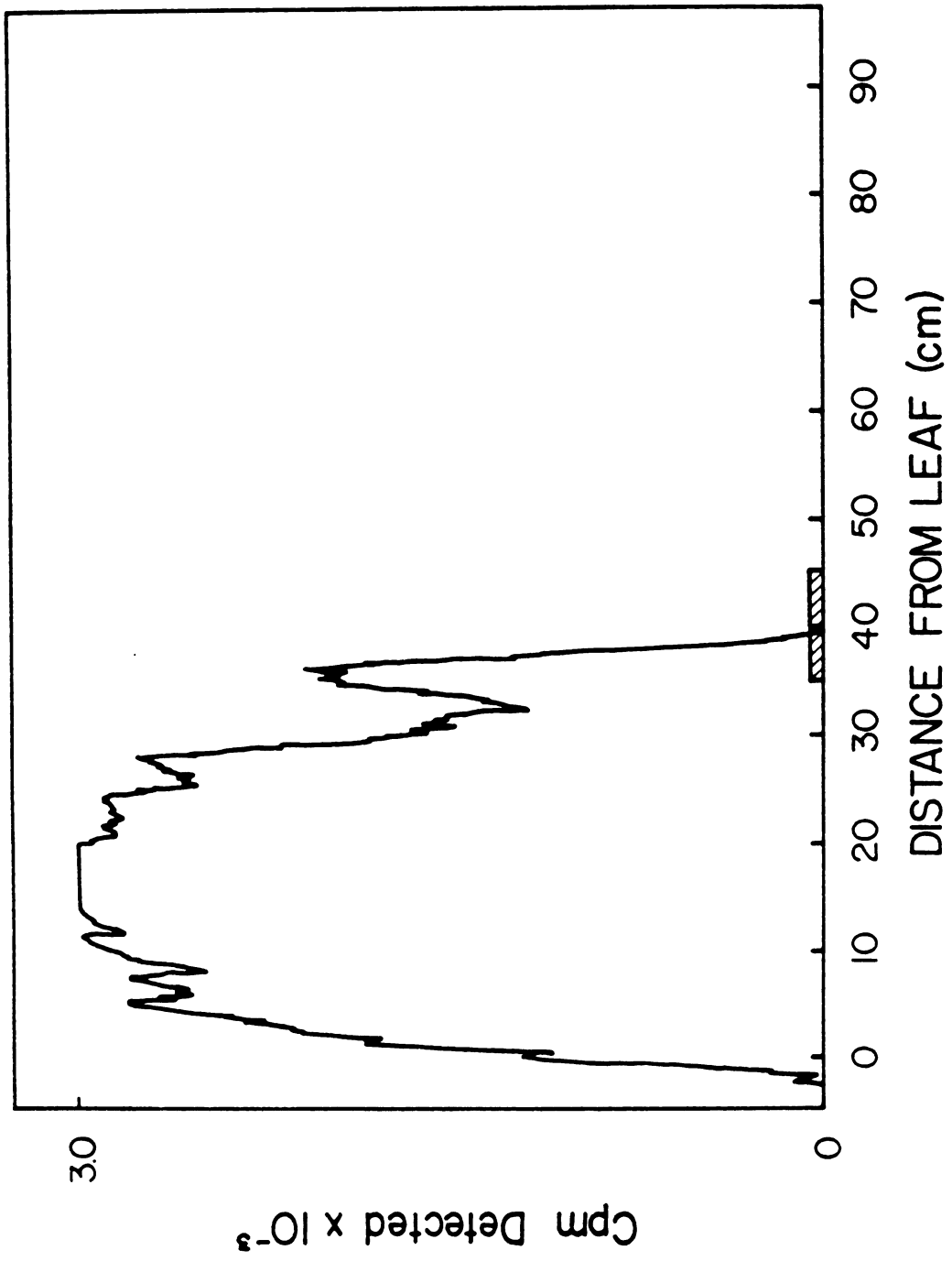
Effect of Leaf Size on Perception of the Inductive Conditions

In order to determine the optimal size of a Pharbitis leaf for photoperiodic perception, plants were classified according to the width of the leaf to be induced prior to trimming. All plants were trimmed and 3 d later were subjected to an identical, single, inductive, photoperiod of 15.5 h. The flowering response is recorded

Figure 3A and B.--Scans of ^{14}C-activity along Pharbitis stems. A 2-cm segment cut each 10 cm was affixed in series to a strip of paper and scanned in a radiochromatigram scanner to rapidly determine the effectiveness of the cold block.

A. Scan of ^{14}C-activity along the stem of a Pharbitis plant treated with an effective cold block. Cross-hatched bars on the abscissa indicate zones of the stems enclosed in the cold block. (p. 67)

B. Scan of ^{14}C-activity along the stem of a control Pharbitis plant not enclosed in a cold block. (p. 68)


Cpm Detected x 10⁻³
3.0
0
0
10
20
30
40
50
60
70
80
90
DISTANCE FROM LEAF (cm)

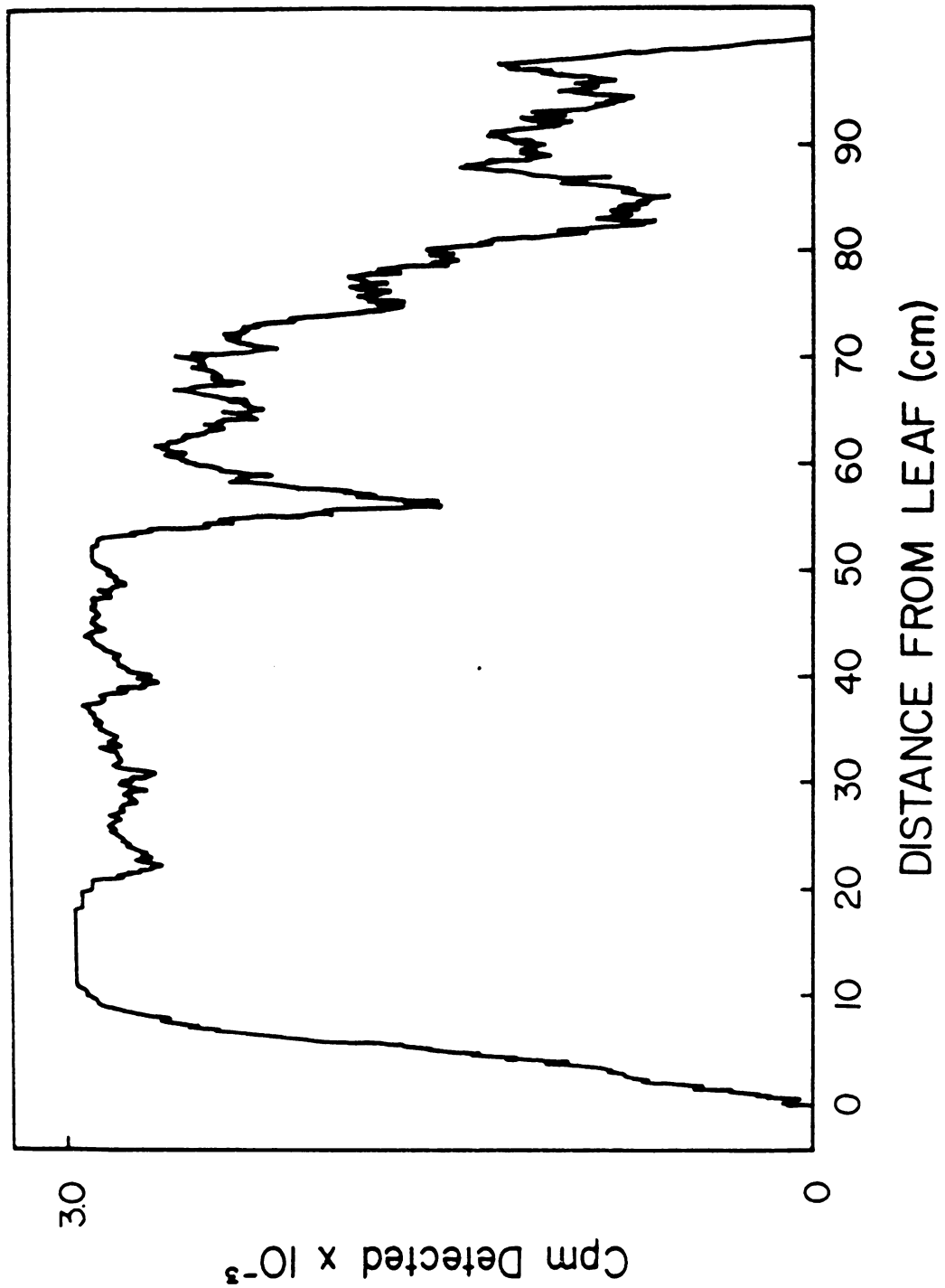
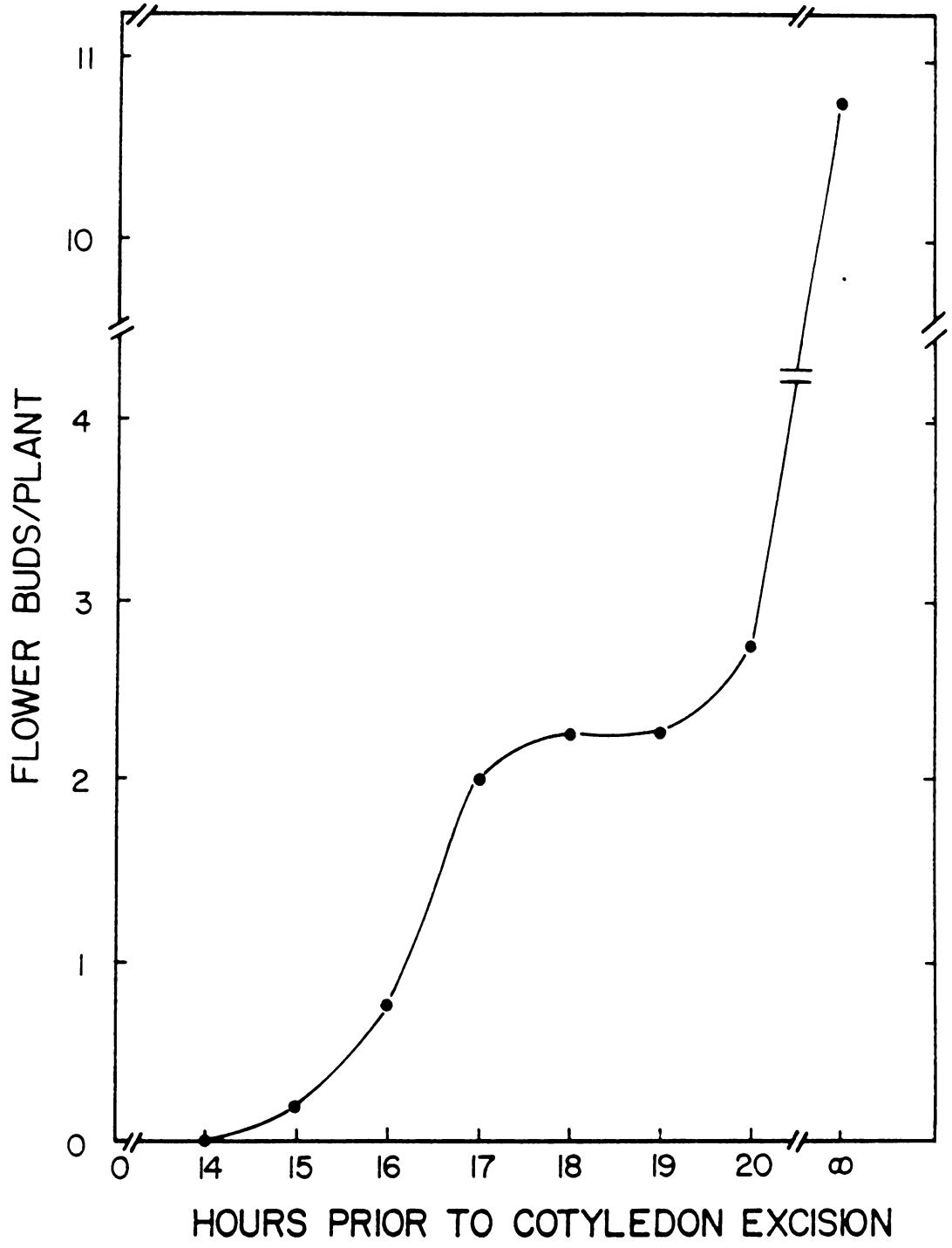

Cpm Detected x 10^{-3}
3.0
0
0
10
20
30
40
50
60
70
80
90
DISTANCE FROM LEAF (cm)

Figure 4.--Movement of floral stimulus out of cotyledons of Pharbitis seedlings. Following a 14 h inductive dark period, the cotyledons were excised between 14 and 20 h after the start of the induction. The flowering of intact control plants was also observed.


FLOWER BUDS/PLANT
0
1
2
3
4
10
11
0
14
15
16
17
18
19
20
∞
HOURS PRIOR TO COTYLEDON EXCISION

in Table 1. It appears that leaves with a diameter in excess of 4.5 cm are optimal for induction. Under the given experimental conditions, the leaves selected for trimming 3 d prior to induction needed to exceed 2.5-3.0 cm at the widest point of the apical half for maximal photoperiodic perception for flowering.

Effect of a Localized Cold Block on ^{14}C-Photosynthate Translocation

^{14}C-assimilate translocation was followed in cold-block and cold-block control plants over different time periods. The cold-block apparatus used in this experiment was the one shown in Figure 1A. Even after 2.75 h (Figure 5A) the radioactive profile clearly indicated that no radioactivity had passed beyond the CB region while significant radioactivity extended much further down the stem in the CB control. The pattern became far more distinct in the 4.25-h and 6-h treatments (Figures 5B and C). It appears that the treatment clearly blocked translocation of ^{14}C-photosynthate. However, this experiment was not adequate to discriminate between the multiple effects that such an apparatus can introduce during the course of the treatment.

Effect of a Localized Cold Block on ^{14}C-Photosynthate Translocation and on Floral-Stimulus Transport

In view of the experimental conditions, one could attribute the treatment effects of the previous experiment to either bending or mechanical contact or both in addition to the low temperature. The present experiment included a bent control as well as plants to monitor treatment effects on floral-stimulus movement. The

TABLE 1.--Effect of leaf size on the perception of the inductive conditions

Width on day cut (cm)	Mean width on day induced (cm)	Flower buds per plant[a]	No. plants with terminal flower buds
1.0[b]	2.9	0.8 ± 0.72	0
2.0[b]	4.2	3.0 ± 0.85	0
3.0[c]	4.6	7.0 ± 0.40	0
4.0[c]	5.2	5.0 ± 0.94	0
5.0[c]	5.9	7.0 ± 0.94	1

[a]Mean and standard deviation of the mean.

[b]5 plants per treatment.

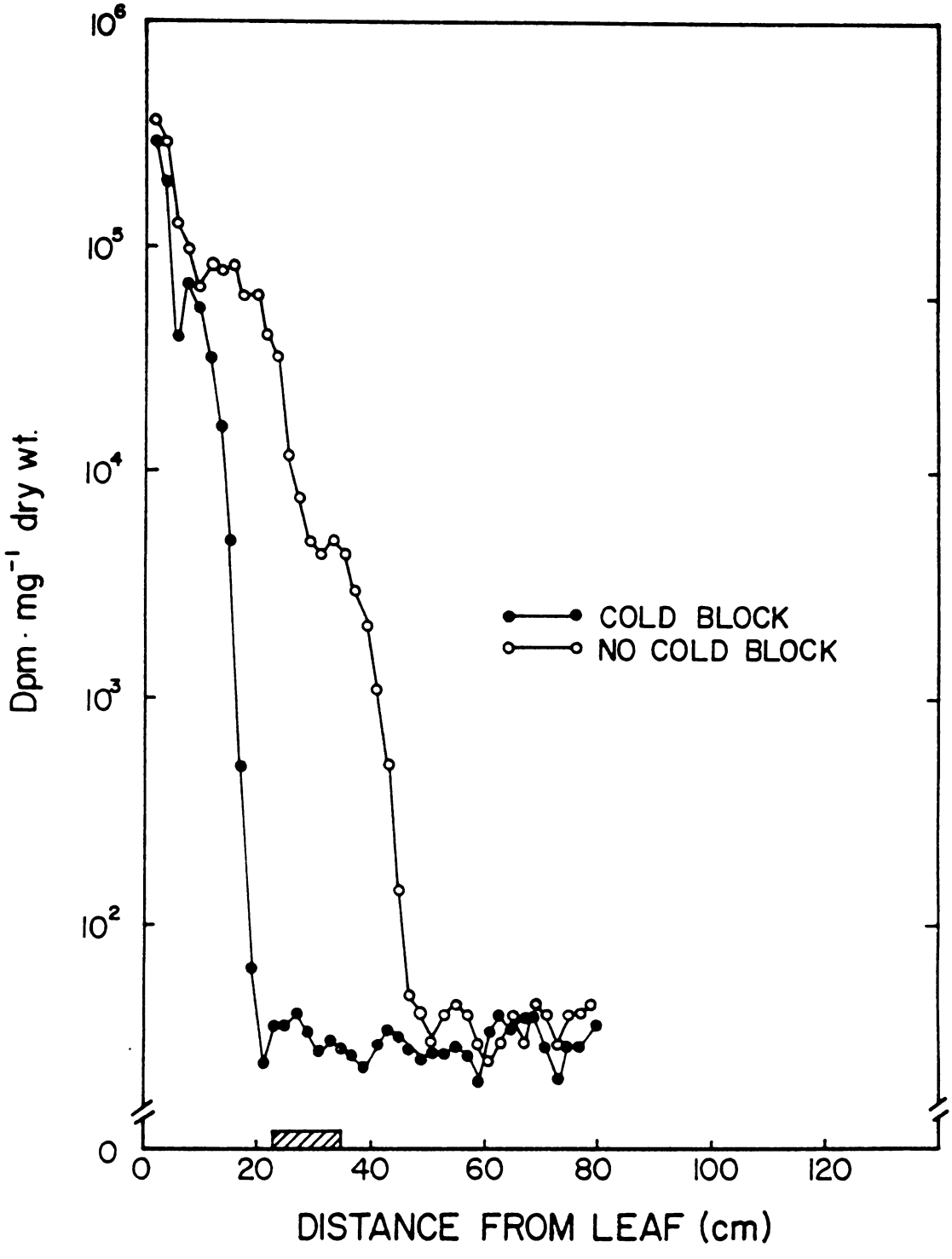
[c]4 plants per treatment.

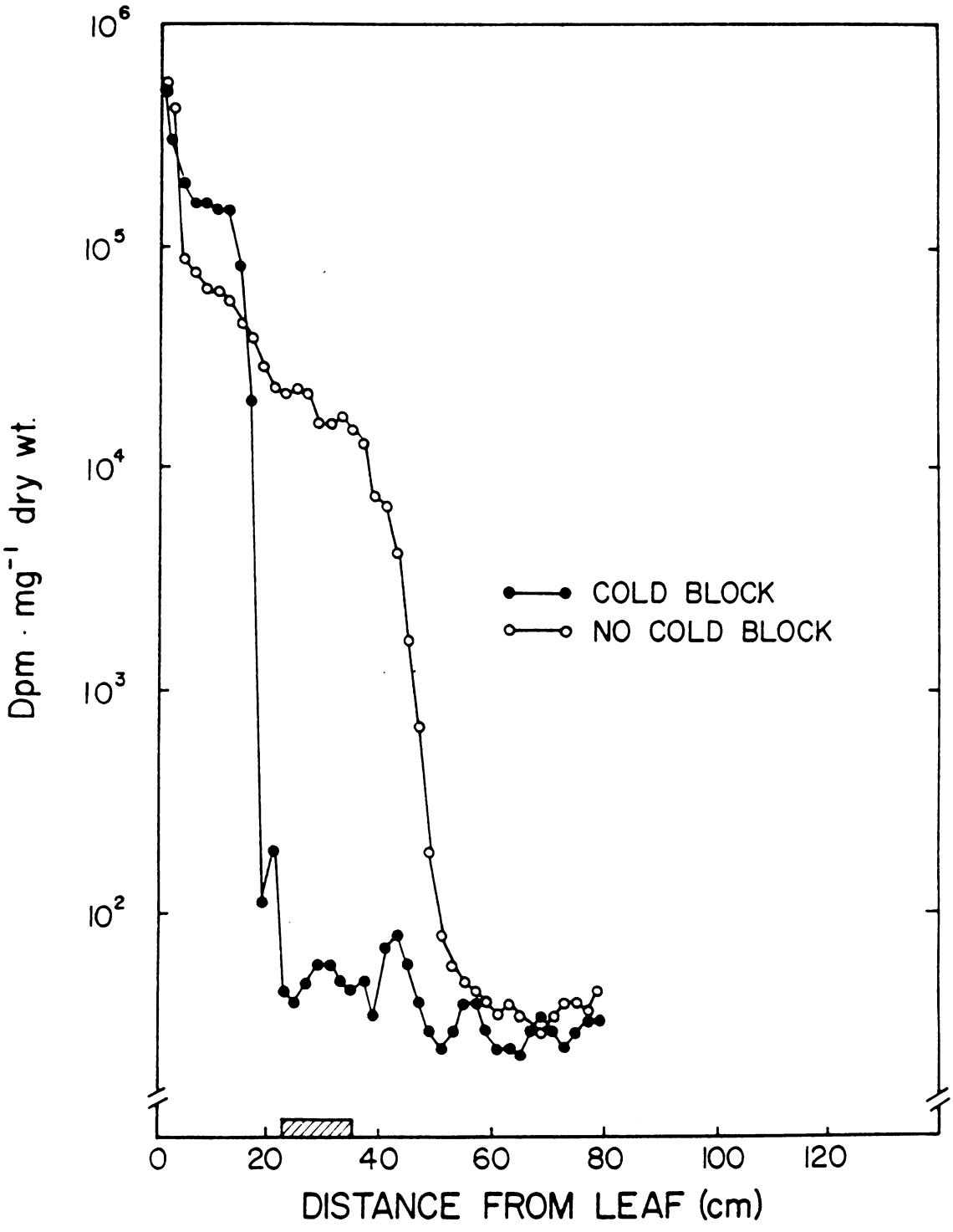
Figure 5A, B, and C.--Distribution of ^{14}C-activity along *Pharbitis* stems in initial cold block after labeling of the source leaves.

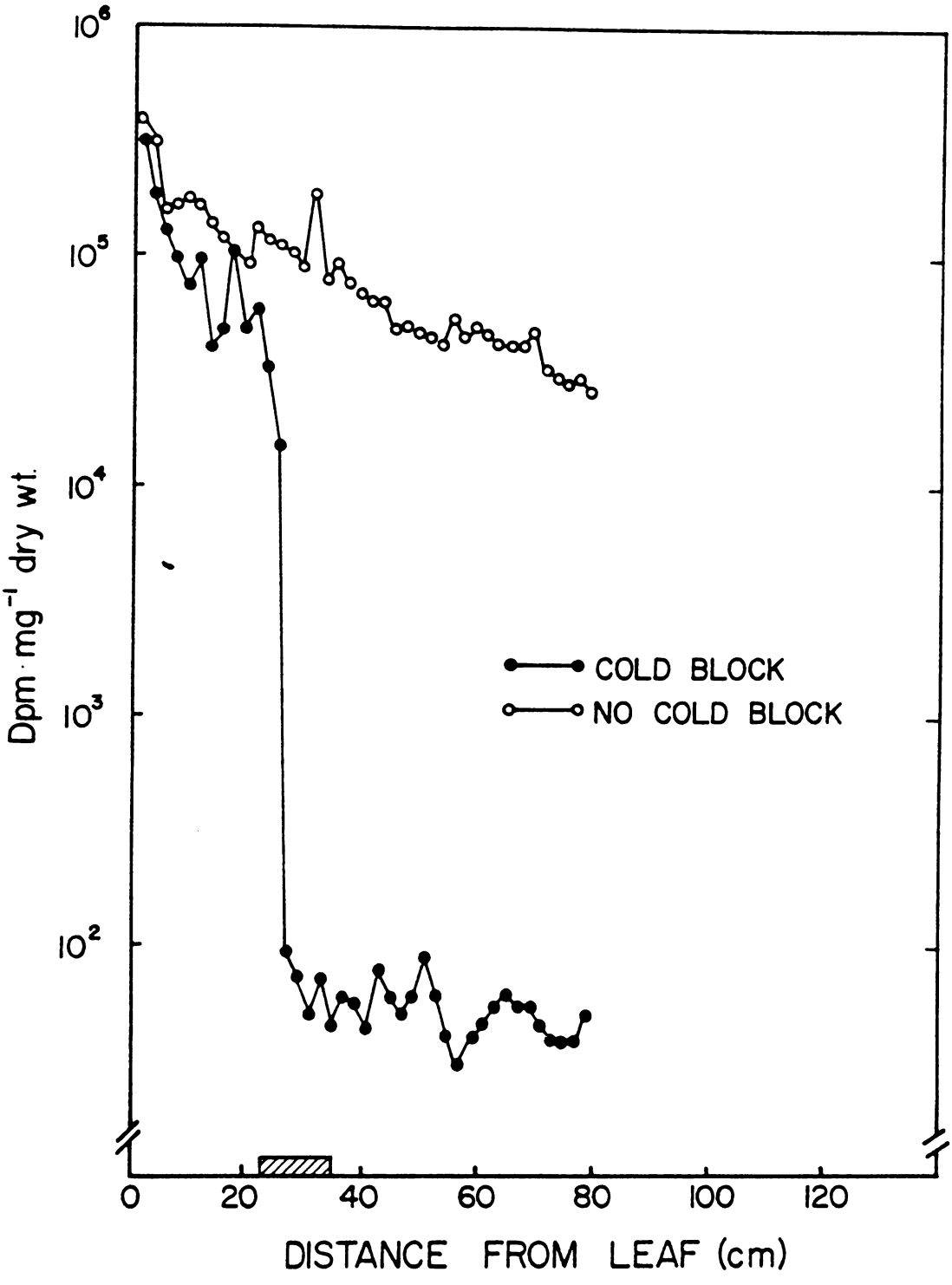
A. Profiles of radioactivity in a sample plant exposed for 2.75 h to low temperature in the cold block depicted in Figure 1A; control plants were kept erect while translocating at 23°C for 2.75 h. (p. 74)

B. 4.25 h. (p. 75)

C. 6 h. Cross-hatched bars on the abscissa indicate zones of the stems enclosed in the cold block. (p. 76)


10^6
10^5
10^4
10^3
10^2
0
Dpm · mg^{-1} dry wt.
COLD BLOCK
NO COLD BLOCK
0
20
40
60
80
100
120
DISTANCE FROM LEAF (cm)


10^6
10^5
10^4
10^3
10^2
Dpm · mg^{-1} dry wt.
COLD BLOCK
NO COLD BLOCK
0
20
40
60
80
100
120
DISTANCE FROM LEAF (cm)


10^6
10^5
10^4
10^3
10^2
Dpm·mg^{-1} dry wt.
COLD BLOCK
NO COLD BLOCK
0
20
40
60
80
100
120
DISTANCE FROM LEAF (cm)

^{14}C-photosynthate profiles at both 4 and 6 h (Figures 6A and B) confirmed the effect of the cold block; however, the warm, bent control, while translocating somewhat better than the cold-block treatment, was clearly inhibited when compared with the unbent control. These results indicated that given the experimental design, it was not possible to discriminate between the low-temperature and the bending effects.

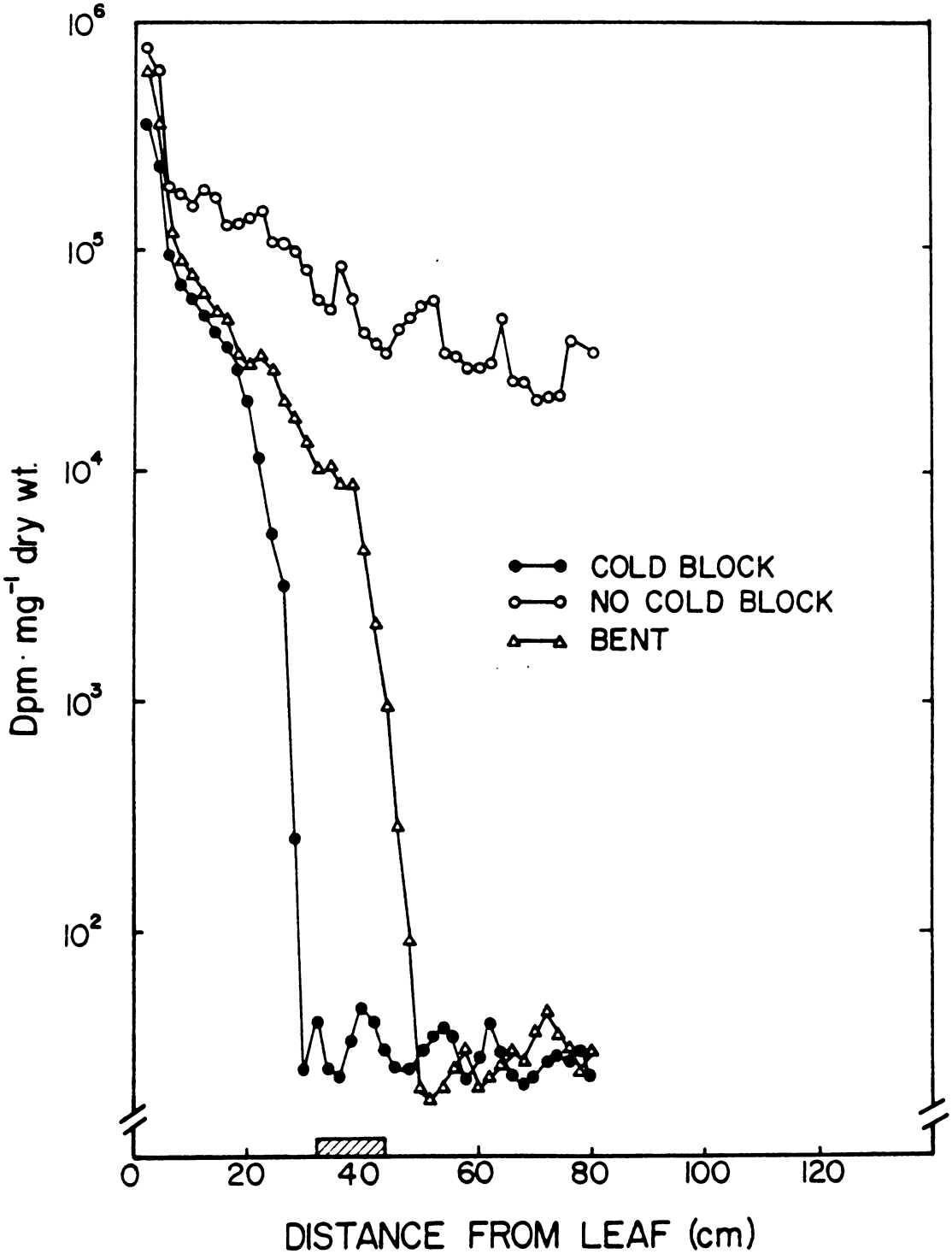
With respect to flowering (Table 2), the mere presence of the cold block for 6 h did not seem to significantly reduce the flowering response when contrasted with the controls without any block. When the stem above the cold block was excised following the 6-h treatments, no flowering resulted, whereas a significant flowering response was achieved when plants without the cold block were excised at the same height. Clearly, the cold block was able to inhibit both the transport of the floral stimulus and the translocation of photosynthate. In the warm, bent control there was significant flowering, although the response was highly variable between plants. This indicated that the bending alone could not account for the entire inhibition.

In an attempt to see if the floral stimulus accumulated above an effective block, and would resume transport following removal of the block, the stem above the cold block was excised at increasing heights above the top of the cold block. When the stem was excised 0 or 3 cm above the block, there was no flowering. Excision at 6 cm above the block resulted in a very slight flowering

Figure 6A and B.--Distribution of ^{14}C-activity along Pharbitis stems in initial cold block and in bent control after labeling of the source leaves.

A. Profiles of radioactivity in a sample plant exposed for 4 h to low temperature in the cold block depicted in Figure 1A; in a cold block control plant kept erect while translocating at 23°C for 4 h; in a bent control plant translocating at 23°C for 4 h. (p. 79)

B. As in A except for a 6 h translocation period. Cross-hatched bars on the abscissa indicate zones of the stems enclosed in the cold block. (p. 80)


10^6
10^5
10^4
10^3
10^2
Dpm · mg^{-1} dry wt.
COLD BLOCK
NO COLD BLOCK
BENT
0
20
40
60
80
100
120
DISTANCE FROM LEAF (cm)

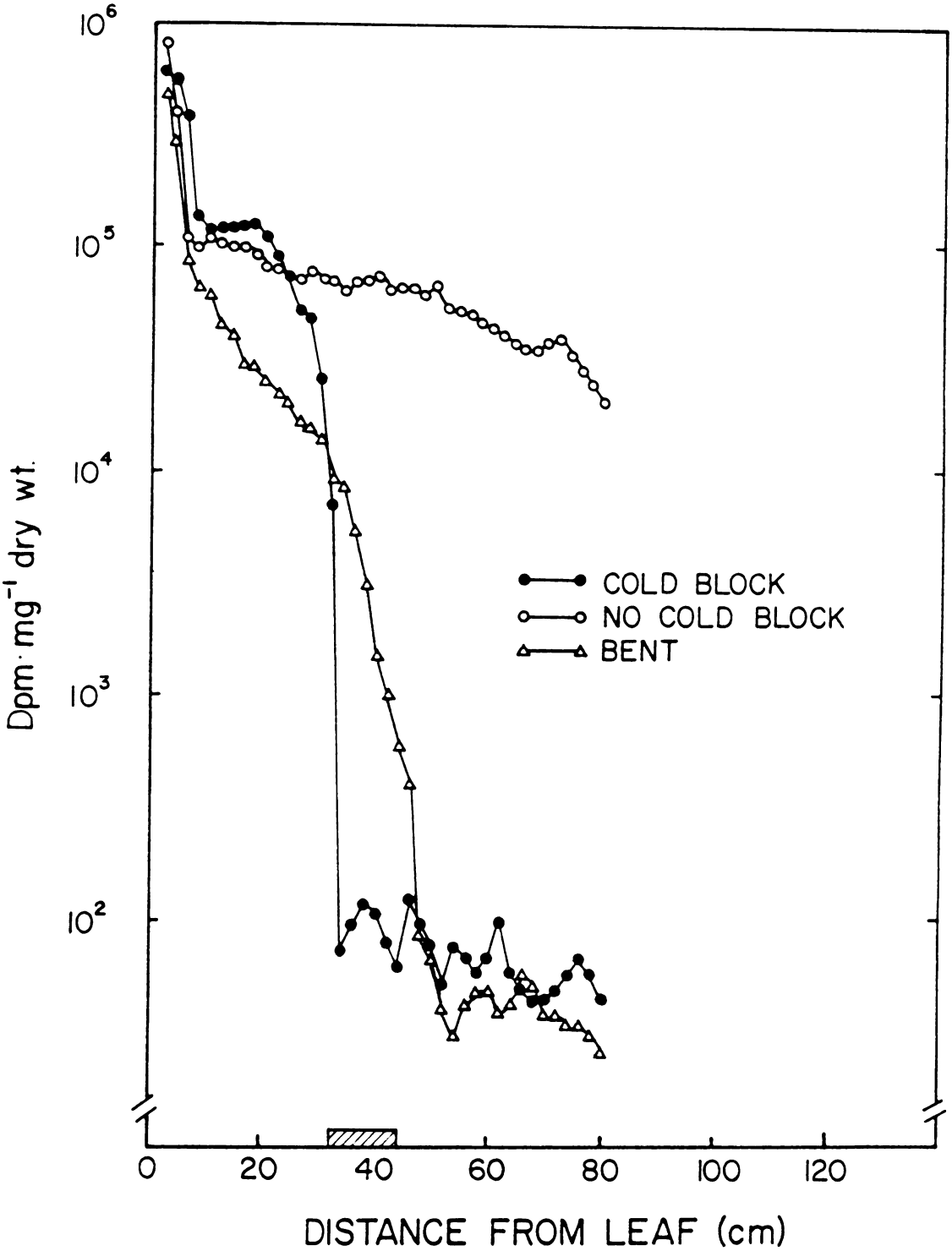

10^6
10^5
10^4
10^3
10^2
Dpm·mg^{-1} dry wt.
COLD BLOCK
NO COLD BLOCK
BENT
0
20
40
60
80
100
120
DISTANCE FROM LEAF (cm)

TABLE 2.--Effect of cold block, bending and excision at various heights above the cold block on movement of the floral stimulus

Treatment	No. Plants with Flower buds	Flower buds per Plant[a]
Intact, erect control[b]	10	15.0 ± 0.76
CB, no excision[c]	6	12.5 ± 2.04
Warm, bent, excised above bend after 6 h[c]	4	5.5 ± 2.70
CB; excised at top of CB after 6 h[b]	0	0
Erect, no CB; excised at same height after 6 h[b]	6	1.7 ± 0.72
CB, excised 3 cm above top of CB after 6 h[b]	0	0
CB, excised 6 cm above top of CB after 6 h[b]	1	0.1 ± 0.09
CB, excised 10 cm above top of CB after 6 h[b]	3	0.3 ± 0.14

[a]Mean and standard deviation of the mean.

[b]10 plants per treatment.

[c]6 plants per treatment.

response while excision at 10 cm above the block resulted in 30% of the plants showing a small flowering response. Apparently the stimulus accumulated in the stem, presumably in the phloem, as transport was blocked.

The general conclusion from the results of this experiment was that bending introduced some inhibitory effect on translocation and that in order to obtain conclusive results, an erect cold-block system was needed.

Effect of an Erect, Localized Cold Block or Simulated-Ice Block on the Transport of the Floral Stimulus

In this experiment an erect cold-block system was devised. In light of the effects of bending there was also concern regarding possible mechanical effects of pressure from the ice in the ice-filled cups surrounding the stems. In one treatment, scintillation-vial caps replaced the ice in an attempt to detect any mechanical effects which may have contributed to the overall transport inhibition.

In general, the flowering response in this experiment was less than optimal as indicated by the extent of flowering in the induced, intact controls (Table 3). In previous experiments it has been noted that often there would be a slight flowering response even when the source leaf was removed immediately following induction. This was specifically tested in this experiment and it was found that when the source leaf was removed just prior to the start of induction, no flowering resulted; when the leaf was removed just

TABLE 3.--Effect of an erect, localized cold block or simulated ice block on the transport of the floral stimulus. Ten plants per treatment.

Treatment	No. plants with flower bud	Flower buds per plant[a]
Noninduced control	0	0
Induced, intact control	10	6.6 ± 0.08
Induced control; leaf removed prior to induction	0	0
Induced control; leaf removed following induction	1	0.1 ± 0.09
6 h CB ; no excision	8	2.5 ± 0.49
6 h simulated ice-block; no excision	8	2.7 ± 0.66
6 h CB; excised above block	0	0
Warm control; excised at same height.[b]	6	1.9 ± 0.39
6 h CB; excised midway between leaf and CB	1	0.1 ± 0.09
Warm control; excised at same height	6	0.3 ± 0.40
6 h CB; excised just below leaf	4	0.4 ± 0.20
Warm control; excised at same height	8	2.8 ± 0.51

[a]Mean and standard deviation of the mean.

[b]Nine replicates only.

following the end of the inductive period a slight flowering response was detected. It appears that some stimulus is already transported prior to the termination of the inductive 16-h dark period. It had been noted by Zeevaart (190) and King and Evans (90) that in Pharbitis transport of the floral stimulus can occur in the dark as well as in the light. The noninduced controls remained, of course, totally vegetative, while the induced controls flowered, but with marked variability. There were plants which carried as few as one flower or as many as 19 per plant.

The presence of the cold block for 6 h with no subsequent excision following its removal reduced the flowering response below the nonblocked controls. An almost identical reduction was observed with the simulated-ice treatment. This indicated that even mechanical effects or sources of pressure may affect phloem transport.

Once again, excision of the stem above the cold block just following the 6-h treatment period resulted in no flowering response. Warm-control plants excised at the same height following 6 h of transport produced flowering in 60% of the plants. Thus, the sum total of the effects resulting from the effects of such an ice-filled cold block was to effectively block transport. However, with this experimental design, it was still not possible to test only the effect of low temperature to the exclusion of all others.

By excising the stem at various heights above the cold block following the 6-h treatment, it was again demonstrated that the floral stimulus accumulated in the stem above the block and the

greater the length of stem that was retained, the greater was the subsequent flowering response.

The inhibitory effects of bending and mechanical stress are really not all that surprising in view of reports in the literature of growth reduction or inhibition resulting from mechanical stress (113, 167). In fact, it is often standard procedure in translocation work to allow the experimental plants to recover following handling, even overnight, prior to actual experimentation (59). In this case, it was concluded that any low-temperature effect could be studied adequately only if a system were devised which introduced only low temperature to the exclusive of all mechanical effects.

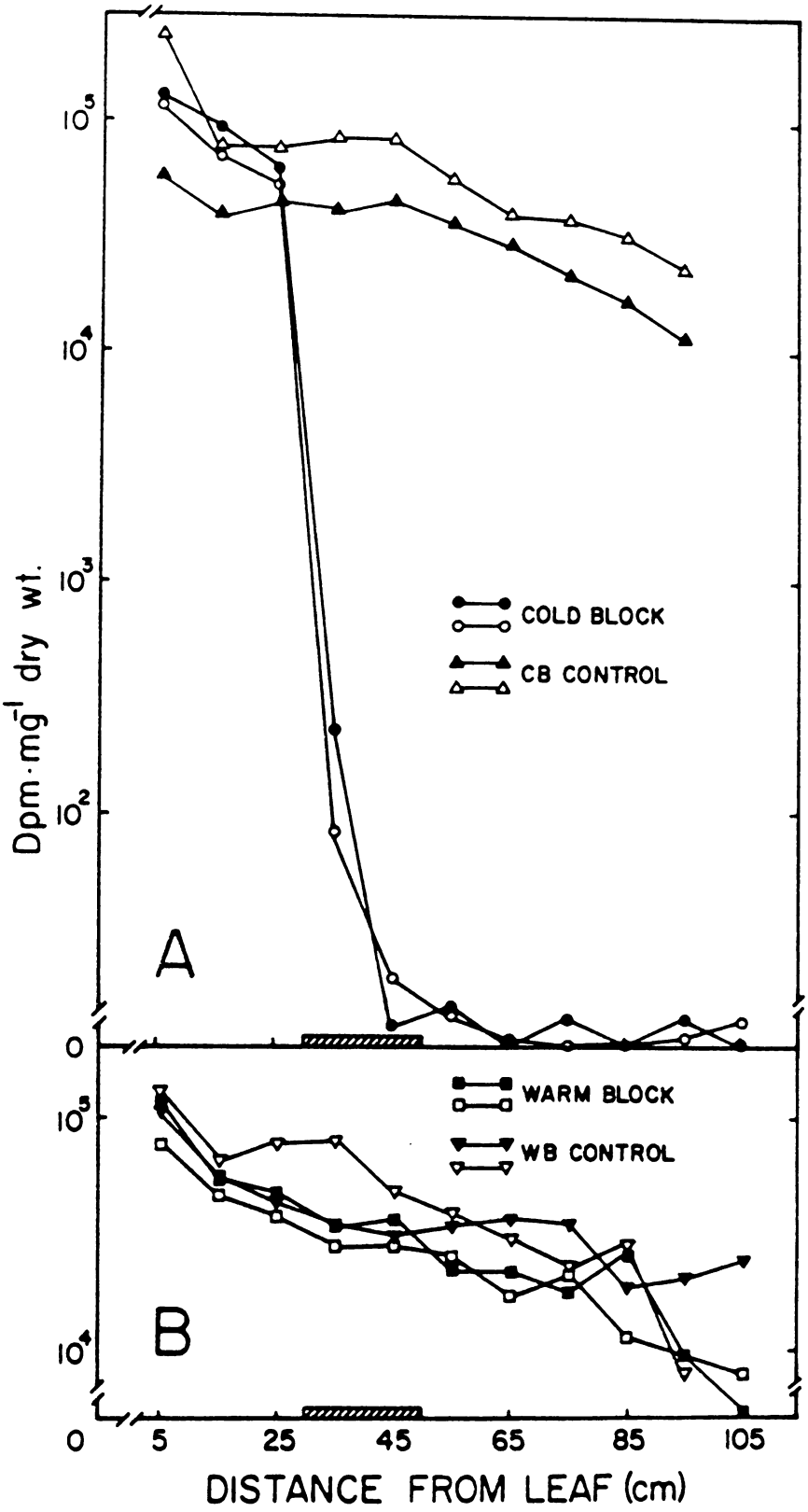
Effect of a Localized Cold Block, Based on Low-Temperature Air, on Translocation of ^{14}C-Photosynthate and on Transport of the Floral Stimulus (90)

A cold block was constructed which introduced only low-temperature air to the treated-stem zone (Figure 1B). No mechanical contact was made with the stem. Both ^{14}C-photosynthate and floral stimulus transport were followed. With this experimental set-up, when source leaves of Pharbitis nil plants were allowed to photosynthesize in the presence of $^{14}CO_2$, ^{14}C-activity accumulated along the entire length of the stem after a 7-h period (CB control). When 20-cm lengths of the stems were exposed to a temperature of 1-2°C (CB), ^{14}C-assimilate transport stopped and virtually no radioactivity moved across the cold zone during the 7-h period (Figure 7A). The analogous experiment with 25°C air circulating around the stems via the

Figure 7A and B.--Distribution of ^{14}C-activity along Pharbitis stems in cold block based on low-temperature air 7 h after labeling of the source leaves.

A. Profiles of radioactivity in two sample plants exposed to low temperature in the cold-block chamber depicted in Figure 1B, and of two control plants labeled simultaneously outside the chamber.

B. Profiles of ^{14}C-distribution in two sample plants kept at 23°C in warm-block chamber, and of two warm controls outside the chamber. Cross-hatched bars on the abscissa indicate zones of the stems enclosed in the chamber.


Dpm·mg⁻¹ dry wt.
10⁵
10⁴
10³
10²
0
COLD BLOCK
CB CONTROL
A
WARM BLOCK
WB CONTROL
B
5
25
45
65
85
105
DISTANCE FROM LEAF (cm)

chamber (WB) produced a ^{14}C-assimilate transport profile identical to the ^{14}C-profile of the WB control group, the stems of which were not enclosed in a chamber (Figure 7B). This indicates that low temperature alone does block phloem translocation in Parbitis stems.

It was further found that in Pharbitis the phloem transport system does not acclimate to the low temperature; at least translocation did not resume during a 12-h test period (Figure 8). This is in contrast to some chilling-insensitive species such as Beta vulgaris (150). Thus, Pharbitis more closely resembles Phaseolus vulgaris, a chilling-sensitive species which at 3°C did not acclimate for at least 9 h (60).

Low temperature applied to a 20-cm zone of the stem between the donor leaf and the receptor bud virtually eliminated the flowering response while the warm block, WB control and CB control treatments all resulted in the same flowering response (Table 4). This demonstrates that the low temperature inhibited transmission of the floral stimulus and that the presence of the chamber itself or the air turbulence did not interfere with translocation. This point is noteworthy in view of the preliminary experiments using the cold block of Figure 1A where gentle bending of the stems alone affected both the ^{14}C-assimilate transport profile and the flowering response using the erect, plastic-cup cold block where, presumably, the pressure of the ice on the stems contributed to the reduction in the flowering response. In light of the varied and often transient responses of translocation to low temperature (61), it is crucial to

Figure 8.--Profile of distribution of ^{14}C-activity along the stem of Pharbitis 12 h after labeling the source leaf. The stem was exposed to low temperature for the duration of the 12 h period. Cross-hatched bar on the abscissa indicates the zone of the stem enclosed in the cold block.

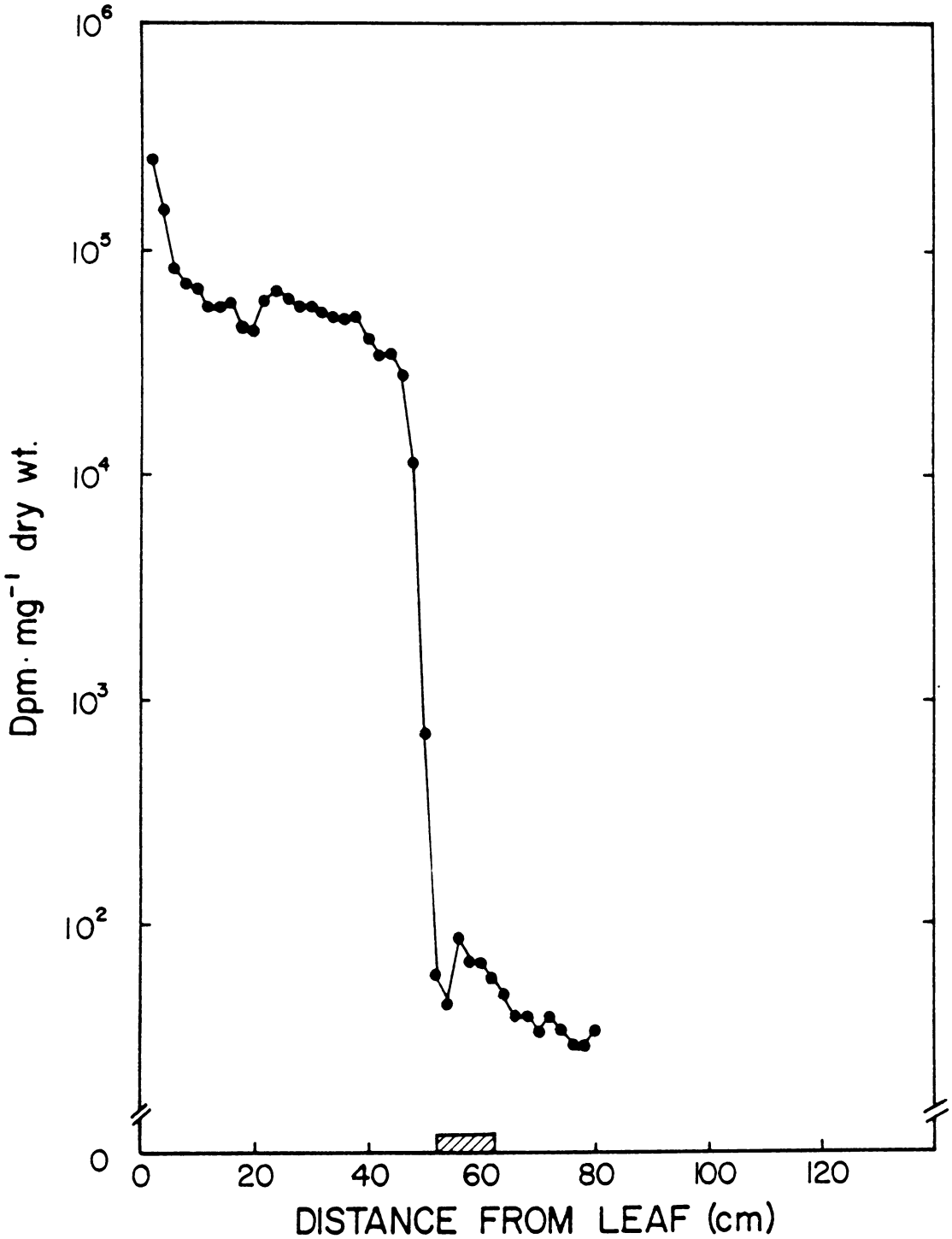

10^6
10^5
10^4
10^3
10^2
0
Dpm · mg^{-1} dry wt.
0
20
40
60
80
100
120
DISTANCE FROM LEAF (cm)

TABLE 4.--Effect of localized chilling of the stem on translocation of the floral stimulus in Pharbitis nil. Ten plants per group, except in cold-block treatment which had only 9 plants. Data of two separate experiments combined

Treatment	No. of Plants with Flower Buds	Flower Buds per Plant[a]	No. of Plants With Terminal Flower Buds
Cold block	4	0.9 ± 0.39	0
CB Control	10	7.0 ± 0.58	2
Warm block	10	7.8 ± 0.71	2
WB control	10	8.3 ± 0.78	0
CB; donor leaf not removed[b]	10	7.0 ± 0.94	0

[a]Mean and standard deviation of the mean.

[b]Stem zone chilled for 6 h.

determine that the responses observed are indeed caused by low temperature, and not by some additional factor(s). When the low-temperature block was removed after 6 h without excising the donor leaf, the flowering response was identical to that of the nontreated controls (Table 4) indicating that the low-temperature effect on the transport system for the floral stimulus was fully reversible.

The small flowering response in the low-temperature treatments (Table 4) can probably be accounted for by the fact that the critical photoperiod of adult plants of P. nil, cv. "Violet" is ca. 11 h (85), i.e., 5 h shorter than the inductive treatment employed in the present experiments. It has been shown that when the distance between donor leaves and receptor buds was 100 cm, removal of the donor leaves after 16 h of darkness resulted in the formation of one flower bud per plant (151). Likewise, in these experiments a sufficient amount of floral stimulus could have moved, prior to chilling of the stem, the 80-cm distance between the donor leaves and receptor buds to evoke a small flowering response, since movement of the floral stimulus in Pharbitis is known to occur equally well in light and in darkness (94).

A possible objection which one might raise with regard to such cold-block experiments is that while chilling a thin stem to 1-2°C, it is inevitable that the xylem water will also be cooled. If, upon reaching the source leaf, this water remains cool enough, it could hamper CO_2 fixation and/or vein loading (as noted in the Introduction, p.28). These effects would produce a similar

^{14}C-assimilate profile to path translocation inhibition. Although the xylem-water temperature near the leaf was not measured by thermocouple, the distance from the top of the cold block to the source leaf was at least 30 cm, a distance over which the xylem water would most probably return to ambient temperature. More convincing, however, are the time-course results of Figures 5A-C and Figure 8 where the radioactive assimilates appear to accumulate above the cold block with time. If either CO_2 fixation or vein loading had been affected, progressive accumulation would not have resulted with time. It appears, therefore, that the phloem-path translocation is experiencing the inhibition.

One final note of interest is that while the flowering data are presented as "number of buds per plant," this measure provides no clue to the sequence of vegetative or floral buds on the individual plants. It was a frequent observation that vegetative and floral buds would alternate. Even if several floral buds were produced at successive nodes, reversion to the vegetative condition for one or more nodes was not uncommon. As many as three or four such alternations was not an extraordinary occurrence. Such observations were made even in the case of plants which ultimately produced a terminal inflorescence, although they were more common in plants which eventually reverted altogether to the vegetative state. A similar observation was made by Imamura and Marushige (84): "After a weak floral stimulus, the shoot apex gives rise to floral primordia in the axils of a few successive nodes. In rare cases one or two vegetative buds may be inserted between flowering nodes." While their observation

was made in response to weak induction, terminal inflorescence production, as noted in the present case is indicative of a strong induction. Nevertheless, these are undoubtedly related observations. Floral differentiation is a complex developmental process. The explanation for the above observation is likely to become apparent only when more is understood of the phenomenon of floral expression and of the stimulus by which it is triggered.

SUMMARY AND CONCLUSIONS

It is generally assumed that both the floral stimulus and assimilates are translocated concurrently in the phloem. This assumption is, among others, based on very similar velocities of translocation of ^{14}C-labeled assimilates and of the floral stimulus in Pharbitis (94), and on the correlation between distribution of ^{14}C-activity following labeling of a Perilla donor leaf and the flowering response (95).

In this present study three methods were devised for applying localized low temperature to the translocation path. The first two methods indicated that mechanical effects contributed to the inhibition of translocation. The results of the last set of experiments, in which a region of stem between the donor leaf and receptor bud was chilled without mechanical perturbation, showed that in Pharbitis translocation of ^{14}C-labeled assimilates as well as the flowering response were simultaneously inhibited by low temperature only. This inhibition is perhaps the result of some physical blockage of the sieve tubes. No recovery of translocation was observed even up to 12 h with the cold block continually present. The inhibitory effect of low temperature on transport of the floral stimulus was shown to be fully reversible following removal of the cold block. Yet, irrespective of the factor(s) which alone or in combination

achieved blockage, the correlation between extent of ^{14}C-translocation blockage and inhibition of flowering always held. Thus, these results give additional support to the contention that floral stimulus and photosynthate move together in the phloem.

LITERATURE CITED

LITERATURE CITED

1. Ballard, L.A.T. and Grant Lipp, A.E.: Juvenile photoperiodic sensitivity in Anagallis arvensis L. supsp. Foemina (Mill.) Schinz and Thell. Aust. J. Biol. Sci. 17, 323-337 (1964)

2. Barrier, G.E. and Loomis, W.E.: Absorption and translocation of 2,4-dichlorophenoxyacetic acid and ^{32}P in leaves. Plant Physiol. 32, 225-231 (1957)

3. Bennett, C.W.: Plant-tissue relations of the sugar-beet curly-top virus. J. Agr. Res. 48, 665-701 (1934)

4. Bennett, C.W.: Influence of contact period on the passage of viruses from scion to stock in Turkish tobacco. Phytopathology 33, 818-822 (1943)

5. Biddulph, O. and Cory, R.: An analysis of translocation in the phloem of the bean plant using THO, ^{32}P and ^{14}C. Plant Physiol. 32, 608-619 (1957)

6. Bieleski, R.L.: Sites of accumulation in excised phloem and vascular tissues. Plant Physiol. 41, 455-466 (1966)

7. Birch-Hirschfeld, L.: Untersuchugen über die Ausbreitungs-geschwindigkeit gelöster Stoffe in der Pflanze. Jahrb. Wiss. Bot. 59, 171-262 (1920)

8. Borthwick, H.A., Parker, M.W., and Heinze, P.H.: Influence of localized low temperature on Biloxi soybean during photoperiodic treatment. Bot. Gaz. 102, 792-800 (1941)

9. Bowen, M.D. and Hoad, G.V.: Inhibitor content of phloem and xylem sap obtained from willow (Salix viminalis) entering dormancy. Planta 81, 64-70 (1968)

10. Bowling, D.J.F., Measurement of the potential across the sieve plates in Vitis vinifera. Planta 80, 21-26 (1968)

11. Bowling, D.J.F.: Translocation at 0° C in Helianthus annuus. J. Exp. Bot. 19, 381-388 (1968)

12. Brown, C.L.: The influence of external pressure on the differentiation of cells and tissues cultured in vitro. In: The Formation of Wood in Forest Trees, pp. 389-404, M.H. Zimmermann, ed. New York: Academic Press 1964

13. Bukovac, M.J. and Wittwer, S.H.: Absorption and mobility of foliar applied nutrients. Plant Physiol. 32 428-435 (1957)

14. Canny, M.J.: The translocation profile: sucrose and carbon dioxide. Ann. Bot. N.S. 26, 181-196 (1962)

15. Canny, M.J.: The mechanism of translocation. Ann. Bot. N.S. 26, 603-617 (1962)

16. Canny, M.J.: Phloem Translocation. London: Cambridge University Press 1973

17. Canny, M.J. and Askham, J.M.: Physiological inferences from evidence of translocated tracer: a caution. Ann. Bot. N.S. 31, 409-416 (1967)

18. Carr, D.J.: On the nature of photoperiodic induction. IV. Preliminary experiments on the effect of light following the inductive long dark period in Xanthium Pennsylvanicum. Physiol. Plant. 10, 249-265 (1957)

19. Cataldo, D.A., Christy, A.L., and Coulson, C.L.: Solution-flow in the phloem. II. Phloem transport of THO in Beta vulgaris. Plant Physiol. 49, 690-695 (1972)

20. Chailakhyan, M.Kh.: New facts in support of the hormonal theory of plant development. Dokl. Acad. Sci. U.S.S.R. 13, 79-83 (1936)

21. Chailakhyan, M.Kh.: Hormone theory of plant development. Moscow: Acad. Nauk. U.S.S.R. (1937)

22. Chailakhyan, M.Kh.: Motion of blossom hormone in girdled and grafted plants. Dokl. Acad. Sci. U.S.S.R. 18, 607-612 (1938)

23. Chailakhyan, M.Kh.: Translocation of flowering hormones across various plant organs. I. Across the leaf. Dokl. Acad. Sci. U.S.S.R. 27, 160-163 (1940)

24. Chailakhyan, M.Kh.: Translocation of flowering hormones across various plant organs. III. Across the root. Dokl. Acad. Sci. U.S.S.R. 27, 373-376 (1940)

25. Chailakhyan M.Kh.: Translocation of flowering hormones in the plant as affected by temperature and narcotics. Dokl. Acad. Sci. U.S.S.R. 31, 949-952 (1941)

26. Chailakhyan, M.Kh.: Photoperiodism of plants with differential light regime for individual leaves. Dokl. Akad. Nauk. S.S.S.R. 54, 739-742 (1946)

27. Chailakhyan, M.Kh.: On the nature of the flower-inhibitory action of leaves. Dokl. Akad. Nauk. S.S.S.R. 55 73-76 (1947)

28. Chailakhyan, M.Kh. and Butenko, R.G.: Translocation of assimilants from leaves to shoots during different photoperiodic regimes of plants. Fiziol. Rast. 4, 450-462 (1957)

29. Chamberlain, I.S. and Spanner, D.C.: The effect of low temperatures on the phloem transport of radioactive assimilates in the stolon of Saxifraga sarmentosa L. Plant, Cell and Environment 1, 285-290 (1978)

30. Child, C.M. and Bellamy, A. W.: Physiological isolation by low temperature in Bryophyllum and other plants. Science 50, 362-265 (1919)

31. Colwell, R.N.: The use of radioactive phosphorus in translocation studies. Am. J. Bot. 29, 798-807 (1942)

32. Coulson, C.L., Christy, A.L., Cataldo, D.A., and Swanson, C.A.: Carbohydrate translocation in sugar beet petioles in relation to petiolar respiration and adenosine 5-triphosphate. Plant Physiol. 49, 919-923 (1972)

33. Crafts, A.S.: Problem of sieve-tube slime. Science 160, 325-327 (1968)

34. Currier, H.B., Esau, K., and Cheadle, V.I.: Plasmolytic studies of phloem. Am. J. Bot. 42, 68-81 (1955)

35. Curtis, O.F.: Studies on solute translocation in plants. Experiments indicating that translocation is dependent on the activity of living cells. Am. J. Bot. 16, 154-168 (1929)

36. Curtis, O.F.: The Translocation of Solutes in Plants. A Critical Consideration of Evidence Bearing upon Solute Movement. New York: McGraw-Hill Book Co. 1935

37. Dixon, H.H. and Ball, N.G.: Transport of organic substances in plants. Nature 109, 236-237 (1922)

38. Duloy, M., Mercer, F.V., and Rothgeber, N.: Studies in translocation. III. The cytophysiology of the phloem of Cucurbita pepo. Aust. J. Biol. Sci. 15, 459-467 (1962)

39. Esau, K., Engleman, E.M., and Bisalputra, T.: What are transcellular strands? Planta 59, 617-623 (1963)

40. Eschrich, W.: Beiträge zur Kenntnis der Wundsiebröhrenentwicklung bei Impatiens holsti. Planta 43, 37-74 (1953)

41. Eschrich, W.: Bidirektionelle Translokation in Siebröhren. Planta 73, 37-49 (1967)

42. Eschrich, W.: Biochemistry and fine structure of phloem in relation to transport. Ann. Rev. Plant Physiol. 21, 193-214 (1970)

43. Eschrich, W.: Bidirectional Transport. In: Phloem Transport, pp. 401-416, Aronoff, S., Dainty, J., Gorham, P.R., Srivastava, L.M., eds. New York: Plenum Press 1974

44. Evans, L.T.: Inflorescence initiation in Lolium temulentum L. II. Evidence for inhibitory and promotive processes involving transmissible products. Aust. J. Biol. Sci. 13, 429-440 (1960)

45. Evans, L.T.: Day-length control of inflorescence initiation in the grass Rottboellia exaltata L.f. Aust. J. Biol. Sci. 15, 291-303 (1962)

46. Evans, L.T.: Flower induction and the florigen concept. Ann. Rev. Plant Physiol. 22, 365-394 (1971)

47. Evans, L.T. and Wardlaw, I.F.: Inflorescence initiation in Lolium temulentum L. IV. Translocation of the floral stimulus in relation to that of assimilates. Aust. J. Biol. Sci. 17, 1-9 (1964)

48. Evans, L.T. and Wardlaw, I.F.: Independent translocation of ^{14}C-labelled assimilates and of the floral stimulus in Lolium temulentum. Planta 68, 310-326 (1966)

49. Evert, R.F.: Phloem structure and histochemistry. Ann. Rev. Plant Physiol. 28, 199-222 (1977)

50. Evert, R.F. and Murmanis L.: Ultrastructure of the secondary phloem of Tilia americana. Am. J. Bot. 52, 95-106 (1965)

51. Fensom, D.S.: An electrokinetic theory of transport. Can. J. Bot. 35, 573-582 (1957)

52. Fensom, D.S., Clattenburg, R., Chung, T., Lee, D.R., and Arnold, D.C.: Moving particles in intact sieve tubes of Heracleum montegazzianum. Nature 219, 531-532 (1968)

53. Ford, J. and Peel, A.J.: Preliminary experiments on the effect of temperature on the movement of ^{14}C-labelled assimilates through the phloem of willow. J. Exp. Bot. 18, 406-415 (1967)

54. Gage, R.S. and Aronoff, S.: Translocation III. Experiments with carbon 14, chloride 36 and hydrogen 3. Plant Physiol. 35, 53-64 (1960)

55. Galston, A.W.: Transmission of the floral stimulus in soybean. Bot. Gaz. 110, 495-501 (1949)

56. Gardner, D.J.C. and Peel, A.J.: The effect of low temperature on sucrose, ATP and potassium concentrations and fluxes in the sieve tubes of willow. Planta 102, 348-356 (1972)

57. Garner, W.W. and Allard, H.A.: Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J. Agr. Res. 18, 553-606 (1920)

58. Geiger, D.R.: Chilling and translocation inhibition. Ohio J. Sci. 69, 356-366 (1969)

59. Geiger, D.R.: Personal communication.

60. Geiger, D.R. and Sovonick, S.A.: Temporary inhibition of translocation velocity and mass transfer rate by petiole cooling. Plant Physiol. 46, 847-849 (1970)

61. Geiger, D.R. and Sovonick, S.A. : Effects of temperature, anoxia and other metabolic inhibitors on translocation. In: Encycl. Plant Physiol., N.S., Vol. I, Transport in Plants, pt. I, pp. 256-286, Zimmermann, M.H. and Milburn, J.A., eds. Berlin: Springer-Verlag 1975

62. Giaquinta, R.T. and Geiger, D.R.: Mechanism of inhibition of translocation by localized chilling. Plant Physiol. 51, 372-377 (1973)

63. Griesel, W.O.: Photoperiodic responses of two Cestrum species and noninterchangeability of their flowering hormones. Plant Physiol. 38, 479-482 (1963)

64. Guttridge, C.G.: Photoperiodic promotion of vegetative growth in the cultivated strawberry plant. Nature 178, 50-51 (1956)

65. Hales, S.: Vegetable Staticks. Reprinted. New York: American Elsevier Inc. 1961, pp. 1-214.

66. Hall, S.M. and Baker, D.A.: The chemical composition of Ricinus phloem exudate. Planta 106, 131-140 (1972)

67. Hammel, H.T.: Measurement of turgor pressure and its gradient in the phloem of oak. Plant Physiol. 43, 1042-1048 (1968)

68. Hamner, K.C. and Bonner, J.: Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. 100, 388-431 (1938)

69. Harder, R.: Notiz über die Abhängigkeit der Ausbildung der Blütenstände von der Größe der photoperiodisch behandelten Blattfläche bei Kalanchoë blossfeldiana. Flora (Jena) 138, 1-10 (1944)

70. Harder, R., v. Witsch, H., and Bode, O.: Über Erzeugung einseitig und allseitig verlaubter Infloreszenzen durch photoperiodische Behandlung von Laubblättern (Untersuchungen an Kalanchoë blossfeldiana). Jahrb. wiss. Bot. 90, 546-591 (1942)

71. Harder, R., Westphal, M., and Behrens, G., Hemmung der Infloreszenzbildung durch Langtag bei der Kurztagspflanze Kalanchoë blossfeldiana. Planta 36, 424-438 (1949)

72. Harel, S. and Reinhold, L.: The effect of 2,4-dinitrophenol on translocation in the phloem. Physiol. Plant. 19, 634-643 (1966)

73. Hartig, T.H.: Ueber die Querscheidewände zwischen den einzelnen Gliedern der Siebrohren in Cucurbita pepo. Bot. Zeit. 12, 51-54 (1854)

74. Hartmann, H.T.: Some effects of temperature and photoperiod on flower formation and runner production in the strawberry. Plant Physiol. 22, 407-420 (1947)

75. Heinze, P.H., Parker, M.W., and Borthwick, H.A.: Floral initiation in Biloxi soybean as influenced by grafting. Bot. Gaz. 103, 518-530 (1942/3)

76. Ho, L.C. and Peel, A.J.: Investigation of bidirectional movement of tracers in sieve tubes of Salix viminalis L. Ann. Bot. N.S. 33, 833-844 (1969)

77. Ho, L.C. and Mortimer, D.C.: The site of cyanide inhibition of sugar translocation in sugar beet leaf. Can. J. Bot. 49, 1769-1775 (1971)

78. Hoad, G.V.: (+) Abscisin II [(+)-dormin] in phloem exudate of willow. Life Sci. 6, 1113-1118 (1967)

79. Hoad, G.V.: Effect of water stress on abscisic acid levels in white lupin (Lupinus ablus L.) fruit, leaves and phloem exudate. Planta 142, 287-290 (1978)

80. Hoad, G.V. and Bowen, M.: Evidence for gibberellin-like substances in phloem exudate of higher plants. Planta 82, 22-32 (1968)

81. Hoddinott, J. and Gorham, P.: Translocation of ^{14}C-labelled assimilates in petioles and phloem loops of Heracleum lanatum. Can. J. Bot. 52, 349-353 (1974)

82. Huber, B., Schmidt, E., and Jahnel, H.: Untersuchungen über den Assimilatstrom. Tharandter Forstl. Jahrb. 88, 1017-1050 (1937)

83. Husain, I., Ph.D. thesis, University of Adelaide, from L.T. Evans, ed., The Induction of Flowering. Ithaca, New York: Cornell University Press, 1969, p. 340.

84. Imamura, S. and Marushige, Y.: The morphology and development of floral primordia. In: Physiology of Flowering in Pharbitis nil, pp. 7-13, Imamura, S., ed. Tokyo: Japanese Society of Plant Physiologists, 1967

85. Imamura, S. and Takimoto, A.: Photoperiodic responses in Japanese morning glory, Pharbitis nil Chois., a sensitive short-day plant. Bot. Mag. 68, 235-241 (1955)

86. Imamura, S. and Takimoto, A.: Transmission rate of photoperiodic stimulus in Pharbitis nil. Bot. Mag. 68, 260-266 (1955)

87. Imamura, S. and Takimoto, A.: Transmission rate of the photoperiodic stimulus across the graft union in Pharbitis nil Chois. Bot. Mag, 69, 23-29 (1956)

88. Imamura, S. and Takimoto, A.: Effect of ringing and incision given to the stem on transmission of photoperiodic stimulus in Pharbitis nil. Bot. Mag. 70, 13-16 (1957)

89. Imamura. S. and Takimoto, A.: Decrement of photoperiodic stimulus in transmission in Pharbitis nil. Bot. Mag. 70, 53-57 (1957)

90. Kavon, D.L. and Zeevaart, J.A.D.: Simultaneous inhibition of translocation of photosynthate and of the floral stimulus by localized low-temperature treatment in the short-day plant Pharbitis nil. Planta 144, 201-204 (1979)

91. Khudairi, A.K. and Hamner, K.C.: The relative sensitivity of Xanthium leaves of different ages to photoperiodic induction. Plant Physiol. 29, 251-257 (1954)

92. Kidwai, P. and Robards, A.W." The appearance of differentiating vascular cells after fixation in different solutions. J. Exp. Bot. 20, 664-670 (1969)

93. King, R.W. and Evans, L.T.: Timing of evocation and development of flowers in Pharbitis nil. Aust. J. Biol. Sci. 22, 559-572 (1969)

94. King, R.W., Evans, L.T., and Wardlaw, I.F.: Translocation of the floral stimulus in Pharbitis nil in relation to that of assimilates. Z. Pflanzenphysiol. 59, 337-388 (1969)

95. King, R.W. and Zeevaart, J.A.D.: Floral stimulus movement in Perilla and flower inhibition caused by noninduced leaves. Plant Physiol. 51, 727-738 (1973)

96. King, R.W. and Zeevaart, J.A.D.: Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol. 53, 96-103 (1974)

97. Kluge, M., Reinhard, E., and Ziegler, H.: Gibberellinaktavität von Siebröhrensäften. Naturwissenshaften 51, 145-146 (1964)

98. Knott, J.E.: Effect of a localized photoperiod on spinach. Proc. Am. Soc. Hort. Sci. (Suppl.) 31, 152-154 (1934)

99. Kursanov, A.L.: Metabolism and the transport of organic substances in the phloem. Adv. Bot. Res. 1, 209-274 (1963)

100. Lang, A.: Physiology of flower initiation. In: Encycl. Plant Physiol. XV/1, pp. 1380-1536, Ruhland, W., ed. Berlin: Springer-Verlag 1965

101. Lang, A. and Melchers, G.: Die photoperiodische Reaktion von Hyoscyamus niger. Planta 33, 653-702 (1943)

102. Lang, A., Chailakhyan, M.Kh., and Frolova, I.A.: Promotion and inhibition of flower formation in a dayneutral plant in grafts with a short-day plant and a long-day plant. Proc. Natl. Acad. Sci. U.S.A. 74, 2412-2416 (1977)

103. Lang, A.: The effect of petiolar temperature upon the translocation of ^{137}Cs in the phloem of Nymphoides peltata. J. Exp. Bot. 25, 71-80 (1974)

104. Lincoln, R.G., Raven, K.A., and Hamner, K.C.: Certain factors influencing expression of the flowering stimulus in Xanthium. Part I. Translocation and inhibition of the flowering stimulus. Bot. Gaz. 117, 193-206 (1956)

105. Lona, F.: Esperienze sulla antoinibizione fotoperiodica e sulla fioritura delle brevidiurne a notta continua. Humus (Milan) 6 (4) 6-10 (1950)

106. Lubimenko, V.N. and Bouslova, E.D.: Contribution à la thèorie du photoperiodisme II. Dokl. Acad. Sci. U.S.S.R. 14, 149-152 (1937)

107. MacRobbie, E.A.C.: Phloem translocation. Facts and mechanisms: a comparative survey. Biol. Rev. 46, 429-481 (1971)

108. Majumder, S.K. and Leopold, A.C.: Callose formation in response to low temperature. Plant and Cell Physiol. 8, 775-778 (1967)

109. Mason, T.G. and Maskell, E.J.: Studies on the transport of carbohydrates in the cotton plant. I. A study of diurnal variation in the carbohydrates of leaf, bark and wood, and the effect or ringing. Ann. Bot. 42, 189-253 (1928)

110. Mason, T.G. and Maskell, E.J.: Studies on the transport of carbohydrates in the cotton plant. II. The factors determining the rate and the directon of movement of sugars. Ann. Bot. 42, 571-636 (1928)

111. Mason, T.G. and Phillis, E.: Further studies on transport in the cotton plant. V. Oxygen supply and the activation of diffusion. Ann. Bot. 50, 455-499 (1936)

112. McNairn, R.B. and Currier, H.B.: Translocation blockage by sieve-plate callose. Planta 82, 369-380 (1968)

113. Metzger, J.D. and Steucek, G.L.: Response of barley (Hordeum vulgare) seedlings to mechanical stress. Proc. Pennsylvania Acad. Sci. 48, 114-116 (1974)

114. Mittler, T.E.: Studies on the feeding and nutrition of Tuberlolachnus salignus (Gmelin) (Homoptera, Aphididae). I. The uptake of phloem sap. J. Exp. Biol. 34, 334-341 (1958)

115. Mittler, T.E.: Studies on the feeding and nutrition of Tuberlolachnus salignus (Gmelin) (Homoptera, Aphididae). I. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. J. Exp. Biol. 35 74-84 (1958)

116. Mortimer, D.C.: Translocation of photosynthetic products in sugar beet petioles. Plant Physiol. 36, Suppl., XXXIV (1961)

117. Mortimer, D.C.: Translocation of the products of photosynthesis in sugar beet petioles. Can. J. Bot. 43, 269-280 (1965)

118. Moshkov, B.S.: Blüte von Kurztagspflanzen in kontinuierlicher Beleuchtung als Resultat von Pfropfungen. Trudy Priklad. Botan. Genetike i Selekstü A 21, 145-156 (1937)

119. Moshkov, B.S.: Transfer of photoperiodic reaction from leaves to growing points. Dokl. Acad. Sci. U.S.S.R. 24, 489-491 (1939)

120. Moshkov, B.S.: On the physiological and surgical removal of leaves during graft action. Dokl. Akad. Nauk. S.S.S.R. 31, 161-162 (1941)

121. Münch, E.: Die Stoffbewegungen in der Pflanze. Verlag Gustav Fischer, pp. 234 Jena (1930)

122. Münch, E.: Durchlässigkeit der Siebröhren für Druckströmungen. Flora (Jena) 136, 223-262 (1943)

123. Nonweiler, T.R.F.: Flow of biological fluids through nonideal capillaries. In: Encycl. Plant Physiol., N.S., Vol. I, Transport in plants, pt. I. Appendix I, pp. 474-477, Zimmermann, M.H., Milburn, J.A., eds. Berlin: Springer-Verlag 1975

124. Ogawa, Y.: Über die Auslösung der Blütenbildung von Pharbitis nil durch niedere Temperatur. Bot. Mag. 73, 334-335 (1960)

125. Parker, J.: Transcellular strands and intercellular particles movement in sieve tubes of some common tree species. Naturwissenschaften 51, 273-274 (1964)

126. Paton, D.M. and Barber, H.N.: Physiological genetics of Pisum. I. Grafting experiments between early and late varieties. Aust. J. Biol. Sci. 8, 231-240 (1955)

127. Peel, A.J. and Weatherly, P.E.: Composition of sieve-tube sap. Nature 184, 1955-1956 (1959)

128. Peel, A.J., Field, R.J., Coulson, C.L., and Gardner, D.C.J.: Movement of water and solutes in sieve tubes of willow in response to puncture by aphid stylets. Evidence against a mass flow of solution. Physiol. Plant. 22, 768-775 (1969)

129. Phillips, D.A. and Cleland, C.F.: Cytokinin activity from the phloem sap of Xanthium strumarium L. Planta 102, 173-178 (1972)

130. Qureshi, F.A. and Spanner, D.C.: The effect of nitrogen on the movement of tracers down the stolon of Saxifraga sarmentosa with some observations on the influence of light. Planta 110, 131-144 (1973)

131. Qureshi, F.A. and Spanner, D.C.: The influence of dinitrophenol on phloem transport along the stolon of Saxifraga sarmentosa. Planta 111, 1-12 (1973)

132. Qureshi, F.A. and Spanner, D.C.: Cyanide inhibition of phloem transport along the stolon of Saxifraga sarmentosa L. J. Exp. Bot. 24, 751-762 (1973)

133. Roberts, R.H. and Struckmeyer, B.E.: The effects of temperature and other environmental factors upon the photoperiodic response of some of the higher plants. J. Agr. Res. 56, 633-677 (1938)

134. Sachs, R.M.: Floral initiation in Cestrum nocturnum. I. A long-short-day plant. Plant Physiol. 31, 185-192 (1956)

135. Salisbury, F.B. and Bonner, J.: The reactions of the photoinductive dark period. Plant Physiol. 31, 141-147 (1956)

136. Schmitz, K. and Willenbrink, J.: Histoautoradiographischer Nachweis ^{14}C-markierter Assimilate in Phloem. Z. Pflanzenphysiol. 58, 97-107 (1967)

137. Schneider-Orelli, O.: Die Miniergänge von Lyonetia clercella L. und die Stoffwanderung in Apfelblätten. Zbl. f. Bact. 24 II, 158-181 (1909)

138. Schumacher, W.: Untersuchungen über die Lokalisation der Stoffwanderung in den Leitbündeln höherer Pflanzen. Jahrb. wiss. Bot. 73, 770-823 (1930)

139. Searle, N.E.: Persistence and transport of flowering stimulus in Xanthium. Plant Physiol. 36, 656-662 (1961)

140. Selim, H.H.A.: Translocation of the floral stimulus in two-branched plants of Perilla. Proc. Kon. Nederl. Akad. Wetensch. Amsterdam. Ser. C 60, 67-69 (1957)

141. Sij, J.W. and Swanson, C.A.: Effect of petiole anoxia on phloem transport in squash. Plant Physiol. 51, 368-371 (1973)

142. Skok, J. and Scully, N.J.: Characteristics and movement of the flowering stimulus from the induced leaf of Xanthium. Bot. Gaz. 116, 142-147 (1954)

143. Spanner, D.C.: The translocation of sugar in sieve tubes. J. Exp. Bot. 9, 332-342 (1958)

144. Spanner, D.C.: Sieve-plate pores, open or occluded? A critical review. Plant, Cell and Environment 1, 7-20 (1978)

145. Stigter, H.C.M. de: Studies on the nature of the incompatibility in a Cucurbitaceous graft. Meded. Landbouwhogeschool Wageningen 56, 1-51 (1956)

146. Stigter, H.C.M. de: Parallelism between the transport of ^{14}C-photosynthate and the flowering response in grafted Silene armeria. Z. Pflanzenphysiol. 55 11-19 (1966)

147. Stout, M.: Translocation of the reproductive stimulus in sugar beets. Bot. Gaz. 107, 86-95 (1945)

148. Swanson, C.A. and Böhning, R.: Translocation of carbohydrates. Plant Physiol. 26, 557-564 (1951)

149. Swanson, C.A. and Whitney, J.B., Jr.: Studies on the translocation of foliar applied ^{32}P and other radioisotopes in bean plants. Am. J. Bot. 40, 816-823 (1953)

150. Swanson, C.A. and Geiger, D.R.: Time course of low temperature inhibition of sucrose translocation in sugar beets. Plant Physiol. 42, 751-756 (1967)

151. Takeba, G. and Takimoto, A.: Translocation of the floral stimulus in Pharbitis nil. Bot. Mag. 79, 811-814 (1966)

152. Takimoto, A., Tashima, Y., and Imamura, S.: Effect of temperature on flower initiation of Pharbitis nil cultured in vitro. Bot. Mag. 73, 377 (1960)

153. Tammes, P.M.L., Vonk, C.R., and Van Die, J.: Studies on phloem exudation from Yucca flaccida Harv. VII. The effect of cooling on exudation. Acta Bot. Neerl. 18, 224-229 (1969)

154. Thaine, R.: Transcellular strands and particle movement in mature sieve tubes. Nature 192, 772-773 (1961)

155. Thaine, R.: A translocation hypothesis based on the structure of plant cytoplasm. J. Exp. Bot. 13, 152-160 (1962)

156. Thaine, R.: Protoplast structure in sieve-tube elements. New Phytol. 63, 236-243 (1964)

157. Thaine, R.: Movement of sugars through plants by cytoplasmic pumping. Nature 222, 873-875 (1969)

158. Thompson, P.A. and Guttridge, C.G.: The role of leaves as inhibitors of flower induction in strawberry. Ann. Bot. N.S. 24, 482-490 (1960)

159. Thrower, S.L.: Translocation of labelled assimilates in the soybean. IV. Some effects of low temperature on translocation. Aust. J. Biol. Sci. 18, 449-461 (1965)

160. Tournois, J.: Anomalies florales du houblon japonais et du chanvre déterminées par des semis hâtifs. C. R. Acad. Sci. 155, 1017-1020 (1911)

161. Tournois, J.: Influence de la lumière sur la floraison du houblon japonais et du chanvre. C. R. Acad. Sci.

162. Tournois, J.: Étude sur la sexualité du houblon. Ann. Sci. Nat. Bot. sér. 9, 19 49-191 (1914)

163. Trip, P. and Gorham, P.R.: Autoradiographic study of the pathway of translocation. Can. J. Bot. 45, 1567-1573 (1967)

164. Trip, P. and Gorham, P.R.: Translocation of sugar and tritiated water in squash plants. Plant Physiol. 43, 1845-1849 (1968)

165. Trip, P., Nelson, C.D., and Krotkov, G.: Selective and preferential translocation of ^{14}C-labeled sugars in white ash and lilac. Plant Physiol. 40, 740-747 (1965)

166. Tsybul'ko, V.S. and Yastrebov, F.F.: Flow of assimilation products in Sorghum under conditions of brief interruption of night by light. Sov. Plant Physiol. 15, 511-515 (1968)

167. Turgeon, R. and Webb, J.A.: Growth inhibition by mechanical stress. Science 173, 961-962 (1971)

168. Ullrich, W.: Zur Sauerstoffabhängigkeit des Transportes in den Siebröhren. Planta 57, 402-429 (1961)

169. Ullrich, W.: Über die Bildung von Kallose bei einer Hemmung des Transportes in den Siebröhren durch Cyanid. Planta 59, 387-390 (1963)

170. Van den Honert, T.H.: On the mechanism of transport of organic materials in plants. Proc. Kon. Nederl. Akad. Wetensch. Amsterdam. 35, 1104-1112 (1932)

171. Van de Pol, P.A.: Floral induction, floral hormones and flowering. Meded. Landbouwhogeschool Wageningen 72-9, 1-89 (1972)

172. Vernon, L.P. and Aronoff, S.: Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch. Biochem. Biophys. 36, 383-398 (1952)

173. de Vries, H.: Ueber die Bedeutung der Circulation und der Rotation des Protoplasmas für den Strofftransport in der Pflanze. Bot. Zeit., 43, 18-26 (1885)

174. Walding, H.F.: Studies in sieve tube translocation. Ph.D. thesis, University of Aberdeen, from P. E. Weatherly, Some aspects of the Münch hypothesis. In: Phloem Transport, Aronoff, S., Dainty, J., Gorham, P.R., and Srivastava, L.M., eds, New York: Plenum Press 1974

175. Wardlaw, I.F.: Temperature and the translocation of photosynthate through the leaf of Lolium temulentum. Planta 104, 18-34 (1972)

176. Watson, B.T.: The influence of low temperature on the rate of translocation in the phloem of Salix viminalis L. Ann. Bot. N.S. 39, 889-900 (1975)

177. Weatherly, P.E.: Some aspects of the Münch hypothesis. In: Phloem Transport, pp. 535-555, Aronoff, S., Dainty, J., Gorham, P. R., and Srivastava, L.M., eds. New York: Plenum Press 1974

178. Weatherly, P.E., Peel, A.J., and Hill, G.P.: The physiology of the sieve tube. J. Exp. Bot. 10, 1-16 (1959)

179. Weatherly, P.E. and Watson, B.T.: Some low-temperature effects on sieve-tube translocation in Salix viminalis. Ann. Bot. N.S. 33, 845-853 (1969)

180. Webb, J.A.: Translocation of sugars in Cucurbita melopepo. IV. The effect of temperature change. Plant Physiol. 42, 881-885 (1967)

181. Webb, J.A.: Translocation of sugars in Cucurbita melopepo. VI. The reversible low-temperature inhibition of ^{14}C-movement and the cold acclimation of phloem tissue. Can. J. Bot. 49, 717-733 (1971)

182. Webb, J.A. and Gorham, P.R.: Translocation of photosynthetically assimilated ^{14}C in straight-necked squash. Plant Physiol. 39, 663-672 (1964)

183. Webb, J.A. and Gorham, P.R.: The effect of node temperature on assimilation and translocation of ^{14}C in the squash. Can. J. Bot. 43, 1009-1020 (1965)

184. Wellensiek, S.J.: The floral hormones in Silene armeria L. and Xanthium strumarium L. Z. Pflanzenphysiol. 63, 25-30 (1970)

185. Willenbrink, J.: Ueber die Hemmung des Stofftransports in den Siebröhren durch lokale Inaktivierung verschiedener Atmungsenzyme. Planta 48, 269-342 (1957)

186. Willenbrink, J.: Zur lokalen Hemmung des Assimilattransports durch Blausäure. Z. Pflanzenphysiol. 55 119-130 (1966)

187. Williamson, R.E.: An investigation of the contractile protein hypothesis of phloem translocation. Planta 106, 149-157 (1972)

188. Withrow, A.P. and Withrow, R.B.: Translocation of the floral stimulus in Xanthium. Bot. Gaz. 104, 409-416 (1943)

189. Zeevaart, J.A.D.: Flower formation as studied by grafting. Meded. Landbouwhogschool Wageningen 58, 1-88 (1958)

190. Zeevaart, J.A.D.: Physiology of flowering. Science 137, 723-731 (1962)

191. Zeevaart, J.A.D.: Effects of the growth retardant CCC on floral initiation and growth in Pharbitis nil. Plant Physiol. 39, 402-408 (1964)

192. Zeevaart, J.A.D.: Bryophyllum. In: The Induction of Flowering, pp. 435-456, Evans, L.T., ed, Ithaca, N.Y.: Cornell University Press 1969

193. Zeevaart, J.A.D.: Physiology of flower formation. Ann. Rev. Plant Physiol. 27, 321-348 (1976)

194. Zeevaart, J.A.D.: Sites of abscisic acid synthesis and metabolism in Ricinus communis L. Plant Physiol. 59 788-791 (1977)

195. Zeevaart, J.A.D. and Lang, A.: The relationship between gibberellin and floral stimulus in Bryophyllum daigremontianum. Planta 58, 531-542 (1962)

196. Zeevaart, T.A.D. and Marushige, K.: Biochemical Approaches. In: Physiology of Flowering in Pharbitis nil, pp. 121-144, Imamura, S., ed. Tokyo: Japanese Society of Plant Physiologists 1967

197. Zeevaart, J.A.D., Brede, J.M., and Cetas, C.B.: Translocation patterns in Xanthium in relation to long day inhibition of flowering. Plant Physiol. 60, 747-753 (1977)

198. Ziegler, H.: Uber die Atmong und den Stofftransport in den isolierten Leitbündeln der Blattstiele von Heracleum mantegazzianum Somm. et Lev. Planta 51, 186-200 (1958)

199. Ziegler, H.: Nature of transported substances. In: Encycl. Plant Physiol., N.S., Vol. I., Transport in Plants, pt. I, pp. 59-100, Zimmermann, M.H., and Milburn, J.A., eds. Berlin: Springer-Verlag 1975

200. Ziegler, H. and Vieweg, G.H.: Der experimentelle Nachweis einer Massenströmung im Phloem von Heracleum montegazzianum Somm. et Lev. Planta 56, 402-408 (1961)

201. Ziegler, H. and Ziegler, I.: Die wasserlöslichen Vitamine in den Siebröhrensäften einiger Bäume. Flora (Jena) 152, 257-278 (1962)

202. Zimmerman, P.W. and Kjennerud, J.: Flowering and other responses induced in Piquera trinervia with photoperiodic treatment. Contrib. Boyce Thompson Inst. 16, 177-189 (1950)

203. Zimmermann, M.H.: Translocation of organic substances in trees. II. On the translocation mechanism in the phloem of white ash (Fraxinus americana L.) Plant Physiol. 32, 399-404 (1957)

204. Zimmermann, M.H.: Translocation of organic substances in trees. III, The removal of sugars from the sieve tubes in white ash (Fraxinus americana L.). Plant Physiol. 33, 213-217 (1958)

205. Zimmermann, M.H.: Longitudinal and tangential movement within the sieve-tube system of white ash (Fraxinus americana L.). Beih. Z. Schweiz. Forstvereins. 30, 289-300 (1960)

206. Zimmermann, M.H.: Translocation of organic substances in trees. V. Experimental double interruption of the phloem in the white ash (Fraxinus americana L.). Plant Physiol. 37, 527-530 (1962)

207. Zimmermann, M.H.: Translocation velocity and specific mass transfer in the sieve tubes of Fraxinus americana L. Planta 84, 272-278 (1969)

208. Zimmermann, M.H. and Brown, C.L.: Trees: Structure and Function. New York: Springer-Verlag 1971