TESTING THE EFFECTS OF SOME PLANT GROWTH REGULATORS ON EMBRYONIC DIAPAUSE OF THE EUROPEAN PINE SAWFLY

D. R. WALLACE AND C. R. SULLIVAN

GREAT LAKES FOREST RESEARCH CENTRE

SAULT STE. MARIE, ONTARIO

REPORT O-X-239

CANADIAN FORESTRY SERVICE

DEPARTMENT OF THE ENVIRONMENT

DECEMBER 1975

Copies of this report may be obtained from

Information Office,
Great Lakes Forest Research Centre,
Canadian Forestry Service,
Box 490,
Sault Ste. Marie, Ontario,
P6A 5M7.

ABSTRACT

Indole-3-acetic acid, gibberelic acid and kinetin were supplied in water solution to cut branchlets of Scots pine, *Pinus sylvestris* L., bearing freshly laid European pine sawfly, *Neodiprion sertifer* (Geoff.), eggs. These plant growth regulators affected the health of the branches over a 31-day experimental period. Kinetin promoted bud formation in the axils of needle fascicles near the cut apical end of the branch. The induction of embryonic diapause in *N. sertifer* was not altered by the plant growth substances.

RÉSUMÉ

En solution aqueuse, l'acide indole-3-acétique, l'acide gibberelique et la kinétine furent fournies à des ramules coupés de Pin sylvestre, *Pinus sylvestris* L., sur lesquels se trouvaient des oeufs récemment déposés de la Tenthrède du Pin d'Écosse, *Neodiprion sertifer* (Geoff.). Ces régulateurs de la croissance des plantes affectèrent la santé des ramules après une période expérimentale de 31 jours. La kinétine provoqua la formation de bourgeons à l'aisselle des fascicules d'aiguilles près du bout apical coupé du ramule. L'induction de diapause embryonnaire chez *N. sertifer* ne fut pas altérée par ces trois substances régulatrices.

TABLE OF CONTENTS

INTRODUCTION

Students of the conifer sawflies, the Diprionidae, frequently remark about the close physical contact between the insect egg and the host plant tissue that results from the oviposition habits of the sawfly females. This oviposition behaviour is a highly specialized process and usually each species has a distinctive egg pattern with characteristics that are determined by the movement sequence of the ovipositing female and the interaction of the morphometries of the female and the conifer needle she has selected (Ghent 1959). In most species attacking pines the eggs are inserted individually into slit-like cavities cut in the mesophyll tissue of the needles. With so intimate a mechanical relationship with such an active plant tissue and with the proximity to the needle vascular elements, it would not be surprising to find that the physiological state of the needle influences the physiological state of the egg and developing embryo. It is a well-documented fact that water is often a vital environmental component of insect eggs and that in many species the eggs must obtain water from their environment to allow successful development (Browning 1967). As long ago as 1740 Reaumur noted that the eggs of sawflies absorb water through their cuticular membranes. Breny (1957) quotes several other authors who describe in a general way how sawfly eggs, including those of the European pine sawfly, *Neodiprion sertifer* (Geoff.), absorb water from their host plants. Our own unpublished data for *N. sertifer* show that the newly hatched larva weighs about twice as much as a ripe ovarian egg and this does not take into account the weight of the fluid and the membranes lost in the hatching process. The dry weight of the newly hatched larva, again without the egg membrane, is about two-thirds to three-quarters of the dry weight of the mature ovarian egg. On a dry weight basis, uncorrected for weight loss during development, the water content increases from about 75% just before oviposition to 375% in the newly hatched larva. The absolute amount of water in the larva is about 3.5 times greater than in the ovarian egg.

The main points are that relatively large amounts of water are incorporated inside the maternal and embryonic envelopes during embryonic development and that because of the location of the egg this water comes from the cell protoplasm and/or the conducting elements of the needle. Usually in the case of sawflies it has been assumed that the egg membranes are permeable to water only and thus the physiological action of the plant sap is that of pure water with the exception of osmotic effects. Extensive investigations of water and solute uptake by cricket eggs (McFarlane 1970; Browning 1967, 1969, 1972; Hogan 1962a, b) have shown that these egg membranes are permeable to substances such as ammonia, urea, and glucose. Furthermore, Hogan (1961, 1964) showed that ammonia, some ammonium compounds and urea influence the rate of termination of the embryonic diapause in the common field cricket, *Teleogryllus commodus* (Walk.). The effectiveness of the ammonium compounds is influenced by the anion present. Only those with organic acid radicals are active and Hogan believed that the function of the organic acid radical is to affect the rate of entry of the ammonia into the egg. In studies of the termination of embryonic diapause in eggs of the false melon beetle, *Atrachya menetriesi* Faldermann,

by immersion in dilute solutions of mercuric chloride, Ando (1971) found that the mercury ions can enter the eggs of that insect if the concentration is great enough. Ivanova-Kasas (1959) found that the eggs of a willow-leaf-gall sawfly, *Pontania capreae* L., are slightly permeable to salts and dyes. Among parasitic Hymenoptera in which the yolk system providing nutrients for embryological development from within the egg may be greatly reduced or even entirely lacking, the maternal and embryonic membranes must be permeable to nutrients from the host (Ivanova-Kasas 1972). A similar condition exists in the paedogenetic Diptera where the nutrients are supplied from the substance of the mother herself without the intermediary of yolk formation (Ivanova-Kasas 1965). Preliminary experiments of our own suggest that some sugars and amino acids may pass into the eggs of *N. sertifer* via the needle tissues (unpublished data). In other work we found that colchicine supplied to egg-bearing shoots killed the eggs of the pine sawfly, *N. nigroscutum* Midd.

The range of substances that can reach the egg surface in the plant juice that supplies the water for swelling is certainly great. Although no direct studies of xylem sap in pine needles are known to us, the work of Barnes (1963a, b) shows that the xylem sap of pine branches is a good source (20 mgl^{-1}) of glutamine and six other amino acids. Zimmermann's (1960) review suggests that the phloem sap probably contains considerable amounts of soluble carbohydrates (10%-25% w/v), especially sucrose (Nelson 1964). The myriad substances that would be present in the "cell sap" of the mesophyll needs no elaboration. The kinds and concentrations of the solutes from all the sources will vary with the season and the physiological state of the tree.

There are some characteristics of the eggs and embryonic development in conifer sawflies that should be recognized before discussing the potential effects of plant solutes passing through the egg membranes. The eggs of conifer sawflies are relatively large, 1-2 mm x ~ 0.5 mm x ~ 0.25 mm (Forsius 1920, Brygider 1952), and have a moderate yolk supply. They develop in ovaries of the polytrophic meroistic type (Smith 1941, Mahowald 1972) and the female's total egg complement may be mature and ready for oviposition at the time of adult emergence from the cocoon. After oviposition the eggs undergo meroblastic cleavage and embryonic development may take from about a week to 5 or 6 weeks, depending on the temperature, for species without an embryonic diapause. When the diapause occurs, the interval from oviposition to larval eclosion may be 10 months.

The possible effects of solutes from the plant sap entering the pine sawfly egg may be considered from two viewpoints, first as nutrient material and second as substances regulating the course of embryonic or subsequent development. When embryological development is completed rapidly and without an embryonic diapause it is not likely that translocation of solutes from the plant into the egg would be of much nutritional consequence when the eggs have a good yolk supply. Perhaps there may be instances when the yolk has been impoverished in some way so that nutrients might become important. On the other hand, when there is an embryonic diapause and the embryo must be sustained for long periods

of time, nutrients from the host plant could become very important. The implications of this possibility and its role in population trends of diprionid species such as *N. sertifer*, *N. taedae* ssp., *N. pratti* ssp., *N. nanulus* ssp., and *N. abietis sensu lato* have not been investigated.

The possibility that solutes from the plant sap could act as developmental regulators is also much more likely in the cases where the egg in the needle is the overwintering stage. The period of cessation of embryogenesis in the fall and its resumption and culmination in the spring tend to coincide with periods of marked physiological change in the host tree. Among substances that might influence the induction and termination of embryonic diapause and are present in changing concentrations over the season are the plant growth substances. The presence of gibberellin-like substances, inhibitors, and cytokinins has been demonstrated in *P. sylvestris* (Kopcewicz 1968a, b, 1970; Rogozińska 1967), where they may influence such processes as seed germination, radicle growth, hypocotyl growth, stem growth, breaking of dormancy, apical dominance, and cambial activity (Dunberg 1974, Zajączkowski 1973). Since the entering and leaving of winter dormancy in the embryo of *N. sertifer* and in its host tree, *P. sylvestris*, are at least fortuitously synchronized, and these events in the tree are related to changes in growth regulators, we decided to test the effects of exogenous growth regulators on the initiation of the embryonic diapause in *N. sertifer*. Three substances were tested: gibberelic acid (K salt) (GA), indole-3-acetic acid (IAA) and kinetin (6-furfuryl-amino-purine) (KIN)[1].

MATERIALS AND METHODS

Neodiprion sertifer adults for mating and oviposition were obtained by incubating cocoons collected near Durham, Ontario, in 1973. Cocoon incubation was at $\sim 20^{\circ}$C and 13 hours light per 24-hour day, beginning on 26 April, 1974. Pairs of adults (1♂ 1♀) were enclosed over freshly prepared *P. sylvestris* branches in lamp chimney assemblies on 5 June, 1974. The *P. sylvestris* branches were cut directly into water from trees in a young plantation (trees $\leq$ 1 m in height) at the Pointe aux Pins Insectary just west of Sault Ste. Marie, Ontario on 3 June, 1974 and were prepared using our scrubbing procedure followed by Javex® (10% v/v) and 95% ethyl alcohol dips. All 1974 growth was removed by cutting carefully at the shoot base. There was good oviposition overnight on 5-6 June and only egg clusters laid in this interval were used in the test.

The rationale of the experimental design was to test the substances individually and in combination by adding them directly to the water supply of the shoot, thereby giving them direct access to the translocation system of the cut shoot. The basic concentration used was

---

[1]The growth regulators used, GA, IAA, and KIN, were obtained from ICN Nutritional Biochemicals Corporation, Cleveland, Ohio.

IAA, 5 mgl^{-1}; GA, 1 mgl^{-1}; KIN, 0.2 mgl^{-1} (Zajączkowski 1973). Two additional concentration levels were used: ten times the basic level and one-tenth the basic level. All possible combinations were made within each concentration level, but none among different concentration levels. Stock solutions were made as follows:

IAA 250 mg in 25 ml absolute ethyl alcohol
GA 500 mg in 25 ml distilled water
KIN 10 mg in 10% (v/v) aqueous hydrochloric acid.

The addition of 1 ml of stock solution to each container of ~ 200 ml water provided the highest concentration level desired. Appropriate serial dilutions were made to provide the two lower concentration levels by adding 1 ml of the diluted stocks to the water in the shoot containers. Suitable controls were also established. Table 1 summarizes the different test and control conditions.

Two egg-bearing shoots were used for each of the seven combinations of plant growth substances at each of the three concentration levels and five for each of the controls for a total of 62 egg clusters. All plant growth substances were added to the water containers on the afternoon of 6 June and the controls were established on the morning of 7 June. The egg-bearing shoots were held at 21±1°C, 70±5% R.H., and 17 hours light per 24-hour day. The light was provided by four 40-watt Sylvania Gro-lux® fluorescent tubes 12-15 in. (30.48-38.10 cm) above the branches. The branch ends in the liquids were not clipped nor were the solutions in the jars changed during the observation period, but distilled water was added to maintain the liquid levels.

An egg-bearing needle was removed from each shoot on 11 June, 16 June, and 21 June, respectively, and fixed in hot modified Kahle's fixative. Later the eggs were dissected from the needles, stained in Grenacher's borax-carmine, and the stage of embryonic development was scored according to the developmental series illustrated by Breny (1957).

On 8 July, 1974 all the shoots were examined and notes were taken on their general health as well as on obvious microbiological growth in the bathing liquids.

RESULTS

On 21 June, about 15 days after the start of the experiment, there were no totally dead shoots and only a few appeared to be dying. Later, at the end of the 31-day experimental period, the control shoots in water alone were all healthy, although there was some accumulation of microorganisms in the water (Table 2). This accumulation was greater than usual because our normal procedure of periodic wiping and trimming of the shoots and transfer to clean containers of water was not followed. Similarly, the containers with control shoots standing in 0.5% ethyl alcohol (5000 ppm alcohol) had some growth of microorganisms in the

Table 1

Summary of experimental and control conditions. An X indicates the inclusion of the substance at the concentration shown. Each horizontal line represents a single condition at each concentration level.

	Level 1			Level 2			Level 3		
	IAA	GA	KIN	IAA	GA	KIN	IAA	GA	KIN
Solutes	50 mgl^{-1}	10 mgl^{-1}	2 mgl^{-1}	5 mgl^{-1}	1 mgl^{-1}	0.2 mgl^{-1}	0.5 mgl^{-1}	0.1 mgl^{-1}	0.02 mgl^{-1}
IAA	X			X			X		
GA		X			X			X	
KIN			X			X			X
IAA+GA	X	X		X	X		X	X	
IAA+GA+KIN	X	X	X	X	X	X	X	X	X
IAA+KIN	X		X	X		X	X		X
GA+KIN		X	X		X	X		X	X

Controls:
a) Water only
b) Water + 1 ml absolute ethyl alcohol (in 200 ml water)
c) Water + 1 ml 10% aqueous HCl (in 200 ml water)
d) Water + 1 ml absolute ethyl alcohol + 1 ml 10% aq. HCl (in 200 ml water)

Table 2

Condition of *P. sylvestris* branchlets bearing *N. sertifer* eggs at end of 31-day experimental period and visual estimation of growth of microorganisms in shoot containers.

Treatment	Shoot no.	Shoot condition	Bud formation[a]	Microbiological growth in solution[b]		
				Black	Slimy	Milky
Control - Water	1	healthy	+	+		
	2	healthy		+		
	3	healthy	+	++		
	4	healthy		+		
	5	healthy		+		
Control - 0.005% aq. HCl	1	basal 2/3 dead		+++		
	2	dead		+++		
	3	poor		++		
	4	basal 1/3 dead		+++	+++	
	5	dead		+++		
Control - 0.5% Ethyl alcohol	1	healthy		<+		
	2	healthy	+	<+		
	3	healthy		<+		
	4	healthy				+
	5	healthy	+			<+
Control - 0.005% aq. HCl - 0.5% Ethyl alcohol	1	dead		+++		
	2	fair		++		
	3	dead		++		
	4	poor			+	
	5	dead		++		
IAA 50 mgl^{-1}	1	poor-fair			+++	+
	2	fair-good				++
5 mgl^{-1}	1	healthy	+			
	2	healthy		++		
0.5 mgl^{-1}	1	healthy		++		
	2	healthy		++		
GA 10 mgl^{-1}	1	dead				+
	2	good	+	<++		
1 mgl^{-1}	1	dead				+
	2	dead		++		+
0.1 mgl^{-1}	1	healthy	+		+	
	2	poor		<++		

(continued)

Table 2 (concluded)

Condition of *P. sylvestris* branchlets bearing *N. sertifer* eggs at end of 31-day experimental period and visual estimation of growth of microorganisms in shoot containers.

Treatment	Shoot no.	Shoot condition	Bud formation[a]	Microbiological growth in solution[b]		
				Black	Slimy	Milky
KIN 2 mgl^{-1}	1	basal 3/4 dead		++++		
	2	good	+	++++	++++	
0.2 mgl^{-1}	1	healthy	+	++		
	2	healthy	++	<++		
0.02 mgl^{-1}	1	healthy	++	+		
	2	healthy	++	++		
IAA + GA 50 + 10 mgl^{-1}	1	dead			+	+++
	2	dead			+	+++
5 + 1 mgl^{-1}	1	dead				+
	2	dead				+
0.5 + 0.1 mgl^{-1}	1	dead		+++		
	2	dead		++		
GA + KIN 10 + 2 mgl^{-1}	1	dead		+++	+++	
	2	dead		+++	+++	
1 + 0.2 mgl^{-1}	1	good		++	++	
	2	dead		++	++	+
0.1 + 0.02 mgl^{-1}	1	good		++		
	2	poor	++		++	
IAA + KIN 50 + 2 mgl^{-1}	1	dead		<++		
	2	dead		++		
5 + 0.2 mgl^{-1}	1	healthy		+++		
	2	healthy		++		
0.5 + 0.02 mgl^{-1}	1	good	+	+		
	2	basal 1/2 dead	++	+		
IAA + GA + KIN 50 + 10 + 2 mgl^{-1}	1	dead		+++		
	2	dead		+++		
5 + 1 + 0.2 mgl^{-1}	1	dead		++		+
	2	dead		++		+
0.5 + 0.1 + 0.02 mgl^{-}	1	healthy		+		
	2	poor		++		

[a] Bud formation: +, 1 bud formed in a needle fascicle axil near apical node where shoot had been decapitated; ++, several buds formed in needle fascicle axil.

[b] Microbiological rowth: +, readily apparent on usual examination; ++, moderate growth, +++, heavy growth; ++++, very heavy growth.

solution, but much less than with water alone. The *P. sylvestris* shoots in the 0.5% alcohol were all healthy in appearance. The addition of hydrochloric acid to make a 0.05% solution (1,850 ppm HCl) resulted in a very heavy growth of microorganisms and the shoots were nearly all dead at the end of the observation period. When the immersing solution contained 1,850 ppm hydrochloric acid and 5,000 ppm ethyl alcohol the growth of microorganisms was not much greater than in the water controls, but the condition of the pine branchlets was almost as poor as with hydrochloric acid alone. The detrimental effect of the hydrochloric acid seems to have been directly upon the shoot and not through vascular occlusion because of enhanced growth of organisms. In both control groups, i.e., water and 0.5% ethyl alcohol, where the shoots remained healthy, some rebudding occurred in the axils of terminal needle fascicles of 40% of the shoots.

The controls in which ethyl alcohol and hydrochloric acid were added to the water represented the concentration of these solutes at the highest concentration level of the experimental plant growth regulator solutions. At the two lower levels the concentrations of ethyl alcohol and hydrochloric acid would have been 0.05% (500 ppm) and 0.0005% (185 ppm) in the intermediate level, and 0.005% (50 ppm) and 0.00005% (18.5 ppm) in the lowest level, respectively. The effect of the hydrochloric acid at the two lower concentrations is unknown.

The addition of indole-3-acetic acid (Table 2) to the solution had a detrimental effect on the health of the shoots at 50 mgl^{-1}, but not at the 5 or 0.5 mgl^{-1}. Growth of microorganisms was greater than in the water or alcohol controls. Rebudding was not obviously different from that of the controls.

Gibberelic acid was also detrimental to shoot health even at the lowest concentration. With this substance there were no obvious effects on rebudding or microbial contamination.

Kinetin at 2 mgl^{-1} appeared to counteract the effect of the hydrochloric acid in the solvent for that regulator as shown by the better condition of the experimental shoots than of the acid controls. The growth of microorganisms in the solution containing the kinetin at this concentration was very heavy. At the two lower concentrations of kinetin the shoots remained healthy and rebudding was obviously stimulated, particularly at 0.02 mgl^{-1}. The results indicate that the hydrochloric acid did not inactivate the kinetin because the two lower concentrations were obtained by serial dilution, and it does not seem likely that the protective effect of the kinetin at 2 mgl^{-1} was caused by a neutralizing of the hydrochloric acid. At least if this were the case the reaction product still had plant activity.

All the shoots were killed by the combinations of indole-3-acetic acid and gibberelic acid, while the combination gibberelic acid and kinetin was only slightly less damaging. In the latter test the growth of microorganisms was enhanced except at the lowest concentration. At this

concentration also the stimulation of bud formation by kinetin was apparent. The combination of indole acetic acid and kinetin killed all the shoots at the highest concentration and once again budding was greater at the lowest concentration level. The bud formation on shoots immersed in solutions of mixed growth regulators was related to the presence of kinetin in the mixture. Survival of shoots treated with all three substances was poor.

The results already presented show that the growth regulator treatments affected the health of the shoots and bud formation, yet examination of the *N. sertifer* eggs showed that embryonic development was no different in the controls than in the treatments, and in both the diapause intervened at the stage normally observed in laboratory tests.

DISCUSSION

Prediapause embryonic development in *N. sertifer* takes place very rapidly at ∿ 21°C, requiring about only 10 days. Our observations show that the shoots were in good condition for this interval or longer and that the plant growth regulators supplied to the shoots affected the shoots but did not prevent the inception of embryonic diapause in *N. sertifer*.

The results of this preliminary test do not suggest that it would be worthwhile to continue this study. It is possible, though, that our negative results are misleading because of certain aspects of our techniques. The application of a growth regulator solution directly to the cut ends of the shoots should have ensured that the solutes were translocated to all parts of the cuttings, but perhaps the growth regulators were partially inactivated by light exposure in the solutions or by microbial growth. The periodic replacement of the solutions would eliminate this possibility and also provide for the elimination of much of the growth of microorganisms that accumulated. The branch ends could be clipped also to improve uptake. Another choice would be to stand the shoots in pure water and to apply the growth regulators in ointment to the decapitated shoot. The active material could be protected by a suitable covering. This approach, however, would introduce more uncertainty about the uptake and translocation of the growth regulators.

Perhaps the *N. sertifer* embryonic diapause is sensitive to pine growth regulators but only to the specific regulators found in pines. These substances in pines are undoubtedly different from some of those used in our test, judging from the indications of specificity described in the literature (Kopcewicz *et al.* 1967, Rogozińska 1967, Kopcewicz 1968a, Zajączkowski 1973) and the fact that kinetin has never been isolated from a natural source. The time and cost of isolating the natural growth regulators from *P. sylvestris* for use in a test are not justified. On the other hand, a wider range of available growth regulators and concentrations could be tested, including exposure to gaseous ethylene or the use of ethylene-releasing substances.

Another factor that should be considered is whether the introduction of the growth regulators was too late. It is possible that by 12-18 hours after oviposition the developmental sequence has been irrevocably established. Tests could be tried with growth regulators applied before oviposition. To check for *any* effects of growth substances tests could be made using embryos that have been fully potentiated for completion of development to larval eclosion.

During some 8 years of work on the embryonic diapause in *N. sertifer* we have examined embryos developing in eggs laid in the laboratory in *P. sylvestris* needles on branches freshly cut from trees growing outside at all seasons of the year. In some instances the natural events of bud swelling and shoot elongation either continued or were initiated in our experiments. We have never observed any alteration in the induction of the embryonic diapause even though the eggs were certainly subject to some manifestation of the changing pattern of natural pine growth regulators. This evidence in conjunction with the current test results make us conclude that host tree growth regulators are not likely involved in the regulation of the diapause.

There is an additional aspect of this experiment that may have application in our work on the embryonic diapause in some species of conifer sawflies and in our sawfly propagation work for the multiplication of biological control agents. In much of this work there is a basic need to keep cut branchlets of pines in healthy condition for long periods of time at warm temperatures in the laboratory. We have already made considerable progress in this respect without using any additives in the water with which the cut branches are supplied. It is noteworthy that kinetin had a protective effect in the current test and also that this substance is known to improve the water balance and delay senescence in cut flowers (Mayak and Halevy 1974). Similarly, the reduction of microbiological growth we observed when ethyl alcohol was added to the water might be exploited in maintaining healthy shoots for long periods of time because the growth of microorganisms in the conducting tissues of the shoots is one factor contributing to shoot degeneration. Other chemicals such as silver nitrate have been used in floral preservatives to reduce bacterial growth (Bravdo *et al.* 1974). Tests should be conducted with some of these substances to determine the best way of using them to prolong shoot life and to determine if these substances have adverse effects on the insects that would be exposed to the chemicals.

REFERENCES

Ando, Y. 1971. Distribution of mercury in the eggs of the false melon beetle, *Atrachya menetriesi* Faldermann (Coleoptera: Chrysomelidae), treated with mercuric chloride. Appl. Ent. Zool. 6: 67-74.

Barnes, R.L. 1963a. Nitrogen transport in the xylem of trees. J. For. 61: 50-51.

Barnes, R.L. 1963b. Organic nitrogen compounds in tree xylem sap. For. Sci. 9: 98-102.

Bravdo, B., Mayak, S., and Gravrieli, Y. 1974. Sucrose and water uptake from concentrated sucrose solutions by gladiolus shoots and the effect of these treatments on floret life. Can. J. Bot. 52: 1271-1281.

Breny, R. 1957. Contribution à l'étude de la diapause chez *Neodiprion sertifer* Geoffr. dans la nature. Mem. Acad. r. Belg., Classe Sci. 30, No. 1679. 86 p.

Browning, T.O. 1967. Water, and the eggs of insects. *In* Beament, J.W.L., and Treherne, J.E., *Eds*. Insects and Physiology. Oliver and Boyd Ltd., Edinburgh & London, p. 315-328.

Browning, T.O. 1969. The permeability of the shell of the egg of *Teleogryllus commodus* measured with the aid of tritiated water. J. Exp. Biol. 51: 397-405.

Browning, T.O. 1972. The penetration of some non-polar molecules in solution through the egg-shells of *Locusta migratora migratorioides* and *Teleogryllus commodus*. J. Exp. Biol. 56: 769-773.

Brygider, W. 1952. In what embryonic stage do the eggs of *Neodiprion* enter the winter diapause? Can. J. Zool. 30: 99-108.

Dunberg, A. 1974. Occurrence of gibberellin-like substances in Norway spruce (*Picea abies* [L.] Karst.) and their possible relation to growth and flowering. Stud. For. Suec. 111. 66 p.

Forsius, R. 1920. Kleinere Beiträge zur Kenntnis der Tenthredinoiden-Eier I. Medd. Soc. Fauna Fl. Fen. 45: 169-184.

Ghent, A.W. 1959. Row-type oviposition in *Neodiprion* sawflies as exemplified by the European pine sawfly, *N. sertifer* (Geoff.) Can. J. Zool. 37: 267-281.

Hogan, T.W. 1961. The action of urea on diapause in eggs of *Acheta commodus* (Walk.) (Orthoptera: Gryllidae). Austr. J. Biol. Sci. 14: 419-426.

Hogan, T.W. 1962a. The absorption and subsequent breakdown of urea by diapausing eggs of *Acheta commodus* (Walk.) (Orthoptera: Gryllidae). Austr. J. Biol. Sci. 15: 362-370.

Hogan, T.W. 1962b. The effect of ammonia on the rate of termination of diapause in eggs of *Acheta commodus* (Walk.) (Orthoptera: Gryllidae). Austr. J. Biol. Sci. 15:538-542.

Hogan, T.W. 1964. Further data on the effect of ammonia on the termination of diapause in eggs of *Teleogryllus commodus* (Walk.) (Orthoptera: Gryllidae). Austr. J. Biol. Sci. 17: 752-757.

Ivanova-Kasas, O.M. 1959. Die embryonale Entwicklung der Blattwespe *Pontania capreae* L. (Hymenoptera: Tenthredinidae). Zool. Jahrb. Anat. Ont. 77: 193-228.

Ivanova-Kasas, O.M. 1965. Trophic connections between the maternal organism and the embryo in paedogenetic Diptera (Cecidomyidae). Acta Biol. Hung. 16: 1-24.

Ivanova-Kasas, O.M. 1972. Polyembryony in insects. *In* Counce, S.J., and Waddington, C.H., *Eds*. Developmental systems: Insects. Vol. 1. Academic Press, London and New York. p. 243-271.

Kopcewicz, J. 1968a. Some physiochemical and biological properties of the gibberellin-like substances from pine (*Pinus sylvestris* L.). Bull. Acad. Pol. Sci. Ser. Biol. 16: 667-672.

Kopcewicz, J. 1968b. Seasonal changes of gibberellin-like substances and growth inhibitors in the apical meristems of pine (*Pinus sylvestris* L.). Acta Soc. Bot. Pol. 37: 579-587.

Kopcewicz, J. 1970. Seasonal changes of auxin-like substances and growth inhibitors in the apical meristems of pine (*Pinus sylvestris*). Zesz. Nauk. Uniw. Mikolaja Kopernika Ser. Biol. 23: 123-130.

Kopcewicz, J., Michniewicz, M., and Kriesel, K. 1967. Dynamics of gibberellin-like substances and growth inhibitors in pine (*Pinus sylvestris* L.) and larch (*Larix decidua* Mill.) in relation to age and season. Bull. Acad. Pol. Sci. Ser. Biol. 15: 427-433.

Mahowald, A.P. 1972. Oogenesis. *In* Counce, S.J., and Waddington, C.H., *Eds*. Developmental systems: Insects. Vol. 1. Academic Press, London and New York, 1-47.

Mayak, S., and Halevy, A.N. 1974. The action of kinetin in improving the water balance and delaying senescence processes of cut rose flowers. Physiol. Plant. 32: 330-336.

McFarlane, J.E. 1970. The permeability of the cricket egg shell. Comp. Biochem. Physiol. 37: 133-141.

Nelson, C.D. 1964. The production and translocation of photosynthate-C^{14} in conifers. *In* Zimmerman, M.H., *Ed.* The formation of wood in forest trees. Academic Press, New York and London. p. 243-257.

Rogozińska, J.H. 1967. The occurrence of cytokinins in Scots pine. Bull. Acad. Pol. Sci. Ser. Biol. 15: 789-794.

Smith, S.G. 1941. A new form of spruce sawfly identified by means of its cytology and parthenogenesis. Sci. Agric. 21: 245-305.

Zajączkowski. S. 1973. Auxin stimulation of cambial activity in *Pinus sylvestris*. I. The differential cambial response. Physiol. Plant. 29: 281-287.

Zimmermann, M.H. 1960. Transport in the phloem. Annu. Rev. Plant Physiol. 11: 167-190.