ABSTRACT

Effects of different concentrations of terbutryne on photosynthesis, respiration, chlorophyll concentration and dry matter accumulation of Elodea canadensis Michx. over a wide range of temperature, under natural and artificial light climates have been described in this thesis.

At a given temperature, effects of terbutryne increased with increased concentration, and at a given concentration phototoxicity increased with rising temperature. There was a highly significant concentration x temperature interaction of terbutryne activity. The difference in the metabolic and physiological responses of E. canadensis to terbutryne treatment under the two light climates were not wide.

At all concentrations and temperatures the maximum inhibitory effects on net photosynthesis were expressed by the second day and on dark respiration by the third day of treatment.

Fifty percent inhibition (I_{50}) in net photosynthesis occurred at the lowest concentration of 0.0125 mg l^{-1} and temperature 10°C and dark respiration at a concentration of 0.025 mg l^{-1} and temperature 20°C. Complete suppression of net photosynthesis (100% inhibition) occurred at the highest concentration of 0.05 mg l^{-1}, at temperatures 20° and 25°C, under natural light conditions, while it did not occur under artificial light conditions at the same concentration and temperature. Complete

suppression of dark respiration never occurred at any concentration and temperature.

Recovery from photosynthetic inhibition of terbutryne started from third day of treatment in all concentrations at most of the temperatures. Recovery was higher at lower concentrations and temperatures. Recovery from respiratory inhibition never occurred.at any concentration and temperature.

Dry weight of the treated plants decreased at all concentrations and temperatures. The general trend was for the dry weight to decrease most with increasing concentration of terbutryne.

Despite some variation, the chlorophyll (a+b) concentration in the tissues of the treated plants remained more or less unaffected and the plants suffered no visible physical damage due to terbutryne treatment over the experimental period.

The relevance of these findings to aquatic weed control in tropical conditions of Bangladesh (i.e. at the equivalent high ambient temperatures) is discussed.

STUDIES ON THE EFFECTS OF VARIABLE TERBUTRYNE CONCENTRATIONS AND TEMPERATURES ON SOME PHYSIOLOGICAL RESPONSES OF ELODEA CANADENSIS MICHX.

BY

ABDUL MATIN

A THESIS SUMITTED TO THE UNIVERSITY OF ST. ANDREWS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BOTANY

UNIVERSITY OF ST. ANDREWS

OCTOBER 1980.

IN THE NAME OF GOD,

THE ALMIGHTY AND MERCIFUL.

TO MY PARENTS

DECLARATION

I hereby declare that this thesis is of my own composition, that it is based on the results of the experiments carried out by me, and that it has not been presented previously in application for a higher degree.

(Abdul Matin)

St. Andrews, December, 1980.

CERTIFICATE

I certify that Abdul Matin has spent seven terms of research under my direction, that he has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967 No. 1, and that he is qualified to submit the accompanying thesis in application for the degree of Master of Science.

D.H.N. Spence

St. Andrews, November, 1981.

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CONTENTS

CONTENTS contd.

DEFINITIONS OF THE TERMINOLOGY USED IN THIS THESIS.

(1) CONTROL: Plants where no terbutryne was added and kept under the same experimental conditions as those of the treatment to compare differences in metabolic and physiological responses of the treated plants.

(2) DAILY BASIS: Variable increase or decrease in the rates of net photosynthesis and dark respiration in the treatments as compared to the control over each light and dark period.

(3) DARK PERIOD: 16-hour duration in darkness starting from 17.00 hour in the evening to 09.00 hour in the following morning.

(4) DARK RESPIRATION: The amount of oxygen consumed in respiration. Decrease in the dissolved oxygen concentration in the medium during dark period.

(5) TERBUTRYNE CONCENTRATION: The amount of herbicide applied to a plant at any one time.

(6) EFFECTS: Variable increase or decrease of the metabolic or physiological responses in the treated plants as compared to control.

(7) INHIBITION: Variable decrease in the evolution of oxygen in photosynthesis (Photosynthetic inhibition) or the consumption of oxygen in respiration (Respiratory inhibition), as compared to control.

(8) INTERACTION: The influence of one factor (temperature) upon the activity of another factor (terbutryne concentration).

(9) LAG PHASE: The period during which little or no change in net photosynthesis or in dark respiration, as compared to control, is apparent.

(10) LIGHT PERIOD: 8-hour duration in light starting from 09.00 hour in the morning to 17.00 hour in the afternoon.

(11) NET PHOTOSYNTHESIS: The amount of oxygen produced in excess over respiration and dissolved in water. Increase in the dissolved oxygen concentration in the medium during light period.

(12) PHYSICAL DAMAGE: As compared to control, the visible changes like discolouration, yellowing, disintegration, decomposition that appear in the treated plants.

(13) RATES: Per gram dry weight (gdw^{-1}) of the experimental plants, mg O_2 produced in net photosynthesis or consumed in dark respiration.

(14) RESPONSE PHASE: The period during which net photosynthesis or dark respiration or both, as compared to control, are variably affected.

(15) TREATMENTS: Plants where terbutryne was added at different concentrations and the effects were measured as difference from control.

I(i). INTRODUCTION

Like most developing countries, the diet of the people in Bangladesh is deficient in protein. Whatever the protein intake is, it is mainly of vegetable origin and is inferior to protein from animal sources. The prospect of obtaining animal protein from sources other than fish in Bangladesh is less promising because of the pressure on land for cereal production and consequent limitation of pasture land (Dacca, 1967).

The fishery resource of Bangladesh is one of the richest in Southeast Asia. As a source of food, the fisheries of the country provide about 80% of the animal protein intake. The people of Bangladesh prefer freshwater fish to marine fish. About 90% of the total fish production of the country is contributed by the inland fisheries. The major types of freshwater fish habitats of the country are the ponds, beels (the natural depressions), baors (the old ox-bow lakes), reservoirs, rivers and paddy fields, during monsoon (Directorate of Fisheries, Bangladesh, 1977).

The climate and soil conditions of Bangladesh are very favourable for fish production, but unfortunately the production has not increased proportionately to the increase in human population and demand. Presence of various aquatic weeds in the fisheries is one of the reasons that thwart efforts in increasing fish production. A large number of ponds, baors and other water bodies are infested with floating and submerged aquatic weeds and it

is the submerged aquatic weeds that make it all the more difficult to practising intensive fish culture (Chokder, 1958).

Clearing the water bodies of aquatic weeds is a prime task in the development of the fisheries. Use of herbicide is one possible method of controlling aquatic weeds and which may have some advantage over the imperfect, time consuming and laborious manual method now in practice in Bangladesh.

Controlling aquatic weeds by herbicidal methods is a technical job. The use of herbicide does not merely end in application at the recommended dose. In using herbicide one needs to understand the chemical structure, formulation, technique and time of application and, above all, the mode of action of the herbicide in an aquatic system.

The mode of action of herbicide under temperate conditions is more or less known, but little known under tropical conditions. The present project was undertaken in the context of the tropical conditions of Bangladesh. The aim of the project was to study how the action of terbutryne on the photosynthesis and dark respiration of aquatic plants is affected by variation in temperature and dose concentration. The report of failure (Robson et al. 1978) to control aquatic weeds by certain dose concentrations of terbutryne under certain environmental conditions provided the background for the project on which the present thesis is based. In controlling aquatic weeds the aim should be to disturb the aquatic system as

little as possible. This can only be done if the most favourable conditions for the greatest herbicidal activity at the minimum dose concentration are known. The need to investigate this aspect of aquatic weed control justifies my undertaking the project.

Application of herbicide to water may immediately or in the long run affect animal life, including fish in it. The degree of effect on aquatic animals may depend on the extent of response of the plants to herbicide. Metabolic responses like decrease in photosynthesis or increase in dark respiration may immediately affect the animal life by unbalancing the dissolved oxygen concentration of water. A physiological response like decrease in plant biomass may upset the aquatic ecosystem through altering the dependant animal life. The necessity of studying the metabolic and physiological responses of plant to herbicide in an aquatic system stem from these possibilities.

Depending on formulation and dose concentration the metabolic and physiological responses of plant are greatly influenced by the environmental conditions. No one factor of the environment operates singly under field conditions. The mode of action of herbicide is often modified by one or two more factors and particularly by temperature and light. In view of the complexity of the field situation, the study of individual factors is the best approach. The effects of individual factors can best be understood if observations are made under more or less controlled conditions, which is why the experiments were done in a greenhouse.

Studies presented in this thesis concern the post-treatment response of Elodea canadensis Michx., in respect of photosynthesis, dark respiration, changes in dry weight and chlorophyll concentration, to different dose concentrations over a wide range of temperatures. The herbicide 'Terbutryne' used in the experiments was chosen in the perspective of submerged weed problems in the ponds of Bangladesh. Terbutryne is known to be effective against a wide variety of aquatic plants including submerged plants. Elodea canadensis Michx. was chosen as the experimental plant because of its close morphological resemblance to Hydrilla verticillata (Roxb.) Royle, a plant commonly found in the ponds of Bangladesh.

The data presented in this thesis come mainly from the experiments carried out in the greenhouse of the Weed Research Organisation in Oxford. Initially, before tackling the question of terbutryne action, a series of experiments was carried out in St. Andrews on the effects on net photosynthesis of E. canadensis Michx., of variation in amounts of PAR (Photosynthetically available radiation). This allowed familiarisation of the apparatus and techniques of oxygen measurement and calculation, for example, saturating irradiance for this species of plant with a non-limiting carbon supply. These experiments are not described in any detail.

The observed effects of terbutryne on the metabolic and physiological processes of E. canadensis Michx. are not strictly comparable to the effects produced under field conditions, but the results obtained, interpretations

made and conclusions drawn from them may help in understanding the influence of environmental factors and the effects of temperature in particular on the activity of terbutryne on naturally occurring plants. Particularly the results of the experiments at temperatures 30^{o} and $35^{o}C$ may help considerably in formulating field trials in the warm waters of Bangladesh although it is appreciated that tropical plants may react differently from the temperate B. canadensis.

SECTION - II

GENERAL INFORMATION

This section provides background information to the present study. After outlining different methods of aquatic weed control, the history and development of relevant herbicides are reviewed, the section concludes with an account of terbutryne as an aquatic herbicide.

II(i). DIFFERENT METHODS OF AQUATIC WEED CONTROL:

There are three methods of weed control viz. mechanical, biological and chemical.

(a) Mechanical methods: These are the oldest methods of weed control. They include uprooting by hand, burning, smothering, ploughing for land weeds, and drying (seasonal fall in water level in shallow ponds) and dewatering (by deliberately lowering the water level in deep ponds); by raking or use of under water weed cutters and dredges which scrape up plants and roots. Controlling weeds by these methods is time consuming, laborious and also uneconomic. Moreover, weeds are not perfectly controlled and the results are not long lasting.

(b) Biological methods: The underlying principle of these methods of weed control is to kill one organism with another. Submerged aquatic weeds like Hydrilla spp., Najas spp., Vallisneria spp., Ceratophyllum spp., Potamogeton spp., etc. may be controlled by blanketing them for 4-6 months with a dense mat of floating weeds like Eichornia spp., Pistia spp., Lemna spp. or a mat of algae. Controlling submerged weeds by this method is not only, once again, time consuming, but creates another problem, that of controlling the floating weeds themselves. Submerged weeds may be controlled by employing herbivorous fish like chinese grass carp (Ctenopharyngodon idella), common carp (Cyprinus carpio), Tilapia spp., or by employing animals like turtle, snail, crab etc. Floating weeds may be controlled by employing insects. Biological

methods of weed control, particularly those employing herbivorous fish, are gaining popularity, because, while mechanical and chemical methods destroy plants, in biological methods the plants are converted to forms useful for human consumption.

(c) Chemical methods: At present, these are the most widely practised method of weed control. In the 1940's, with the invention of hormone weed killers, chemical weed control became very popular. Using herbicides, weed may be controlled very quickly and efficiently. A wide variety of herbicides is now available, which may be used in controlling terrestrial as well as aquatic weeds. Both time and labour can be saved by this method. However, success depends on technical knowledge, proper equipment and, above all, the efficient and timely use of herbicides.

Advantages and disadvantages of chemical methods:

(1) Advantages - The use of herbicide, in controlling weeds, has some advantages over the traditional and mechanical methods;

(i) firstly, it helps in saving time and labour and

(ii) secondly, the wide variety of herbicides available and the differences in their properties and mode of action make them a versatile set of tools for regulating plant growth. They can be used to select desirable from undesirable species, to reduce plant growth without killing the plants or to eradicate all vegetations for varying periods of time.

(2) Disadvantages - There are some disadvantages in chemical methods;

(i) firstly, desired plants may be killed with undesired ones.

(ii) secondly, the ecological balance may be upset for longer if regrowth is delayed or eliminated,

(iii) thirdly, the residues of herbicides and metabolites in water and soil may affect the higher members of the food chain in the long run.

II(ii). HISTORY AND DEVELOPMENT OF HERBICIDE:

To produce more and more food for the ever increasing population, man's effort has been to develop and improve cultivation, fertilizer and seeds on the one hand, and to fight weeds and other pests on the other. Before the introduction of chemical weed control, four measures were adopted to eradicate or limit the spread of weeds. These were manual weeding, crop rotation, ploughing and various methods of preventing weed seeds being dispersed in crop seeds. Although hand weeding is still widely practised in many parts of the world, and particularly in the tropics, the cost of weeding has greatly increased during this century as the cost of labour has increased and selective weed control by hand has become more and more uneconomical. The present day agriculturists are seeking easy, cheap and efficient ways of dealing with weeds.

For ages man has used sea salt to kill unwanted plants, and only in about 1900 did he start using purified chemicals for selective weed control. The history of chemical weed control began with the work of Julius Sacks (1859-1887), a Germany botanist, who wrote that minute substances, which he named "chemical Messengers", were related to the flowering behaviours of begonias and squash. This is one of the earliest works on translocation of growth-regulating substances. Charles Darwin, in his book "Power of Movement in Plants", published in 1900 mentioned growth-regulating substance. Darwin further mentioned "In several respects, light seems to act on plants in nearly the

same manner as it does in animals by means of the nervous system ..... the effect ..... is transmitted from one part to another".

The chemical messenger or plant hormone was identified as indoleacetic acid in 1933. Synthesis of indoleacetic acid and the discovery that external application could cause dramatic effects on plant growth led to the manufacture of related compounds. The most important of these from the weed-control standpoint is 2,4-dichlorophenoxyacetic acid for which the techniques for chemical synthesis were developed and first reported by Pokorny (1941). Zimmerman and Hitchcock (1942) first reported 2,4-D as a plant growth regulating substance. Marth and Mitchell (1944) established the selectivity of 2,4-D, and Hamner and Tukey (1944) applied 2,4-D successfully in field weed control.

The synthesis and use of 2,4-D as a herbicide opened the new era of weed control and revolutionised the science of herbicide development. Since then herbicides have been developed and produced every year in large numbers and the science of weed control has developed more in the last forty years than in the previous hundred centuries.

II(iii). HERBICIDES: FORMULATION AND CLASSIFICATION

Chemicals that are used for retarding growth, deforming or killing undesirable plants are called herbicides.

Formulation: Herbicides are not sold as pure chemicals. They are formulated, that is combined with appropriate solvents, diluents and/or surfactants to form an effective product. The purpose of formulating a herbicide is,

(1) to enable the user to readily disperse the herbicide so that a relatively small amount of herbicide may be uniformly distributed over a rather large area,

(2) to enhance phytotoxicity of herbicide e.g. by improving uptake or reducing evaporation,

(3) to improve storage.

Formulations may either be liquids (solutions, emulsions or suspensions), granules or pellets made with clay, diatomite or other inert material. The liquids are usually diluted with water and sprayed, while the granules and pellets are applied without further dilution. Apart from the concentration of active ingredient the composition of the formulation is rarely revealed by the manufacturers.

Herbicide classification: Herbicides differ very much in the way they act and the effects they have on plant. Herbicides therefore may be classified on the basis of one or more common characteristics such as; chemistry (molecular composition and configuration etc.), biological effect (mode of action, selectivity etc.), application (pre-plant,

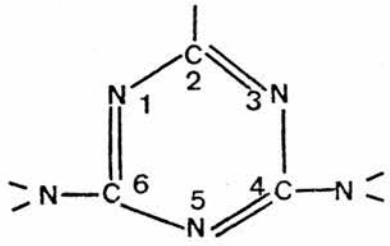
pre-emergence, post-emergence etc.) or use (control of annual, perennial, grass, broadleaved plants, herbaceous, woody plants etc.). Detailed classification is beyond the scope of the present work. The classification of Ashton and Crafts (1979), and Fryer and Makepeace (1977), has been followed here. According to their systems herbicides may be classified, on the basis of physiological behaviour and mode of action, into two classes:

(1) Contact herbicides - are those herbicides that kill a plant or the part of it with which they come in contact. Contact herbicides kill plants by acute toxicity. Acute toxicity infers "rapid kill", usually within minutes or a few hours after the herbicide contacts the plant, or its cells or tissue;

(2) Translocated herbicides - also called systematic herbicides, are those herbicides that do not kill plants rapidly, but enter the plant itself, and are then moved to some other part where they have effect, and generally interfere with the normal functioning of one or more of the physiological or metabolic processes of the cell. The functions most commonly disrupted are cell division, cell development, chlorophyll or plastid formation, photosynthesis, respiration, nitrogen metabolism and enzyme activity. Translocated herbicides kill by chronic toxicity. Chronic toxicity infers "slow action" with the death of the plant occurring after a prolonged period of time such as days, weeks and even months.

II(iv). TRIAZINE HERBICIDES:

The name heterocyclic is used in chemistry to designate a ring structure composed of atoms of different kinds. The chemical structures of the substituted diamino-s-triazine herbicides are centered about a common six-membered ring composed of three nitrogen N atoms and three carbon C atoms arranged symmetrically (asymmetrically in metribuzin) about the ring, with amino NH_2 groups attached to the carbon atoms in positions 4 and 6 of the ring. A six-membered ring structure with two or more nitrogen atoms in the ring is called "azine"; when the number of nitrogen atoms is three and they are symmetrically arranged, the ring structure is designated "s-triazine". The chemical structure common to members of the diamino-s-triazine herbicide family (Anderson, 1977) is as follows.



4, 6-diamino-s-triazine.

The first preparation of what is now known as simazine was made by Hofmann (1885). Further chemicals in the triazine group were synthesized by Pearlman Banks (1948). However, triazine compounds were developed and tested for their herbicidal properties in Switzerland and in the early 1950's certain triazines were introduced as

herbicidal chemicals by Geigy Agricultural Chemicals of Switzerland. Several triazine chemicals have herbicidal properties and are sold in the market under different trade names, but simazine is by far the best known.

Described by Anderson (1977) following are some of the common characteristics of triazine herbicides,

(1) used for pre and post emergence weed control;

(2) most effective against broadleaved weeds; do not control established annual or perennial weeds, or deep rooted perennial weeds;

(3) readily absorbed by soil and resist leaching;

(4) readily absorbed by roots; certain members are absorbed by leaves and other parts of the shoot;

(5) readily translocated upward to the leaves via the transpiration stream following root absorption; these herbicides are not translocated from leaves; they accumulate in the leaves following foliar absorption;

(6) the mode of action of triazine herbicides is the inhibition of photosynthesis, achieved through interference with CO_2 fixation and the splitting of water into oxygen and hydrogen (Hill reaction);

(7) these herbicides are very persistent in soil; soil persistence ranges from less than 3 months to more than 12 months, depending on the herbicide involved, the dose applied, and environmental conditions such as rainfall, organic matter and temperature;

(8) methoxy and methylthio-diamino-s-triazines are detoxified principally by means of microbial action; other members are detoxified primarily by hydrolysis.

II(v). MODE OF ACTION OF TRIAZINE HERBICIDES:

Inhibition of photosynthesis is the most pronounced physiological effect expressed by the triazine group of herbicides, but the full mechanism of inhibition is still unknown. Current information on inhibitors of photosynthetic reactions indicates that herbicides interfere with the light reactions of the photosynthetic process and more specifically their site of action is within photosystem II at the hydrolysis of water step (Hill reaction).

The process of photosynthesis is carried out within the chloroplast in two distinct phases, namely; (1) light dependent phase, and (2) dark dependent phase. The light dependent phase of photosynthesis again consists of two distinct systems, commonly called photosystem I and photosystem II, acting in conjunction with one another.

In photosystem I, the chlorophyll molecules are raised momentarily to an excited state on absorption of a photon or quantum of far red light, enabling the chlorophyll molecules to serve as electron donors. In photosystem I, the organic electron acceptor ferredoxin within the grana of the chloroplast accepts electrons from the light energized chlorophyll molecules and in turn reduces NADP. In photosystem II, the chlorophyll molecules are also raised to an excited state by absorption of a photon or quantum of light, and electrons from the energized chlorophyll molecules pass to an organic electron acceptor,

Ferredoxin. In photosystem II the chlorophyll molecules immediately replace their lost electrons by hydrolysing water (splitting water molecules to form hydrogen (H^+) and hydroxyl (OH^-) ions and taking one electron from the hydroxyl group). Four hydroxyl ions then interact to form two molecules of water ($2H_2O$) and one molecule of oxygen (O_2). The gaseous oxygen molecules then escape into the atmosphere as free oxygen. The hydrolysis of water (splitting of water) during photosynthesis is referred to as the Hill reaction.

Disruption of the Hill reaction by herbicides has been demonstrated by many workers, using various techniques, in isolated chloroplasts, unicellular algae and in intact plants. Exer (1958), Moreland et al. (1958, 1959) showed that simazine inhibited the Hill reaction in isolated chloroplasts. Indirect evidence of a photosynthetic block was provided by Gast (1958) when he found that starch synthesis in Coleus blumei was blocked by simazine, but the addition of sucrose counteracted the effect. Roth (1958) observed photosynthetic inhibition in Elodea spp. Ashton et al. (1960), Gysin and Knusil (1960), Avron (1960), Exer and Good (1961), Moreland and Hill (1962), Bishop (1962), Funderburk and Lawrence (1964), van Oorschot (1966, 1968, 1970), Imbamba (1970), van Rensen (1971), Johannes and Luedemann (1972), Robson et al. (1976) all reported inhibition of photosynthesis. Moreland and Hill (1962) showed that the inhibition of the Hill reaction by simazine was reversible. That is, when chloroplasts were treated with 6×10^{-5} or 4×10^{-5}M simazine the inhibition was 50 and 85% respectively; however, when they were

washed following herbicide treatment values of 96 and 94% respectively were obtained. Bishop (1962) suggested that inhibition of the Hill reaction always implies a poisoning of the mechanism of oxygen production. Working with Scenedesmus spp. and other hydrogenase-containing algae, he observed that oxygen evolution can be practically eliminated by specific inhibitors of this step while the primary photochemistry and carbon dioxide reduction may continue at a relatively high rate. All these findings are consistent with the concept that triazine herbicides inhibit the oxygen-evolving system of photosynthesis.

In photosystem II, hydrogen ion (H^+) of the split water molecule indirectly reduces the co-enzyme nicotinamide adenine dinucleotide phosphate (NADP) to form $NADPH_2$ which in turn serves as an essential electron donor in the conversion of carbon dioxide (CO_2) to sugar ($C_6H_{12}O_6$) in the 'dark reaction' of photosynthesis. The blockage of light reactions of photosynthesis by triazine herbicides should result in a lack of reducing power which is required for carbon dioxide fixation (Ashton and Zweig, 1958; Ashton et al., 1960). Most workers in this field agree that triazine herbicides act at the reducing side of photosystem II by limiting the availability to the plant of chemical energy in the form of ATP and of reducing power in the form of NADPH. The inhibition of carbon dioxide fixation by several triazine herbicides in a variety of plants has been reported. Ashton and Zweig (1958) found that simazine inhibited carbon dioxide fixation in beans. Ashton et al. (1960) found that simazine, trietazine and

simeton drastically inhibited carbon dioxide fixation of beans in light. Good (1962) reported that ATP formation (photophosphorylation) was inhibited by triazine herbicides in isolated chloroplasts when flavin mononucleotide (FMN) was used as an electron acceptor but not when N-methylphenazonium (PMS) was used as an electron acceptor. This was confirmed by Shimabukuro and Swanson (1968). van Oorschot, 1964 (Simeton in bean), Funderburk and Carter, 1965 (atrazine in beans), Couch and Davis, 1966 (atrazine in maize, cotton and soyabean). Sikka and Davis (1969), using prometryne in cotton and soyabean observed similar inhibition of carbon dioxide fixation. Zweig and Ashton (1962), Couch and Davis (1966), and Sikka and Davis (1969), observed no such effect on dark reactions.

The effect of triazine herbicides on several biochemical events other than photosynthesis has been reported. In intact plants, atrazine (Funderburk and Davis, 1963; Olech, 1966), and ipazine (Nasyrova et al., 1968) have been reported to inhibit respiration. On the other hand, Davis (1969) and Olech (1967), reported that atrazine had no effect on respiration. While Roth (1958), observed stimulation of dark respiration in Elodea spp., Ahston and Uribe (1962) observed no effect of atrazine on the respiratory rates of excised embroys of red kidney bean plants. Working with isolated mitochondria, Foy and Penner (1965) reported that atrazine inhibited respiration but Davis (1968) observed no effect. In excised barley roots using various substrates, Palmer and Allen (1962)

observed a general trend of stimulation of respiration. Olech (1966) concluded that the inhibition of respiration was indirect and caused by a lower level of assimilates as a result of inhibition of photosynthesis.

Several investigators have reported that triazine herbicides reduce the amount of glucose, fructose, and/or sucrose in a variety of plants. Glabiszewski et al. (1966, 1967), Swietochowski et al. (1966), Ploszynski and Zurawski (1967), Timofeeva (1967) observed such effects.

Nitrogen metabolism has been reported to be affected by triazine herbicides. Some investigators have observed that subtoxic levels of triazine herbicides increase growth and the amount of nitrogen and/or protein in the plant. Gast and Grob (1960) observed that atrazine or simazine increased the protein content of maize. De Vries (1963) demonstrated an increase in nitrogen uptake by maize when treated with simazine and those plants showing nitrogen deficiency symptoms recovered when simazine was applied. Bartley (1957) reported that simazine increased the growth and green colour of maize. Ashton (1965) concluded that in spite of several investigations of this problem there are still differences of opinion about whether the triazine herbicides increase the amount of nitrogen in plants and it is often not clear how these are related to the herbicidal properties of the compounds.

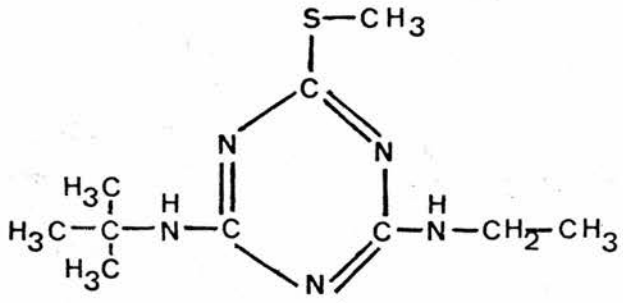
On the mode of action of herbicides Ashton and Crafts (1973) concluded, "The mechanism of action of triazine herbicides in higher plants is a blockage of photosynthesis. More specifically their site of action

is within photosystem II at the photolysis of water step.".

II(vi). TERBUTRYNE AS AN AQUATIC HERBICIDE:

Interest in herbicides for the control of freshwater weeds arose when the traditional methods of control became impractical and uneconomical. Although most aquatic herbicides have been developed from chemicals used to control weeds in agriculture, there are some which have been chosen specifically for aquatic weed control. However, throughout the world there are probably no more than twenty chemicals used for aquatic weed control.

The use of triazine herbicides for aquatic weed control is a recent development. Members of this group used for aquatic weed control are terbutryne, ametryne, desmetryne, prometryne, cyanatryne. Of these herbicides terbutryne is the most promising (Marks, 1974) and is being used in some twenty countries (Ashton and Raynaert, 1974) as an aquatic herbicide. It is available in the market either as a wettable powder (50% ai) or 1% granular formulation. The commercial name of wettable powder is IGRAN^{TM} and that of granular formulation is CLAROSAN. The molecular weight of terbutryne is 241 and its solubility in water is 58 p.p.m. at temperatures of 20-25^{o}C (Kearney and Kaufman, 1975 and Fryer and Makepeace, 1977). The chemical formula of terbutryne is 2-methylthio-4-ethylamino-6-tert-butylamino-s-triazine. The chemical structure of terbutryne (Anderson, 1977) is as follows.



Chemical structure of terbutryne.

The efficiency of a herbicide depends on the dose rate applied. At a dose rate of 0.025 to 0.05 mg l^{-1}, terbutryne has a strong effect on algae and waterplants in temperate climates (Ashton and Reynaert, 1974). Terbutryne is an almost total algicide (Newbold, 1975) and it is effective against a wide range of submerged weeds (Ashton and Reynaert, 1974; Blamson, 1977) and is especially useful against filamentous algae (Marks, 1974; Robson et al., 1976). Most emergent aquatic macrophytes, however, are not affected by this compound (Ashton and Reynaert, 1974; Newbold, 1975).

In aquatic systems terbutryne is mostly absorbed through the leaves, and is also absorbed through roots when the chemical settles down on mud. The mode of action of terbutryne, as with other triazines, is the inhibition of photosynthesis and when added to water cause a depletion of the oxygen level present (Roth, 1958; Johannes and Luedemann, 1972; Marks, 1974; Robson et al., 1976).

Terbutryne is presistent in soil (Newbold, 1975; Fryer and Makepeace, 1977). van der Weiz et al. (1971) recorded a half-life of 25 days when this chemical was

applied at 0.1 mg l^{-1}. Newbold (1975) recorded 0.18 mg l^{-1} associated with the organic layers of mud 133 days after treatment at 0.1 mg l^{-1}.

Detailed information on the effects of terbutryne on aquatic ecosystems is scanty. Possible ecological effects, as have been observed with other triazine herbicides, may include, (1) depletion of oxygen level, (2) decrease in pH level, (3) increase in bacterial population leading to increased biological oxygen demand (BOD), (4) increase in nutrient content of water, (5) encouraging growth of non-susceptible plants, (6) increase in detritus level, (7) reduction of zoo-plankton population, and (8) fish kill. Summarising the effects of terbutryne in aquatic systems, Ashton and Reynaert (1974) concluded that terbutryne treatment has no, or minimal, detrimental effects on the local environment. They also concluded that temporary shifts of local population densities are bound to occur as they would if weeds were cut or allowed to decompose naturally; zoo-plankton population may be affected by the reduction in primary production level; fish may be in distress due to sharp drops in oxygen level. Marks (1974) observed no adverse effect on water fauna. Newbold (1975) reported that terbutryne does not significaly affect most invertebrates. Robson et al. (1978) concluded that terbutryne does not create a "Biological desert".

There have been no recorded problems of triazine, including terbutryne, toxicity to fish when the compounds have been used at prescribed rates (Ashton and Reynaert, 1974) but there are reports of triazine accumulation in

fish by van der Weij (1971). While Lawrence et al. (1963) and Maier-Bode (1972), using C^{14} labelled levelled simazine, observed that the chemical accumulated in fish to levels approximating to that of the level found in treated water, they also observed that most C^{14} simazine was in the vicera and when such fish were removed and placed in freshwater the simazine level fell away steadily and was negligible after four weeks. On the basis of these findings of Lawrence et al. (1963), and of Maier-Bode (1972), Ahston and Reynaert (1974) concluded that, having similar properties to simazine, terbutryne may have the same effect on fish. Ashton and Reynaert (1974) noted that the *LC_{50} for rainbow trout in a fish toxicity experiment was 3.5 mg l^{-1} and concluded that terbutryne dose concentration up to 0.1 mg l^{-1} may not be toxic to fish, even under long term exposure.

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*LC_{50} the concentration at which 50% of the experimental organisms die.

III(i). MATERIALS AND METHODS

A preliminary series of experiments were carried out in St. Andrews and later in Oxford on the effects, on net photosynthesis of E. canadensis and other aquatic plants, of variation in the amounts of P.A.R. The St. Andrews experiments were carried out between February and April, 1979 and the Oxford experiments in October, 1979. The experiments are described, although not in detail, under the heading 'Light' on page 29.

The main series of experiments, investigating effects of terbutryne on photosynthesis and respiration of E. canadensis under varied temperature and light conditions were carried out in the greenhouse of the Weed Research Organisation (W.R.O.) in Oxford. Experiments under natural light conditions, at temperatures of 5° to 20°C were done between July and September, 1979, and at temperatures 20° to 35°C between May and June, 1980.

To compare effects of the natural, fluctuating light with those of constant saturating light on terbutryne activity in E. canadensis some experiments at temperatures of 7° to 20°C were also carried out under artificial light conditions between October and November, 1979. Details of the method are given below.

(1) Plant material: As far as possible intact plants with roots were collected. In the laboratory, the plants were washed free of mud, sand, and other debris and all but the smallest epiphytes and then the plants were stored in a plastic bucket with tap water at the

temperature of the greenhouse, for 2 to 3 days before use. Fresh collections of plants were made every week and treated in the same way. On the day of use the plants were taken out of water, shaken gently to drain off water as far as possible, and then a bundle of shoots, with roots, weighting 10 grams was placed in each experimental jar.

(2) Experimental set up: Experiments were carried out in 3 l sweet jars with 3 l water and 10 grams wet weight of E. canadensis. Tap water was used as the medium in all experiments. Mounted on a hanging platform, the sweet jars were placed in a 81 x 71 x 60 centimeter fibreglass tank which acted as a water bath. The fibreglass tank was filled with tap water up to the neck of the sweet jars. As and when required, water was added to the tank to maintain its level. Two sets of sweet jars were used and great care was taken to clean them of algal growth, marl deposit and herbicide residues by scraping, and by washing with repeated rinses of water, before using them for the next experiment.

(3) Gross and net photosynthesis: Effects of terbutryne on the photosynthetic and dark respiratory activity of E. canadensis were measured by recording changes in the dissolved oxygen concentrations of water in the experimental jars. Dissolved oxygen measurements were taken once at the beginning of the light period at 09.00 hours and again at the close of the light period at 17.00 hours. An EIL portable oxygen meter, Model 1520 was used in measuring dissolved oxygen concentrations in

percentage saturation. Readings for dissolved oxygen concentrations obtained from the meter were multiplied by the conversion factor for a particular temperature to obtain dissolved oxygen in mg l^{-1}. Dissolved oxygen in mg l^{-1} thus obtained was finally converted to units of mgO_2/gdw/h. Mention should be made here that to avoid supersaturation in dissolved oxygen concentration, plants were subjected to a sixtyfour hour dark period before the start of the experiment, to reduce level of oxygen in the experimental jars.

(4) Terbutryne concentrations: Studies of the effects of different concentrations of terbutryne on the photosynthetic and respiratory activity of E. canadensis was one of the major objectives of the experiment. A wettable powder formulation of terbutryne with 50% active ingredient was used in the experiments. Terbutryne concentrations at the rate of 0.0125, 0.025 and 0.05 mg l^{-1} was applied. Terbutryne concentrations of 0.05 mg l^{-1} was the manufacturers recommended dose. To compare effects in different treatments, a control set with no terbutryne was added in the experiment. A strong solution (50 mg l^{-1}) was prepared and from the stock solution an appropriate volume was added to the experimental jars to make up the desired concentration. Before adding the herbicide a volume of water, equal to the volume of the stock solution to be added for a particular concentration, was removed from the jar. There were four replicates for each treatment, including control. The experimental jars were numbered and arranged in a randomised block design before the start of the

experiment.

(5) Temperature: A main aim of the experiments was to assess the effects of temperature on the extent of action of terbutryne on E. canadensis. Accordingly the experiment was designed to run at temperatures of 5, 10, 15, 20, 25, 30 and 35°C. Temperature of water in the water bath was kept constant by using a water heater and water cooler. An additional cooler was used during experiments at low temperature. However, under artificial light conditions, because of the strong heat from the lamps, it was not possible to maintain temperature at 5°C, even using two coolers; 7°C was the lowest that could be maintained under artificial light conditions and was accepted as the minimum.

(6) Light: In St. Andrews the studies on photosynthesis of E. canadensis in relation to P.A.R. were carried out in an apparatus developed by E.D. Allen (Allen & Spence, 1981), while in Oxford they were done in 3 l sweet jars. Although each apparatus differed much in construction and efficiency, they served the ultimate purpose of measuring the saturating light intensity for the maximum net photosynthesis rate (Pmax) of E. canadensis. The rates of net photosynthesis of E. canadensis at different light intensities were calculated from the amount of oxygen produced per unit volume (corrected to gram dry weight) of plant per hour. In measuring differences in dissolved oxygen concentrations of water during experiments, a Beckman 39553 O_2 electrode was used in St. Andrews, while in Oxford an EIL oxygen meter (Specification described under the heading 'Gross and net photosynthesis' on page 27.

Artificial light was provided by five 150 w lamps hanging vertically downward from an iron frame, mounted on top of the tank. Interference of other sources of light was blocked by covering the whole set-up with black polythene. In St. Andrews, the photon flux density was measured with a Lamdameter, Model No. L1 185 which gave readings in $\mu E\ m^{-2}s^{-1}$. In Oxford irradiance between 400 and 700 nm was measured with a Comark D.C. microvoltameter, Model 1221 which gave readings in microvolts. By multiplying the readings in the meter with a conversion factor (imv = 88 W/m^2), irradiance in W/m^2 was obtained. With five lamps the maximum irradiance at the water surface was estimated to be about 480 W/m^2. These irradiance readings were converted to photon flux densities by the factors set out in Maberly (1981).

(7) Chlorophyll concentration and dry weight of plant: Chlorophyll (a+b) concentration was analysed in acetone extract of 3 g fresh plant tissue according to the method of Vollenweider (1974). Chlorophyll concentrations in post treated plant tissues were analysed to compare changes due to terbutryne activity during experiments. A Pye Unicam, Model SP30 UV, spectrophotometer was used to analyse chlorophyll extracts.

After treatments, plants were dried to constant weight in an oven at a temperature of $105^{o}C$. Rates of net photosynthesis and dark respiration are expressed in terms of dry weight.

(8) Herbicide residues in water and plants: At the close of the experiment 7 g fresh plant was taken from each

treatment and were handed over to the chemistry section of the Weed Research Organisation for herbicide residue analysis in water and plants. Analytical results are expressed as ppm w/v for water and ug/7 g fresh weight of plants.

(9) Results, duration of experiments and analytical treatment: Results of the preliminary experiments in St. Andrews and in Oxford on photosynthesis in relation to irradiance have not been used or included in the herbicide experiments. Only the light intensity data have been used in formulating the herbicide experiments under saturated light conditions and in comparing its effect on herbicidal activity of terbutryne with the unsaturated fluctuating natural light conditions.

Each herbicide experiment lasted for five days. Each light period started at 09.00 hours with an eight hour day length; the dark period began at 17.00 hours. Each experiment ran for one whole day and four hours of a second day, when terbutryne was added. This point is clearly marked on the abscissa of the graphs in Fig. 1.1 (data in Appendix I) and marks the start of treatment. Days of treatment start from the day of addition of terbutryne and are marked Day 1 as shown in the graphs of Fig. 1.1. Since photosynthetic rates were calculated from oxygen electrode measurements made at the beginning and end of the daily light period, the mean photosynthetic rate on Day 1 integrates four hours without terbutryne and four hours with terbutryne. This fact should be borne in mind when converting photosynthetic rates for that day.

No attempt has been made to eliminate from the calculations the amount of oxygen produced by epiphytic algae or oxygen consumed in dark respiration by epiphytic algae and bacteria. All results presented here have been calculated on the basis of net oxygen changes in experimental jars. With clean material, epiphytes should have negligible effect on the experiments.

Results have been expressed as rates (mgO_2/gdw/h) of net photosynthesis and dark respiration and as percentage difference from control and statistical analysis has been applied to the results. Standard errors are displayed in Figs. 2.2, 3.2 and Tables 4.1 and 4.2. Differences between control and herbicide treatments, and the interaction between concentration and temperature have been tested by analysis of variance, and results are presented in Appendix V. Analysis of variance was chosen because it was considered an appropriate method for attributing variance due to the number of factors involved in the experiments.

It should be mentioned here that although the results of the experiment at a temperature of 20°C under natural light conditions carried out in April, 1980, have been shown in the Results section, they have not been included in the analysis of variance tests.

III(ii)(a). RESULTS

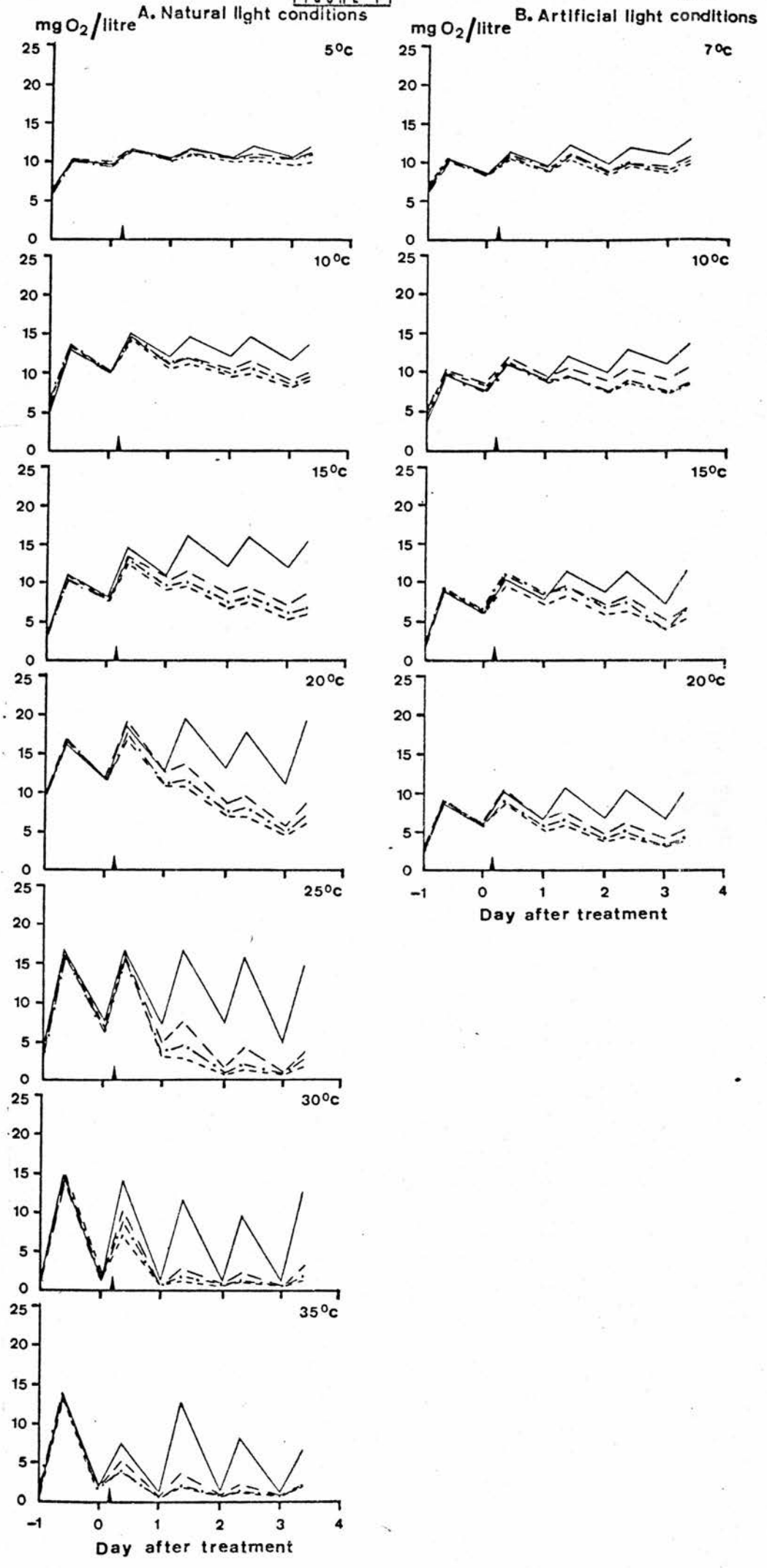
Fig. 1.1 illustrates a general picture of the experiments as a whole including length of the experiments, day and time of application of terbutryne, concentrations of terbutryne, temperature range and light conditions. Although constructed on the basis of unanalysed data (Appendix I), Fig. 1.1 shows the trend of response of *B. canadensis*, in respect of net photosynthesis and dark respiration, to different concentrations of terbutryne and temperature under the two light conditions. It appears from the figure that with the rise of temperature while in the controls net photosynthesis and dark respiration increased, in the treatments they decreased due to the effect of terbutryne. Activity of terbutryne on net photosynthesis and dark respiration of the treated plants became more and more effective with increase in concentrations and temperatures. Effects of terbutryne treatment, although not very clear, excepting at higher temperatures on the day of treatment, became clear from the second day.

FIGURE 1.1

(opposite)

Effects of different concentrations of terbutryne on diurnal fluctuation of dissolved oxygen concentration (mg l^{-1}) in solutions containing E. canadensis, at different temperatures, under (A) Natural light conditions in a greenhouse (July to September, 1979 and April to June, 1980) and (B) Artificial light conditions (October to November, 1979). Eight hour light (09.00 to 17.00) and sixteen hour (17.00 to 09.00) dark period. Terbutryne added on day one of experiment. Concentration of terbutryne mg l^{-1}: (—), 0.00 Control; (– –), 0.0125; (–·–), 0.025; (– – –), 0.05 and (▲), day of terbutryne application.

FIGURE 1·1


A. Natural light conditions
B. Artificial light conditions
mg O2/litre
mg O2/litre
5°c
7°c
10°c
10°c
15°c
15°c
20°c
20°c
25°c
30°c
35°c
0
5
10
15
20
25
–1
0
1
2
3
4
Day after treatment
Day after treatment

METABOLIC EFFECTS

(i) Net photosynthesis:

Tables 1.1 and 1.2 present the mean rates over four days in net photosynthesis of *E. canadensis* treated with different concentrations of terbutryne at different temperatures, under natural and artificial light conditions. From the data in the tables, Fig. 2.1, constructed by plotting rates of net photosynthesis against temperature, illustrates the effect of terbutryne concentration and temperature on the rates of net photosynthesis of *E. canadensis*. It can be seen from the figure that over the temperature range 5^{o} or 7^{o} to $20^{o}C$ under natural and artificial light conditions respectively, the rates of net photosynthesis in the controls and in the treatments increased, although the increase in the treatments was much lower than the controls. The increase in net photosynthesis in the control was significant (p 0.05, see Appendix IV Table A), while in the treatments, excepting in 0.025 treatment under artifical light conditions, it was not significant (p 0.05). Over the temperature range 20^{o} to $35^{o}C$ under natural light conditions the rates of net photosynthesis in the controls and in the treatments decreased. The fall in the rates of net photosynthesis in the controls was not significant while in the treatments it was significant.

Tables 1.3 and 1.4 present mean changes in net photosynthesis of the treated plants as percentage of control, on a daily basis. Fig. 2.2, constructed by plotting changes in net photosynthesis against temperature, illustrates

TABLE 1.1. Mean rates over four days in net photosynthesis of *E. canadensis* in treatments with different concentrations of terbutryne at different temperatures under Natural light conditions.

Concentration of terbutryne mg l^{-1}	Rates of net photosynthesis $mgO_2/gdw/h$							
	1979 Experiments				1980 Experiments			
	Temperature °C							
	5	10	15	20	20	25	30	35
0.00 (Control)	1.17	0.99	1.79	2.41	2.01	1.91	2.22	1.38
0.125	0.79	0.68	0.90	1.24	1.25	1.08	0.87	0.47
0.025	0.69	0.58	0.84	0.86	0.90	0.73	0.53	0.26
0.05	0.58	0.67	0.67	0.62	0.60	0.53	0.37	0.21

TABLE 1.2. Mean rates over four days in net photosynthesis of E. canadensis in treatments with different concentrations of terbutryne at different temperatures under Artificial light conditions.

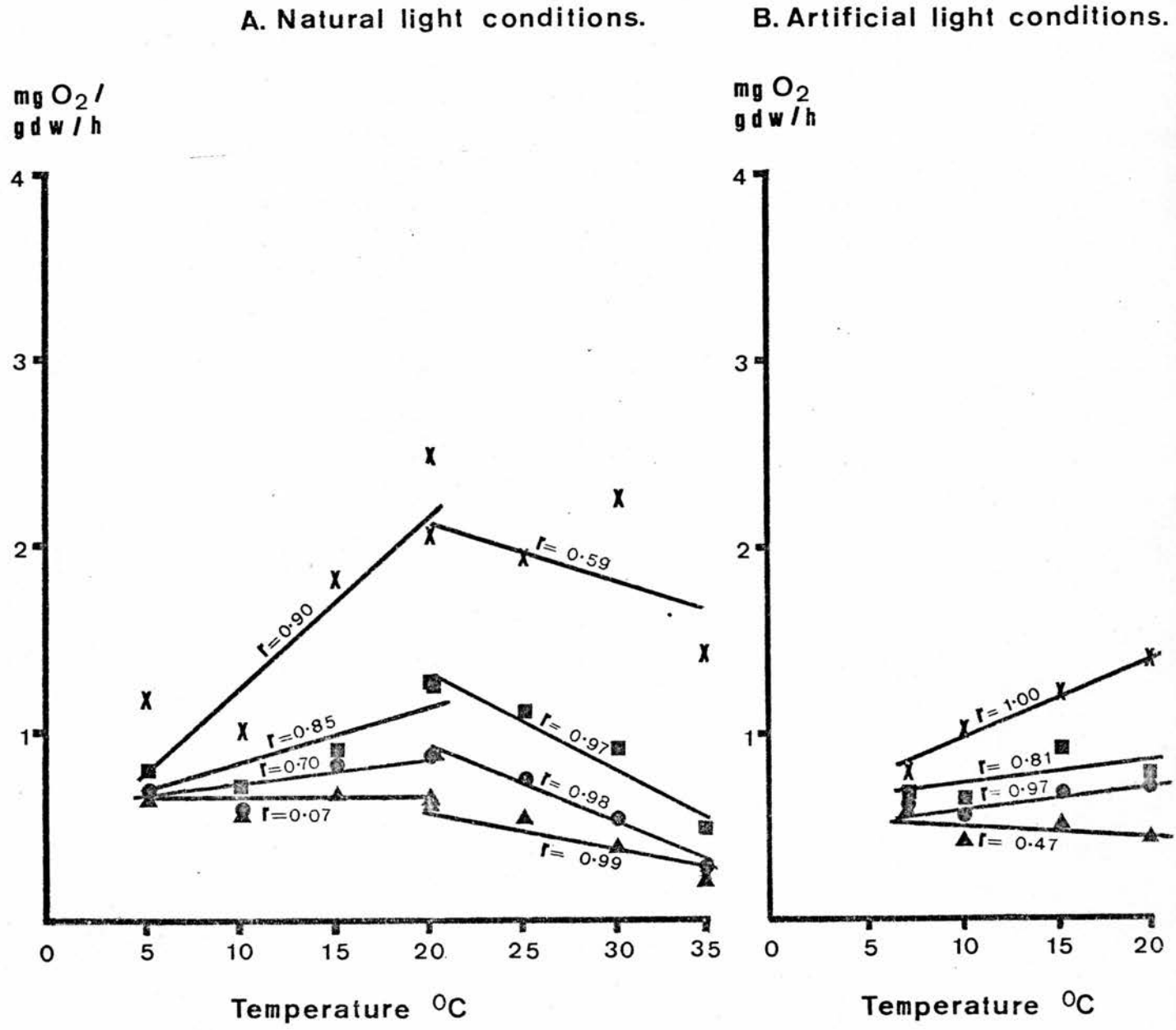
Concentration of terbutryne mg l^{-1}	Rates of net photosynthesis $mgO_2/gdw/h$			
	Temperature ^oC			
	7	10	15	20
0.00 (Control)	0.77	0.97	1.16	1.33
0.0125	0.65	0.62	0.88	0.77
0.025	0.58	0.54	0.65	0.68
0.05	0.57	0.38	0.48	0.42

FIGURE 2.1

(opposite)

Fitted regression lines of the rates of net photosynthesis (mgO_2/gdw/h) of E. canadensis, treated with different concentrations of terbutryne, at different temperatures, exposed to (A) Natural and (B) Artificial light conditions. Concentration of terbutryne mg l^{-1}: (X), 0.00 control; (■), 0.0125; (●), 0.025 and (▲), 0.05.

FIGURE 2·1


A. Natural light conditions.
B. Artificial light conditions.
mg O2 / gdw / h
mg O2 gdw / h
4
3
2
1
0
5
10
15
20
25
30
35
r=0·90
r= 0·59
r=0·85
r=0·70
r=0·07
r= 0·97
r= 0·98
r= 0·99
r= 1·00
r= 0·81
r= 0·97
r= 0·47
Temperature °C
Temperature °C

photosynthetic response of E. canadensis at different concentration of terbutryne and temperature.

It appears from the figure that on the first day of terbutryne application the photosynthetic response of E. canadensis to terbutryne concentrations and temperatures was variable. Under natural light conditions at the highest concentration of 0.05 mgl^{-1} net photosynthesis decreased by more than 50% at temperatures of 30° and 35°C and at other temperatures, with the exception at temperature of 25°C where it was slightly higher than the control, the decrease in net photosynthesis ranged from 6 to 17%. At the same concentration under artificial light conditions, with the exception at 7°C, where net photosynthesis was slightly higher than the control, at other temperatures the decrease net photosynthesis ranged from 19 to 25%. At a concentration of 0.025 mgl^{-1} under natural light conditions, with the exception at a temperature of 25°C where net photosynthesis was slightly higher than the control, at other temperatures the decrease in net photosynthesis ranged from 2 to 48%. At the same concentrations under artifical light conditions net photosynthesis was higher than the control at all temperatures. At the lowest concentration of 0.0125 mgl^{-1} treatment under natural light conditions, the general trend for net photosynthesis was to decrease at most temperatures although the decrease, wherever it occurred, ranged from 1 to 25%. Under artificial light conditions at this concentration net photosynthesis was higher than the controls at all temperatures.

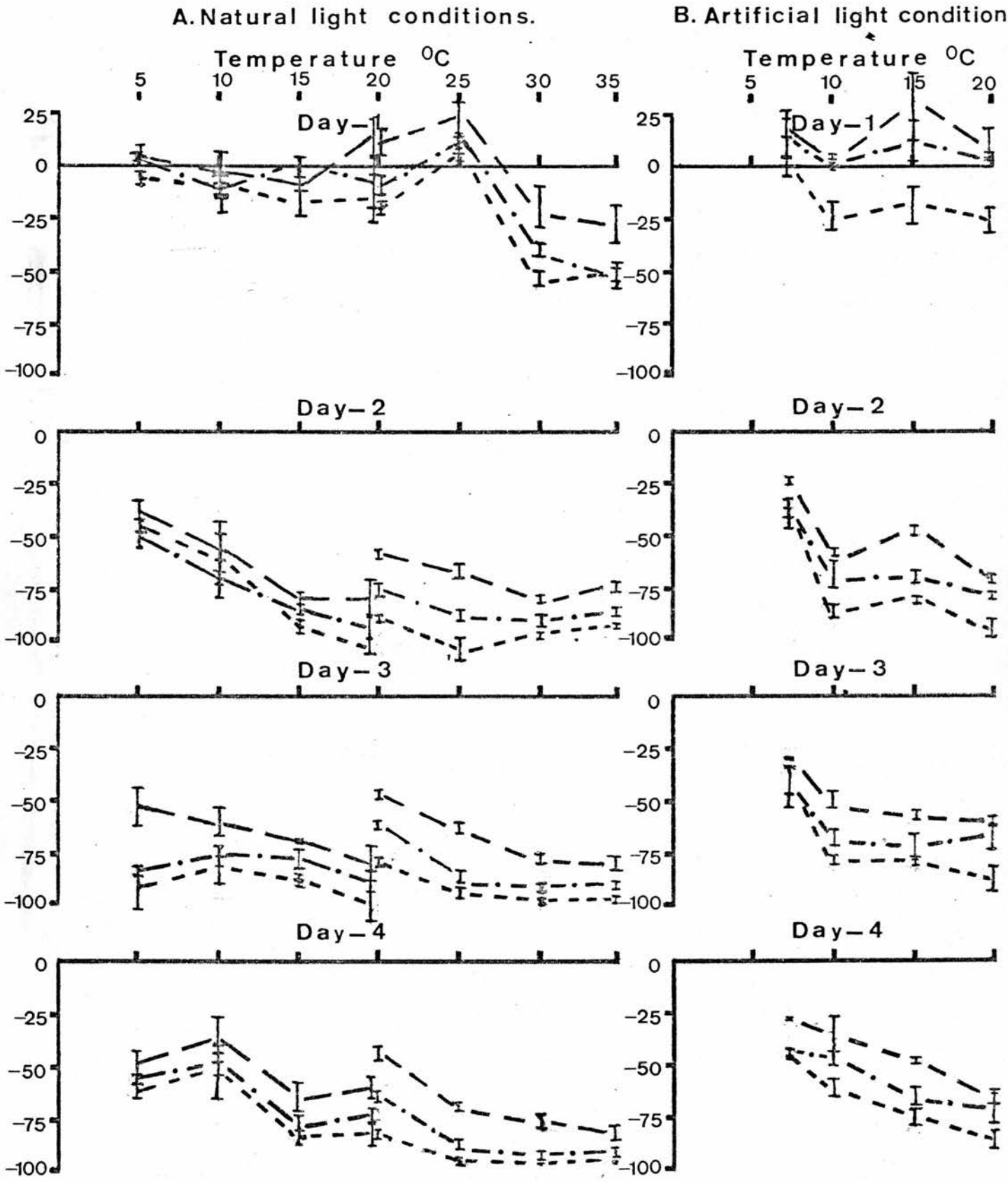
TABLE 1.3. Mean changes in net photosynthesis of E. canadensis, expressed as percentage of control, on a daily basis, in treatments with different concentrations of terbutryne at different temperatures under Natural light conditions.

Day	Concentration of terbutryne mg l^{-1}	Changes in net photosynthesis as percentage of control							
		1979 Experiments				1980 Experiments			
		Temperature °C							
		5	10	15	20	20	25	30	35
1	0.0125	+3.00	-1.00	-8.00	+17.00	+11.00	+26.00	-22.00	-24.00
	0.025	-2.00	-10.00	-3.00	-8.00	-9.00	+14.00	-42.00	-48.00
	0.05	-6.00	-8.00	-17.00	-16.00	-20.00	+8.56	-55.00	-51.00
2	0.0125	-38.00	-55.00	-78.00	-81.00	-58.00	-66.00	-79.00	-70.00
	0.025	-48.00	-69.00	-84.00	-95.00	-75.00	-88.00	-92.00	-87.00
	0.05	-45.00	-61.00	-92.00	-104.00	-88.00	-106.00	-96.00	-93.00
3	0.0125	-64.00	-62.00	-71.00	-81.00	-46.00	-63.00	-79.00	-82.00
	0.025	-81.00	-75.00	-78.00	-90.00	-61.00	-85.00	-91.00	-90.00
	0.05	-91.00	-82.00	-85.00	-85.00	-104.00	-79.00	-95.00	-92.00
4	0.0125	-46.00	-37.00	-63.00	-58.00	-44.00	-68.00	-75.00	-79.00
	0.025	-53.00	-46.00	-75.00	-72.00	-63.00	-86.00	-90.00	-89.00
	0.05	-61.00	-49.00	-82.00	-81.00	-81.00	-94.00	-95.00	-93.00

TABLE 1.4. Mean changes in net photosynthesis of *E. canadensis*, expressed as percentage of control, on a daily basis, in treatments with different concentrations of terbutryne at different temperatures under Artificial light conditions.

Day	Concentration of terbutryne mg l^{-1}	Changes in net photosynthesis as percentage of control			
		Temperature °C			
		7	10	15	20
1	0.0125	+15.00	+4.00	+34.00	+10.00
	0.025	+18.00	+3.00	+10.00	+1.00
	0.05	+3.00	-25.00	-19.00	-24.00
2	0.0125	-23.00	-62.00	-47.00	-69.00
	0.025	-37.00	-71.00	-70.00	-76.00
	0.05	-33.00	-83.00	-77.00	-93.00
3	0.0125	-27.00	-53.00	-57.00	-59.00
	0.025	-41.00	-68.00	-70.00	-66.00
	0.05	-34.00	-77.00	-77.00	-83.00
4	0.0125	-25.00	-35.00	-44.00	-63.00
	0.025	-42.00	-44.00	-61.00	-67.00
	0.05	-40.00	-60.00	-71.00	-84.00

FIGURE 2·2


A. Natural light conditions.
B. Artificial light conditions.
Temperature °C
5
10
15
20
25
30
35
Day–1
Day–2
Day–3
Day–4
25
0
–25
–50
–75
–100
Changes in net photosynthesis as % of control.
Changes in net photosynthesis as % of control.

It can also be seen from Tables 1.3 and 1.4 that at most of the temperatures under both light conditions the general trend for photosynthetic response of E. canadensis to terbutryne treatment was that the maximum inhibitory effects were expressed in all concentrations on the second day after terbutryne application. However, at temperatures of 5^{o}, 10^{o} and 25^{o}C under natural light conditions and 7^{o} and 15^{o}C under artificial light conditions the maximum inhibitory effects were expressed on the third day after terbutryne application.

The effect of terbutryne concentrations and temperatures on photosynthesis of E. canadensis is also apparent from Fig. 2.2. Although the effect of terbutryne concentration and temperature is not clear on the day of terbutryne application, their effect became clear from the second day after terbutryne application. It appears from the figure that at a given temperature net photosynthesis decreased more as concentration of terbutryne increased, and at a given concentration of terbutryne net photosynthesis decreased more as temperature increased. It further appears from the figure that the inhibitory effects were very roughly proportional at all temperature levels. However, irrespective of temperature the decrease in net photosynthesis was always greatest at the highest concentration.

Table 1.5 presents residue analysis data in water and plants. It appears from the table that although not very strictly applicable, the general trend for absorption and accumulation of terbutryne by plants was to increase with concentration and temperature. At a given temperature

TABLE 1.5. Terbutryne residues in water and *E. canadensis* after four day experiments under natural light conditions, at different temperatures.

Concentration of terbutryne mg l^{-1}	Temperature °C	Terbutryne Residues in	
		Water (ppm.)	Plant (ug/g fresh wt.)
1	2	3	4
0.0125	5	0.014	0.67
	10	0.009	0.36
	15	0.009	0.33
	20	0.010	0.20
	25	0.009	0.30
	30	0.012	0.65
	35	0.011	1.03
0.025	5	0.029	0.78
	10	0.012	0.35
	15	0.012	0.34
	20	0.019	0.29
	25	0.020	0.51
	30	0.020	1.14
	35	0.015	2.00
0.05	5	0.053	1.31
	10	0.026	0.45
	15	0.026	0.65
	20	0.034	0.34
	25	0.037	0.71
	30	0.030	1.97
	35	0.025	3.34

TABLE 1.6. Percentage recovery from photosynthetic inhibition by *E. canadensis* at different temperatures, under natural and artificial light conditions.

Temperature °C	Concentration of terbutryne mg l^{-1}	Recovery in percentages	
		Natural light conditions	Artificial light conditions
5/7	0.0125	28.00	no recovery
	0.025	35.00	no recovery
	0.05	33.00	no recovery
10	0.0125	40.00	43.00
	0.025	39.00	38.00
	0.05	40.00	28.00
15	0.0125	19.00	21.00
	0.025	11.00	12.00
	0.05	10.00	7.00
20	0.0125	29.00	8.00
	0.025	24.00	11.00
	0.05	22.00	10.00
20	0.0125	20.00	-
	0.025	18.00	-
	0.05	9.00	-
25	0.0125	no recovery	-
	0.025	no recovery	-
	0.05	no recovery	-
30	0.0125	5.00	-
	0.025	2.00	-
	0.05	1.00	-
35	0.0125	3.00	-
	0.025	1.00	-
	0.05	no recovery	-

absorption and accumulation in the plants increased as terbutryne concentration increased from 0.0125 to 0.05 mg l^{-1}, and at a given concentration of terbutryne absorption and accumulation increased as temperature increased.

Table 1.4 presents percentage recovery from photosynthetic inhibitory effects of terbutryne by E. canadensis at different terbutryne concentrations and temperatures. In general, from the third day after application of terbutryne, as compared to the second day, the dissolved oxygen concentration in the treated jars increased, indicating that the plants were recovering from photosynthetic inhibition. With the exception at temperatures of 25°C under natural light conditions and 7°C under artificial light conditions recovery, although with varying degrees, occurred in all concentrations and temperatures under both light conditions. Although any relationship between terbutryne concentrations and temperatures for recovery can not be found from the table, it appears that recovery was more frequently higher at lower concentrations and temperatures.

(ii) Dark respiration:

Tables 2.1 and 2.2 present mean rates over three days in dark respiration of E. canadensis treated with different concentrations of terbutryne at different temperatures, under natural and artificial light conditions. Fig. 3.1, constructed by plotting the rates of dark respiration against temperature, illustrates the effect of terbutryne concentrations and temperatures on the rates of dark respiration of E. canadensis. It appears from the figure

TABLE 2.1. Mean rates over three days in dark respiration of E. canadensis in treatments with different concentrations of terbutryne at different temperatures under Natural light conditions.

Concentration of terbutryne mg l^{-1}	Rates of dark respiration $mgO_2/gdw/h$							
	1979 Experiments				1980 Experiments			
	Temperature °C							
	5	10	15	20	20	25	30	35
0.00 (Control)	0.46	0.47	0.75	1.14	0.72	1.02	1.10	0.78
0.0125	0.38	0.45	0.56	1.01	0.70	0.83	0.50	0.31
0.025	0.38	0.43	0.61	0.87	0.58	0.63	0.34	0.18
0.05	0.40	0.48	0.58	0.81	0.49	0.54	0.28	0.16

TABLE 2.2. Mean rates over three days in dark respiration of E. canadensis in treatments with different concentrations of terbutryne at different temperatures under Artificial light conditions.

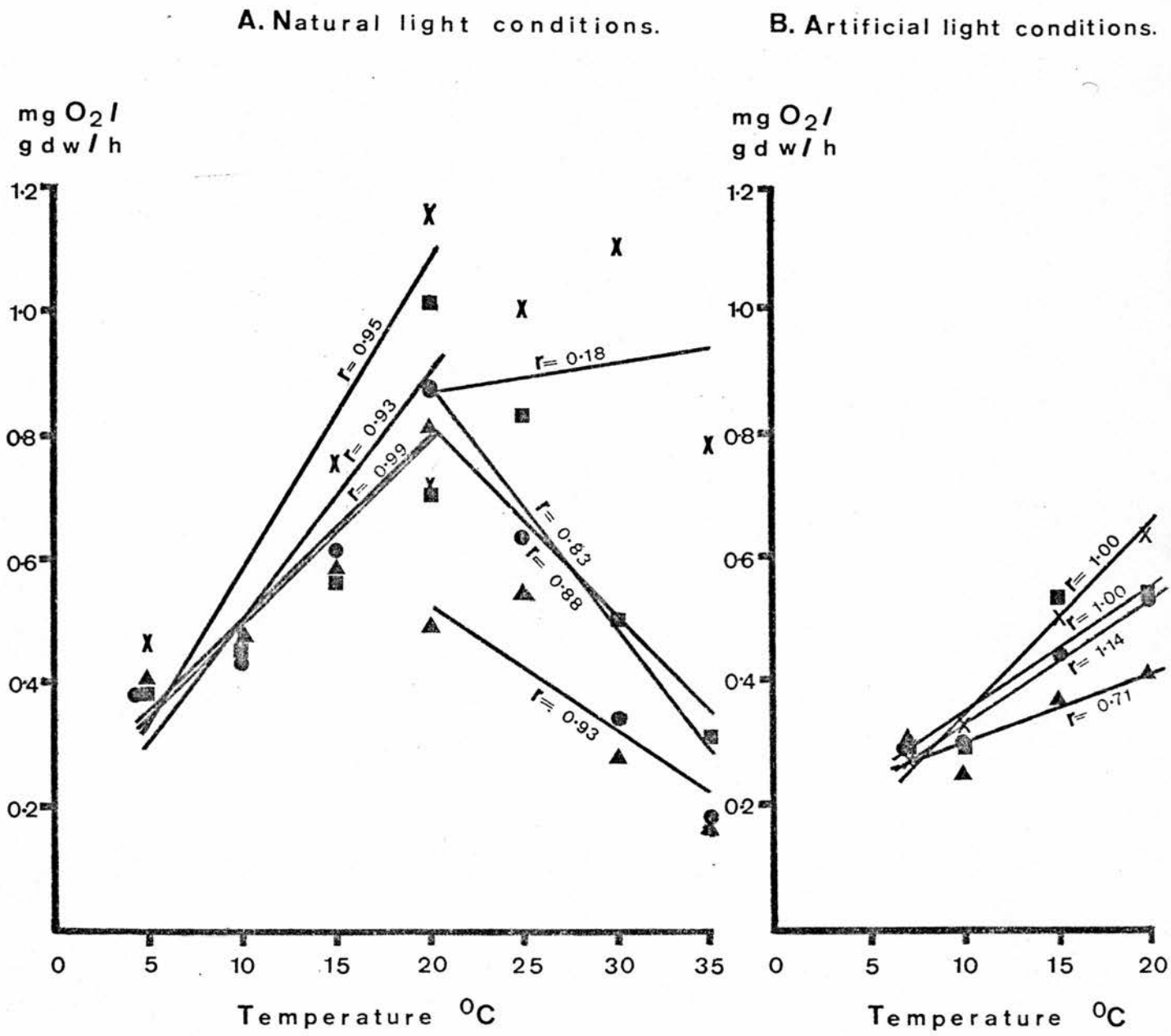
Concentration of terbutryne mg l^{-1}	Rates of dark respiration $mgO_2/gdw/h$			
	Temperature ^{o}C			
	7	10	15	20
0.00 (Control)	0.27	0.33	0.50	0.63
0.0125	0.29	0.29	0.53	0.54
0.025	0.29	0.30	0.44	0.53
0.05	0.31	0.25	0.37	0.40

FIGURE 3.1

(opposite)

Fitted regression lines of the rates of dark respiration (mgO_2/gdw/h) of E. canadensis, treated with different concentrations of terbutryne, at different temperatures, exposed to (A) Natural and (B) Artificial light conditions. Concentration of terbutryne mg l^{-1}: (X), 0.00 control; (■), 0.0125; (●), 0.025 and (▲), 0.05.

FIGURE 3·1


A. Natural light conditions.
B. Artificial light conditions.
mg O2 / g d w / h
1·2
1·0
0·8
0·6
0·4
0·2
r= 0·95
r= 0·93
r= 0·99
r= 0·18
r= 0·83
r= 0·88
r= 0·93
0
5
10
15
20
25
30
35
Temperature °C
mg O2 / g d w / h
r= 1·00
r= 1·00
r= 1·14
r= 0·71
0
5
10
15
20
Temperature °C

that over the temperature range 5° or 7° to 20°C under natural and artificial light conditions respectively, the rates of dark respiration in the controls and in the treatments increased although the increase in the treatments was much lower than in the controls. Excepting in 0.05 mg l^{-1} treatment under artificial light conditions, the increase in dark respiration was significant ($p < 0.05$, see Appendix IV Table B) in other treatments and in the controls under both light conditions. Over the temperature range 20° to 35°C under natural light conditions the rates of dark respiration in the control continued to increase, although not significantly; in the treatments the rates decreased. The decrease in the rates of dark respiration in 0.0125 mg l^{-1} was not significant but in the other two treatments it was significant.

Tables 2.3 and 2.4 present mean changes in dark respiration of treated plants, as percentage of control, on a daily basis. Fig. 3.2, constructed by plotting changes in dark respiration against temperature, illustrate respiratory response of *E. canadensis* to terbutryne concentrations and temperatures.

It appears from the figure that on the first day of terbutryne application, as compared to the controls, dark respiration was higher in most of the treatments up to a temperature of 25°C under natural light conditions and in all concentrations and temperatures under artificial light conditions. Although any relationship between terbutryne concentration and increase in dark respiration on the first day of treatment can not be found from the data in Tables

TABLE 2.3. Mean changes in dark respiration of *E. canadensis*, expressed as percentage of control, on a daily basis, in treatments with different concentrations of terbutryne at different temperatures under Natural light conditions.

Day	Concentration of terbutryne mg l^{-1}	Changes in dark respiration as percentage of control							
		1979 Experiments				1980 Experiments			
		Temperature °C							
		5	10	15	20	20	25	30	35
1	0.0125	-2.00	+13.00	-3.00	+24.00	+32.00	+39.00	-18.00	-16.00
	0.025	-2.00	+13.00	+14.00	+14.00	+21.00	+36.00	-34.00	-39.00
	0.05	+2.00	+31.00	+11.00	+4.00	+19.00	+37.00	-43.00	-43.00
2	0.0125	-22.00	-13.00	-30.00	-17.00	-15.00	-24.00	-79.00	-71.00
	0.025	-24.00	-21.00	-26.00	-29.00	-33.00	-56.00	-92.00	-89.00
	0.05	-13.00	-15.00	-29.00	-34.00	-44.00	-76.00	-96.00	-92.00
3	0.0125	-33.00	-17.00	-42.00	-38.00	-19.00	-66.00	-81.00	-83.00
	0.025	-33.00	-23.00	-40.00	-52.00	-39.00	-89.00	-93.00	-91.00
	0.05	-33.00	-19.00	-45.00	-55.00	-61.00	-96.00	-96.00	-94.00

TABLE 2.4. Mean changes in dark respiration of E. canadensis, expressed as percentage of control, on a daily basis, in treatments with different concentrations of terbutryne at different temperatures under Artificial light conditions.

Day	Concentration of terbutryne mg l^{-1}	Changes in dark respiration as percentage of control			
		Temperature °C			
		7	10	15	20
1	0.0125	+15.00	+12.00	+31.00	+19.00
	0.025	+19.00	+15.00	+21.00	+22.00
	0.05	+22.00	0.00	+3.00	+2.00
2	0.0125	+5.00	-17.00	+11.00	-17.00
	0.025	0.00	-17.00	-7.00	-20.00
	0.05	+2.00	-28.00	-20.00	-38.00
3	0.0125	+7.00	-28.00	-12.00	-39.00
	0.025	0.00	-28.00	-35.00	-47.00
	0.05	+29.00	-48.00	-48.00	-69.00

FIGURE 3.2

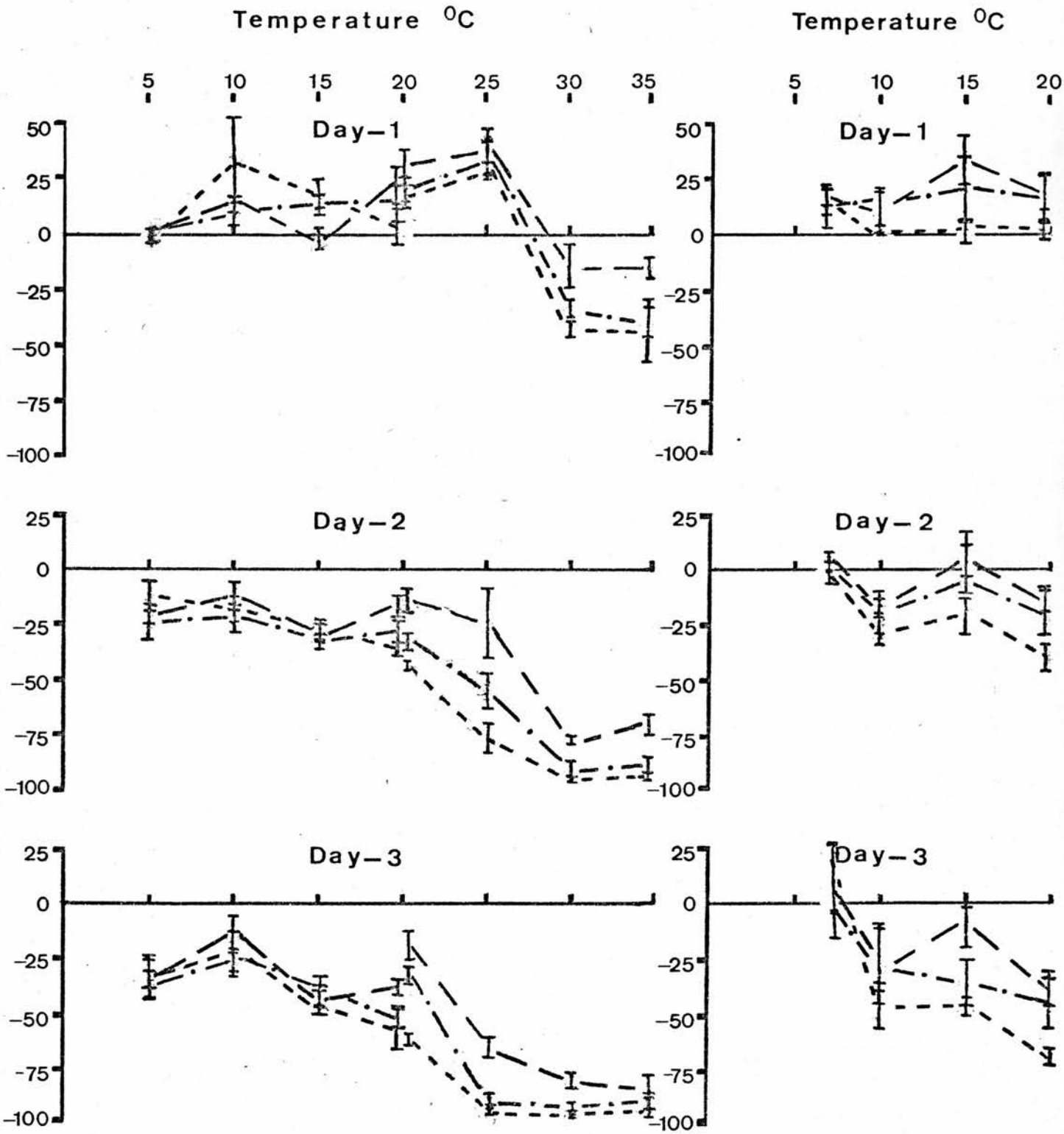
(opposite)

Mean changes in dark respiration of E. canadensis, expressed as percentage of control, on a daily basis, in treatments with different concentrations of terbutryne, at different temperatures, under (A) Natural and (B) Artificial light conditions. Concentration of terbutryne mg l^{-1}: (– –), 0.0125; (–.–), 0.025 and (– – –), 0.05.

FIGURE 3·2

A. Natural light conditions.

B. Artificial light conditions.



2.3 and 2.4, it can be seen that there was a trend for dark respiration to increase more in lower concentrations.

At temperatures 30° and 35°C under natural light conditions dark respiration decreased in all treatments from the first day of terbutryne application (Fig. 3.2). With the exception (Fig. 3.2) at temperatures 7° and 15°C under artificial light conditions, dark respiration at all concentrations and temperatures decreased from the second day of terbutryne application under both light conditions. Although dark respiration in 0.0125 mg l^{-1} at temperature 15°C under artificial light condition decreased on the third day it was still higher even on the third day in 0.0125 and 0.05 treatments at temperatures 7°C under artificial light conditions.

It also appears from Fig. 3.2 that increasing concentrations of terbutryne and temperatures affected dark respiration in the same way as they did net photosynthesis.

(iii) Ratio of net photosynthesis (nP) to dark respiration (dR):

Table 3.1 presents the ratio of net photosynthesis to dark respiration (nP: dR) under natural and artificial light conditions. Fig. 4.1, constructed by plotting nP: dR against temperature, illustrates the effect of terbutryne concentrations and temperatures on nP: dR. It appears from the figure that over the temperature range 5° or 7° to 20°C under natural and artificial light respectively the nP: dR fell in the treatments including the controls. The fall in the nP: dR in the treatments was more rapid than in the controls. The fall in the nP: dR in the control under natural light conditions was not significant ($p > 0.05$,

see Appendix IV Table C), but it was significant under artificial light conditions. The fall in nP: dR was significant ($p < 0.05$) in all treatments under both light conditions. Over the temperature range 20^{o} to $35^{o}C$ under natural light conditions, the nP: dR still continued to fall in the control and in 0.0125 and 0.025 mg l^{-1} treatments although not significantly, in 0.05 mg l^{-1} treatment it went up. The rise in nP: dR in 0.05 mg l^{-1} treatment was not significant.

TABLE 3.1. Ratio of net photosynthesis to dark respiration (nP: dR) of E. canadensis in treatments with different concentrations of terbutryne, at different temperatures under Natural and Artificial light conditions.

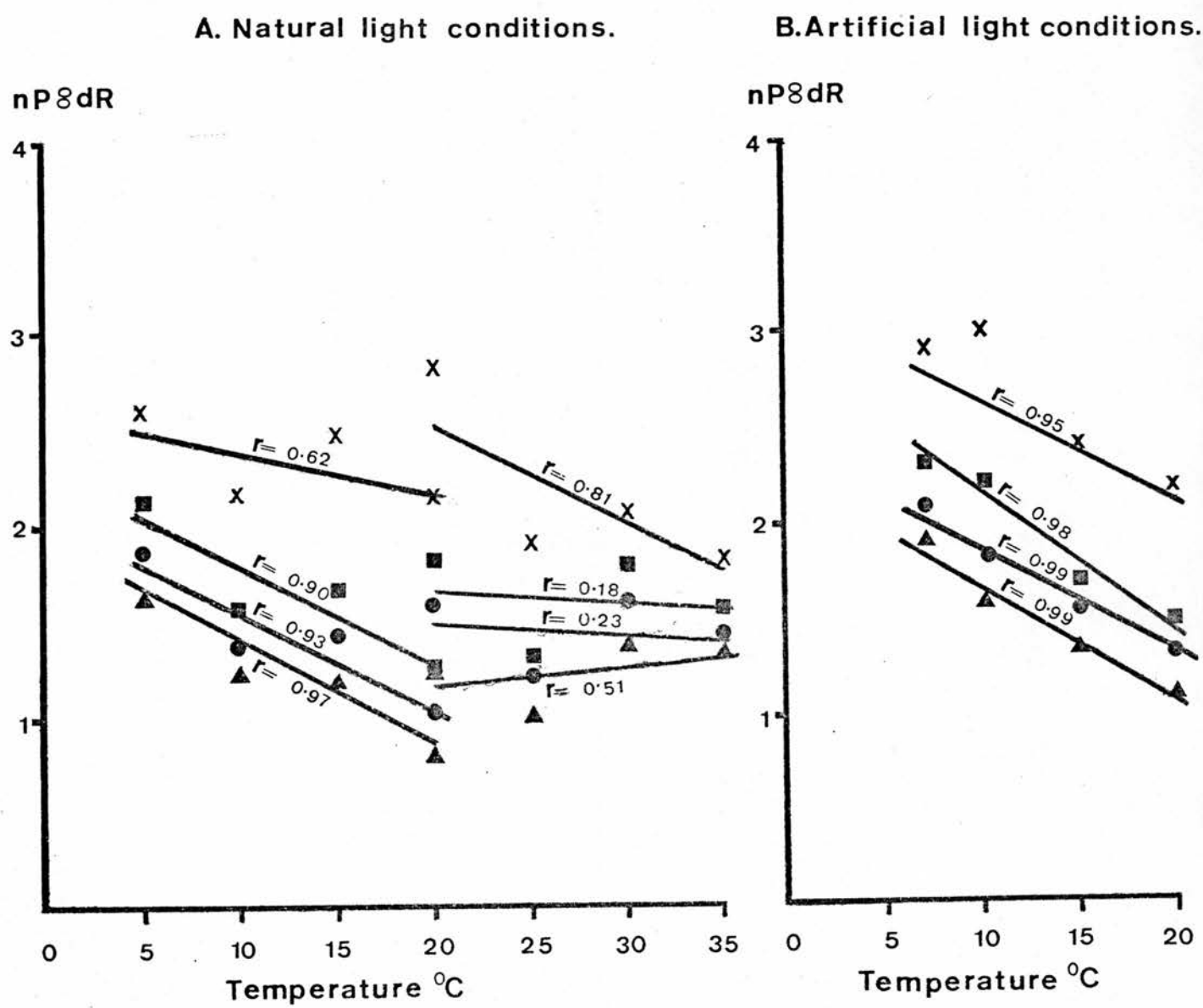
Concentration of terbutryne mg l^{-1}	Temperature °C	Net photosynthesis: Dark respiration	
		Natural light	Artificial light
0.00 (Control)	5/7	2.54	2.85
	10	2.11	2.94
	15	2.39	2.32
	20	2.11	2.11
	20	2.79	
	25	1.87	
	30	2.02	
	35	1.77	
0.0125	5/7	2.08	2.24
	10	1.51	2.14
	15	1.61	1.66
	20	1.23	1.42
	20	1.78	
	25	1.30	
	30	1.74	
	35	1.52	
0.025	5/7	1.81	2.00
	10	1.35	1.80
	15	1.38	1.48
	20	0.99	1.28
	20	1.55	
	25	1.16	
	30	1.56	
	35	1.44	
0.05	5/7	1.60	1.84
	10	1.21	1.52
	15	1.15	1.30
	20	0.76	1.06
	20	1.22	
	25	0.98	
	30	1.32	
	35	1.31	

FIGURE 4.1

(opposite)

Fitted regression lines of the ratio of net photosynthesis to dark respiration (nP: dR) of *E. canadensis*, treated with different concentrations of terbutryne, at different temperatures, under (A) Natural and (B) Artificial light conditions. Concentration of terbutryne mg l^{-1}: (X), 0.00 control; (■), 0.0125; (●), 0.025 and (▲), 0.05.

FIGURE 4.1


A. Natural light conditions.
B. Artificial light conditions.
nP8dR
nP8dR
4
3
2
1
r= 0·62
r= 0·81
r= 0·90
r= 0·18
r= 0·23
r= 0·93
r= 0·51
r= 0·97
r= 0·95
r= 0·98
r= 0·99
r= 0·99
0
5
10
15
20
25
30
35
Temperature °C
Temperature °C

III(ii)(b). PHYSIOLOGICAL EFFECTS:

(i) Dry weight:

Tables 4.1 and 4.2 present mean decrease in dry weight of *E. canadensis* treated at different concentrations of terbutryne and temperatures under natural and artificial light conditions respectively. As compared to the controls the decrease in dry weight in the treatments at all temperatures under natural light conditions was significant ($p < 0.05$), while under artificial light conditions it was significant only at temperatures of 7° and $20^{\circ}C$. Although any relationship between decrease in dry weight and terbutryne concentrations can not be found from the data in the tables, it can be seen, however, that there was a trend for dry weight to decrease more with increasing concentrations of terbutryne.

(ii) Chlorophyll concentration:

Table 5.1 presents chlorophyll (a+b) concentration in plant tissue of *E. canadensis*, treated at different concentrations of terbutryne and temperatures. It appears from the table that despite some ups and downs, changes in the chlorophyll (a+b) concentration in tissue of the treated plants during experiment was nothing significant.

TABLE 4.1. Mean decrease in dry weight of *E. canadensis*, expressed as percentage of control in treatments with different concentration of terbutryne at different temperatures under Natural light conditions.

Concentration of terbutryne mg l^{-1}	Decrease in dry weight as percentage of control							
	1979 Experiments				1980 Experiments			
	Temperature °C							
	5	10	15	20	20	25	30	35
0.0125	6.0 ± 1.0	13.0 ± 9.0	4.0 ± 4.0	9.0 ± 2.0	27.0 ± 6.0	16.0 ± 2.0	11.0 ± 2.0	15.0 ± 3.0
0.025	13.0 ± 5.0	13.0 ± 7.0	14.0 ± 2.0	10.0 ± 5.0	31.0 ± 3.0	11.0 ± 2.0	9.0 ± 5.0	16.0 ± 7.0
0.05	10.0 ± 5.0	17.0 ± 3.0	14.0 ± 6.0	14.0 ± 6.0	30.0 ± 1.0	11.0 ± 1.0	13.0 ± 2.0	18.0 ± 2.0

TABLE 4.2. Mean decrease in dry weight of E. canadensis, expressed as percentage of control in treatments with different concentrations of terbutryne at different temperatures under Artificial light conditions.

Concentration of terbutryne mg l^{-1}	Decrease in dry weight as percentage of control			
	Temperature °C			
	7	10	15	20
0.0125	8.00 ± 7.00	9.00 ± 2.00	14.00 ± 4.00	9.00 ± 3.00
0.025	11.00 ± 4.00	8.00 ± 5.00	5.00 ± 8.00	18.00 ± 9.00
0.05	17.00 ± 6.00	12.00 ± 7.00	3.00 ± 1.00	10.00 ± 3.00

TABLE 5.1. Mean concentration (mg/g fresh weight) of chlorophyll (a+b) tissues of *E. canadensis* treated with different concentrations of terbutryne at different temperatures.

Concentration of terbutryne mg l^{-1}	Temperature ^{o}C	Concentration of chlorophyll (a+b) mg/gm
0.00 (Control)	5	0.84
0.0125		0.77
0.025		0.74
0.05		0.71
0.00 (Control)	10	0.71
0.0125		0.78
0.025		0.66
0.05		0.66
0.00 (Control)	15	0.72
0.0125		0.69
0.025		0.78
0.05		0.78
0.00 (Control)	20	0.63
0.0125		0.69
0.025		0.51
0.05		0.62
0.00 (Control)	25	0.34
0.0125		0.34
0.025		0.33
0.05		0.25
0.00 (Control)	30	0.25
0.0125		0.27
0.025		0.26
0.05		0.24
0.00 (Control)	35	0.41
0.0125		0.57
0.025		0.56
0.05		0.49

III(iii)(a). INTRODUCTION

In this discussion, the general mode of action of the herbicide 'Terbutryne' is outlined and the effects of concentrations and temperatures are discussed. First, however, a distinction is made between effects on photosynthesis and respiration.

In aquatic systems, the increase in dissolved oxygen concentration of water during daylight is a measure of photosynthesis. Terbutryne is a photosynthetic inhibitor so when it is added to water containing aquatic plants, it interferes with their oxygen evolving system, the rate of photosynthesis drops and, at rates which depend on concentration and temperature, the dissolved oxygen concentration of water stops increasing.

The decrease in dissolved oxygen concentration of aquatic systems by night is a measure of respiration. After terbutryne treatment of green plants, the fall in the level of dissolved oxygen in the dark i.e. the rate of respiration drops. The decrease in dark respiration, observed in the present experiments, is arguably due to an indirect effect of lower levels of photosynthetic assimilates following photosynthetic inhibition. Like photosynthesis, the decrease in respiration is affected by terbutryne concentration and temperature.

Quantitative differences in net photosynthesis, dark respiration, the ratio of net photosynthesis to dark respiration, dry weight, chlorophyll concentration and the physical damage of the treated plants are compared with

those of the controls and recognised as effects of terbutryne. Effects of factors like terbutryne concentrations, temperatures and light on the activity of terbutryne in E. canadensis, either separately or in combinations of several factors, are discussed as are the interactions between them.

Results are analysed, interpreted and compared with those of other workers in this field and conclusions are reviewed at the end of the discussion in terms of their possible application in the tropical conditions of Bangladesh.

III(iii)(b). DISCUSSION

Net photosynthesis:

Day of terbutryne addition:-

The phytotoxic symptoms of a herbicide may appear within a few minutes or hours e.g. acting through inhibition of photosynthesis or respiration, to several days or weeks e.g. observed as chlorosis, necrosis or death (Thompson et al., 1970). They also pointed out that to kill a plant a certain threshold concentration of herbicide at the site of action must be maintained for a certain period of time. Thus the length of time required for the action of a herbicide may not only vary with the symptom chosen, but also depends on herbicide formulation, concentration, stage of plant growth, environmental factors or a combination of one or more of these variables.

As observed in the present experiments the photosynthetic response of E. canadensis to terbutryne concentrations and temperatures on the first day of treatment was widely variable. Although the general trend for photosynthetic response of E. canadensis to terbutryne, particularly in higher concentrations was to decrease at most temperatures under both light conditions, it also increased in some treatments at certain temperatures. Excepting at the highest concentration of 0.05 mg l^{-1} at temperatures of 30° and 35°C, where inhibition was more than 50%, the decrease in net photosynthesis in the treatments at certain temperatures was 1 to 25% and still at other temperatures net photosynthesis was even higher than the control (1 to

34%).

The short light period that followed treatment, although may be a possibility for the partial expression of photosynthetic inhibition, it is more probable that the apparent less inhibition was due to integration of the photosynthetic rates for four hour period without terbutryne. The apparent increase in net photosynthesis in some treatments at certain temperatures under both light conditions also might have been due to integration of the photosynthetic rates for the four hour period without terbutryne. Since dissolved oxygen concentration in the treated jars was not measured immediately before terbutryne application it is difficult to say whether terbutryne concentrations completely or partially inhibited photosynthesis within four hours following treatment.

Response phase:

During the 'response phase', at all concentrations and temperatures the maximum inhibitory effects of terbutryne on net photosynthesis were expressed from the second day and on dark respiration with the exception at a temperature of 7^{o}C from the third day of treatment. Inhibition occurred even in the treatments where there was initial increase in net photosynthesis and dark respiration, on first day of terbutryne treatment

Terbutryne concentration:

The response of a plant to an exogenous chemical is a function of chemical structure, formulation, technique and time of application and concentration. With

some chemicals selectivity follows closely on dose levels. Each herbicide has its own critical minimum dose level, at which it is toxic to certain plants but not to others. Higher concentration will, probably, be toxic to most plants, both weeds and crops. The concentration of a herbicide at a vital location in the plant at any time may determine the herbicide's effectiveness. A given rate of application of a herbicide may not give the same degree of control of weeds in every case. Even on the same weed a treatment that gives complete control under one set of conditions may be a total failure under other conditions.

There are reports of varying degrees of phytotoxicity in both aquatic and land plants due to different herbicide concentration. In a greenhouse experiment on Lemna and Elodea plants treated with 0.12, 0.50 and 1.0 mg l^{-1} of simazine, Sutton et al. (1969) observed that the highest concentration (1.0 mg l^{-1}) caused the greatest decrease in dissolved oxygen content, while the lowest concentration (0.12 mg l^{-1}) resulted in least decrease. Marks (1974) reported that a concentration of 0.05 mg l^{-1} of terbutryne controlled submerged as well as floating vascular plants, but the concentration had to be raised to 0.10 mg l^{-1} to control Vaucheria spp. Similar observations were made by Robson et al. (1976). Payne (1974) reported that Hydrilla spp. and Potamogeton spp. were controlled at a terbutryne concentration of 0.025 mg l^{-1}, but that the concentration had to be raised to 0.25 mg l^{-1} to control Ceratophyllum spp. Ashton and Raynaert (1974) concluded that, depending on the problem and situation,

terbutryne concentrations between 0.025 to 0.05 mg l^{-1} may be needed to control weeds.

In the case of land plants, Kozlowski et al. (1967), in a number of field trials with simazine, atrazine, propazine, prometryne, prometone, and ipazine applied at different rates under different temperatures and humidity conditions, observed that at any given temperature, an increase in herbicide concentration increased herbicide phytotoxicity. Minshall (1969), demonstrated that increased concentration of atrazine resulted in an increased uptake of herbicide by tomato plants. Vostral et al. (1970) observed that absorption of ^{14}C ring-labelled atrazine by soybean plants increased with increase in herbicide concentration, absorption time and root temperature.

In the present experiments, photosynthetic inhibition in E. canadensis occurred with varying degrees at all concentrations tested. At a given temperature the greatest inhibition always occurred at the highest concentration. From the data for herbicide residue analyses presented in Table 1.5, it appears that the general trend for absorption of herbicide by plants was to increase as terbutryne concentration increased from 0.0125 to 0.05 mg l^{-1}. Greater inhibiton was possibly due to increased absorption and accumulation of herbicide within the plants. The results of the experiments strongly suggest that at a given temperature an increase in terbutryne concentration will lead to greater inhibitory effects on photosynthesis.

Temperature:

The influence of temperature on the activity of herbicides, including triazine, is well documented, but is mostly related to land crops and land weeds. Information concerning aquatic plants or weeds is scanty. The recommended practice of Lewis (1977) and Thompson (1978) to apply simazine to north temperate aquatic systems between April 1 and May 15 indicates that water temperature should be reasonably high. In field trials carried out between 1973 and 1977, Robson et al. (1978) observed that terbutryne did not kill aquatic plants until the water temperature had risen to above 15°C. All these observations indicate that temperature is an important factor for the activity of triazine herbicide in aquatic systems.

Burnside and Behrens (1961) observed that increasing soil temperature from 59° to 86°F (15° to 30°C) caused increasing simazine toxicity to corn. They also observed a highly significant simazine rate x temperature interaction and temperature had an influence on the response of corn. Kozlowski et al. (1967) using simazine and other triazine herbicides at different rates on germinating pine seeds, observed marked effects of temperature on herbicide activity. They further observed that temperature greatly accelerated herbicide activity and a highly significant dose x temperature interaction. Houseworth and Tweedy (1971) observed interactions of light, temperature and moisture on terbutryne toxicity to cucumber and oat plants, and concluded that environmental conditions

favouring rapid growth result in increased phytotoxicity.

From the results of the experiments and the observations of other workers described in the preceeding paragraphs, it may be said thatlike other triazine herbicides, the phytotoxicity of terbutryne is temperature dependent. At a given concentration, inhibitory effects of terbutryne will increase with rising temperature.

Penner (1971) from his experiment on effects of temperature on phytotoxicity and root uptake of linuron by corn (Zea mays L.) and soybean (Glycine max L.) seedlings and atrazine by soybean seedlings, pointed out that increased phytotoxicity with increasing temperature could result from increased herbicide uptake, greater translocation of herbicide from root to shoot, reduced capacity of the enzymes to detoxicate the herbicide, or rate changes in the aforementioned factors which would result in a greater concentration of herbicide within the plant. Using ^{14}C-labelled atrazine on Agropyron repens L; Muzik and Mauldin (1964) proved that uptake and translocation of root-fed atrazine increased as temperature increased. Treating detopped tomato plants with propazine, prometon and atrazine, Minshall (1969) also observed that increasing soil temperature from 10° to $30^{\circ}C$ increased rate of exudation.

In the present study the effects of terbutryne on net photosynthesis increased more or less steadily at all concentrations up to a temperature of $20^{\circ}C$. The inhibitory effects did not increase very much when the temperature was further raised from 20° to $35^{\circ}C$ (Fig. 2.2).

At a terbutryne concentration of 0.05 mg l^{-1} complete suppression (100% inhibition) occurred at temperatures of 20° and 25°C, but this did not occur at 30° and 35°C. It is very difficult to explain the reasons for less inhibition at those two temperatures from the present experimental evidence. Greater inhibition due to higher absorption and accumulation of herbicide cannot be justified here because absorption and accumulation of terbutryne was higher at temperatures of 30° and 35°C than at 20° and 25°C (Table 1.5).

Reviewing the literature on temperature effects on herbicide activity, Currier and Dybing (1959) concluded that warm, but not excessive, temperature promoted penetration through its effects on physico-chemical processes like increased diffusion, lower viscosity, etc.; and physiological processes like photosynthesis, phloem translocation, accumulation of metabolites, protoplasmic streaming and growth. Currier and Dybing's proposition was established by Mulder and Nalewaja (1978), when they found that atrazine toxicity to barley shoots increased as temperature increased from 10° to 17°C but no further increase in atrazine toxicity took place when temperature was increased from 17° to 24°C. Similar observations were made by Penner (1971), who found that atrazine toxicity to soybean in nutrient solution increased when temperature increased from 15° to 20°C but that no further increase in phytotoxicity occurred when the temperature was increased further to 30°C.

Results of the present experiments suggest that, in

general, the photosynthetic inhibition of terbutryne was greatly accelerated by temperature. At a given concentration, increasing photosynthetic inhibition with rising temperature was due to increased absorption of terbutryne by the plants (Table 1.5). Increased inhibitory effects due to a terbutryne concentration x temperature interaction is apparent from Fig. 2.2. Statistical analysis of the results also suggest that this interaction was highly significant (Appendix V, Tables A and C).

Recovery:

The partial recovery from photosynthetic inhibition from the third day after treatment, perhaps occurred because the chloroplasts, which are the primary site of action, were only partially damaged and could start functioning again. Perhaps, too, plants were able to reach an equilibrium between terbutryne build-up and chloroplast activity and recovered from inhibition by using reserve carbohydrate. The recovery was better at lower concentrations and temperatures, possibly because the inhibitory effects were less severe and the reserve carbohydrate was not fully used up. At higher concentrations and temperatures the inhibition was not only severe, but the reserve carbohydrate was also used up through increased rates of respiration during the lag phase to provide extra energy needed to bring unbalanced cell processes back into order. As a consequence the plants had little or no chance of recovery.

Recovery from photosynthetic inhibition of both land

and aquatic plants is well reported. Robson et al. (1976) observed signs of recovery in ametryne-treated Rhizoclonium hieroglyphicum after two weeks of treatment. Similar observations were made by Robson et al. (1978) in field trials with terbutryne. Sutton et al. (1965) observed, in field trials, that oxygen content in simazine treated water decreased but returned to the original level after two days. In a greenhouse experiment on common duck weed, Elodea spp. and parrotfeather, treated with different concentrations of simazine, Sutton et al. (1969) observed that after the initial reduction, there was a rise in the dissolved oxygen content of water. They did not explain this post-treatment rise.

Ashton, Bisulputara and Risley (1966) in Chlorella spp., Schiff, Zeldin and Rubman (1976) in Euglena spp., Leoppky and Tweedy (1969) in Chlamydomonas spp. and Arvik, Ayzak and Zimdahl (1973) in unicellular algae reported recovery even after seven days treatment with no serious damage to chloroplast structure or to the photosynthetic apparatus as a whole.

Recovery by land plants from triazine inhibition have been reported by Boyer (1976), Hamilton (1964), Jensen et al., (1977), Lund-Höie (1969), Montgomery and Freed (1961), Moreland et al. (1959), Schlue (1976), Shimabukuro and Swanson (1969), Thompson et al. (1970) and van Oorschot (1965, 1969).

Reviewing the literature on recovery from photosynthetic inhibition, Montgomery and Freed (1964) concluded that the metabolism of the triazine herbicides by plants

is a general phenomenon. They went on further to say that although there is a good correlation between resistance and extent of metabolism, even the highly susceptible plants have a limited capacity for degrading these chemicals.

In the same perspective Thompson et al. (1970) concluded that the degree of injury depends on the condition of the plants. If the plants have high carbohydrate reserve, maintenance of a herbicide at a toxic concentration for 2 or 3 days reduces the carbohydrate reserves, but once the herbicide concentration is reduced, plants with good carbohydrate reserves utilize part of the remainder to recover. However, if the carbohydrate reserves of a plant is very low at the time of herbicide application, this reserve may be reduced to such a low level during the period in which a toxic concentration is maintained that the plant cannot recover even when the herbicide concentration is reduced.

Light:

The effects of light on herbicide treated land plants and weeds is well reported. Reports concerning aquatic plants are very scanty. Most of the reports on light effect indicate that high intensity increases the degree and rapidity of injury from triazine herbicides including terbutryne (Allen et al. 1963; Ashton 1963 and 1965; Figuerola and Furtick 1972; Houseworth and Tweedy 1971; Thompson et al. 1970; van Oorschot 1974 and Wills et al. 1963). In contrast, Gingrass (1966) and van Rensen (1971) observed in short term experiments with unicellular algae that inhibition was greater under low light intensity.

TABLE 6.1. Mean rates of net photosynthesis (mgO_2/gdw/h) of E. canadensis under different light intensities, at 20°C in St. Andrews.

Intensity of light $uEM^{-2}S^{-1}$	w/m^2 (approximately)	Rates of net photosynthesis of E. canadensis mgO_2/gdw/h
17	4	0.024
38	8	0.076
125	27	0.104
255	55	0.127
380	82	0.133
495	107	0.137
610	132	0.137

TABLE 6.2. Mean rates of net photosynthesis ($mg)_2$/gdw/h) of E. canadensis under different light intensities, at 20°C in Oxford.

Intensity of light w/m^2	Rates of net photosynthesis of E. canadensis mgO_2/gdw/h
127	0.12
206	0.24
324	0.36
394	0.38
480	0.38

Reviewing the literature of the effects of light on herbicide activity, Muzik (1976) concluded that the action of many herbicides is affected by light intensity. The influence of light, although obviously important, is extremely difficult to separate (except under controlled conditions) from the influence of other environmental factors, particularly temperature.

Effects of terbutryne at different light intensities were not studied in the present investigation, but a comparison was attempted between effects of terbutryne on E. canadensis in constant and saturating artificial light and in variable and sometimes non-saturating natural light. Comparison is restricted to 20°C since no experiment was done beyond that temperature under artificial light conditions. Saturating light intensity for E. canadensis was accepted as about 480 w/m^2 (Table 6.1 and 6.2). Natural light intensity during the experimental period July to October, 1979 (Appendix VI) ranged from 135 to 635 w/m^2 and the average light intensity during an 'experimental day' was estimated as about 395 w/m^2. For the experimental period April to June, 1980 the light intnesity ranged from 127 to 725 w/m^2 and the average light intensity was estimated as about 435 w/m^2.

It appears from the results presented in Table 1.3 that in 1979 experiments under natural light conditions, although complete suppression of net photosynthesis (100% inhibition) occurred at the highest concentration of 0.05 mg l^{-1} and at a temperature of 20°C, it did not occur at the same concentration and temperature in a repeat

experiment in 1980. At a concentration of 0.05 mg l^{-1} and temperature 20°C net photosynthesis decreased by about 88% (Table 1.3) under natural light conditions (1980 experiment) and by about 93% (Table 1.4) under artificial light conditions (1979 experiment).

In the given experimental conditions, effects of light on the activity of terbutryne cannot be separated from the effects of temperature; but it can be said that the overall differences in the inhibitory effects of terbutryne under the two systems of light were not wide.

The effects of light on the activity of terbutryne in B. canadensis observed in the present experiments correspond in general with the views of Muzik (1976).

Dark respiration:

The triazine herbicides have been reported to affect several biochemical events other than photosynthesis. Respiration has been reported to be increased, decreased or not to be affected. In the present experiments, in general, dark respiration was stimulated on the first day of terbutryne application while, with some exceptions under artificial light conditions, from the second day it decreased (Fig. 3.2). Terbutryne concentrations and temperature affected dark respiration in the same way as they did net photosynthesis. To avoid repetition the effects of concentration and temperature will not be discussed here. Discussion will be limited to explain the possible reasons of stimulation and subsequent decrease in dark respiration following terbutryne application.

Although in the absence of biochemical analysis it

cannot be said with certainty the sudden increase in dark respiration on the first day of treatment indicates a direct interference of terbutryne activity with the respiratory system of the plants. Stimulation in dark respiration could have resulted either from, (1) the metabolic processes of the cell being unbalanced during the lag phase, or (2) the demand for extra energy needed to bring cell processes back into order or equilibrium.

The subsequent decrease in dark respiration may be an indirect effect of low accumulation of photosynthesis in the plants as a consequence of photosynthetic inhibition caused by terbutryne. The slow and gradual decrease in dark respiration with time at any given concentration and temperature strongly suggest the above views.

At temperatures 30^{o} and $35^{o}C$ due to high photosynthetic inhibition on the first day there was a 50% decrease in dissolved oxygen concentration in the medium (Table 1.3). The decrease, rather than stimulation, of dark respiration in the treatments on the first day, at those two temperatures, appear to have been caused primarily by low concentration of dissolved oxygen concentration among other factors.

Literature concerning the effects of triazine herbicides on the respiration of aquatic plants is very scanty. The information available is mostly related to land plants and weeds. However, the effects of terbutryne on dark respiration of *E. canadensis*, as observed in the present experiments are comparable with its effects on land plants and weeds.

Ashton (1960) reported that some of the triazines caused an immediate increase in respiration. In short term experiments (1-2 hours), Funderburk and Davis (1963) observed that simazine caused an immediate increase in respiration of corn plant, but in long term experiments respiratory rates of all plants (resistant and sensitive) was reduced 7 and 11 days after treatment. Metcalf and Collins (1978) reported that simazine stimulated respiration of celary cells in short term experiments, but in long term experiments it eventually stopped respiration completely. Palmer and Allen (1962) observed a general trend of stimulation in respiration of excised barley roots treated with simazine. Roth (1958) reported similar stimulatory effects of triazines on the respiration of Blodea spp.

Deeva (1967), Lotlikar et al. (1968), Moshkatov (1967) and Voinilo (1967) suggest that the initial stimulation in respiration is due to triazines acting as an uncoupler. For the subsequent decrease, however, Olech (1966) concluded that inhibition of respiration is indirect and caused by a lower level of assimilates as a result of the inhibition of photosynthesis. With regard to the effect of dissolved oxygen concentration on dark respiration, Owens and Maris (1964) from their experiments with Callitriche spp., Hippuris spp. and Ranunculus spp. concluded that a decrease in dissolved oxygen concentration limits respiration of submerged aquatic plants.

Effects of terbutryne on dark respiration of E. canadensis observed in the present experiments correspond

with the observations described in the preceeding paragraphs. After initial stimulation dark respiration will decrease in terbutryne treatments. Dose concentration and temperature will affect the degree of stimulation and the subsequent decrease in dark respiration.

The ratio of net photosynthesis to dark respiration (nP: dR):

Terbutryne is known as a photosynthetic inhibitor, but in the present experiments dark respiration has also been found to be affected. After initial stimulation dark respiration in all concentrations and temperatures decreased with time. It has already been concluded in the previous section that the inhibitory effects on dark respiration were indirect; that dark respiration decreased due to lack of photosynthetic assimilates and low levels of dissolved oxygen concentration in the medium as a consequence of photosynthetic inhibition.

The fall in the nP: dR, over the temperature range 5^{o} or 7^{o} to $20^{o}C$, in the control was due to higher rates of dark respiration. While over the given temperature range in the treatments, the rates of dark respiration also increased the fall in the rates of net photosynthesis was much more rapid than the fall in the rates of dark respiration so that fall in nP: dR in the treatments was due mainly to a decrease in net photosynthesis.

The fall in the nP: dR, over the temperature range 20^{o} to $35^{o}C$, in the control continued and was still due to higher ranges of dark respiration. Over the same range

of temperature, while nP: dR in 0.0125 mg l^{-1} and 0.025 mg l^{-1} still continued to fall because of higher rates of dark respiration, in 0.05 mg l^{-1} it went up. This rise in nP: dR in the 0.05 treatment was due to decrease in dark respiration. The decrease in the rates of dark respiration was due mainly to the limitation of dissolved oxygen concentration in the medium as a consequence of photosynthetic inhibition (discussed on p. 76).

These observations are in agreement with those of Tieszen (1970), who from his experiment with simazine on rye, concluded that effects on net photosynthesis were more pronounced than those on respiration.

Dry weight:

Dry weight of E. canadensis decreased at all concentrations and temperatures. This decrease in dry weight was presumably caused by a lack of photosynthetic assimilates as a consequence of photosynthetic inhibition and the using up of reserve carbohydrate through respiration.

Bastin and Davis (1967) in corn and Johnsongrass (atrazine), Graham and Buchholtz (1968) in chippewa soybean (atrazine), Jenesen et al. (1977) in different species of grass (atrazine, cyanazine and cyprazine), Kozlowski et al. (1967) in pine seedlings (simazine, atrazine, propazine, prometryne, prometon and ipazine), Pinthus (1972) in wheat seedlings (terbutryne), Robson et al (1976) in Cladophora glomerata (terbutryne) and Singh and West (1967) in oats (simazine) observed decrease in dry weight of the treated plants. All these authors concluded that decrease

in dry weight was due to decrease in carbohydrate content as a consequence of photosynthetic inhibition in triazine treated plants.

From the results of the present experiments it can be said that terbutryne treatment reduces the dry weight of E. canadensis. Dry weight may decrease more with increasing concentrations.

Chlorophyll concentration and physical damage to plants:

Data in Table 5.1 show that terbutryne barely affected the chlorophyll (a+b) concentration of the treated plants.

There are conflicting reports on the effect of triazine herbicides on chlorophyll content and nitrogen uptake. Bartley (1957) reported that simazine increased the growth and green colour of maize. De Vries (1963) demonstrated an increase in nitrogen uptake by simazine treated maize. Bastin and Davis (1967) reported an increased percentage of total nitrogen in atrazine treated corn plants. Gast and Gorb (1960) observed that atrazine and simazine increased protein content of maize. On the other hand, Freeman et al. (1966) reported that atrazine reduced both chlorophyll a and chlorophyll b in raspberries. Wheeler and Hamilton (1968) reported that atrazine treated wheat, corn and sorghum showed a marked reduction in chlorophyll.

From the results of the present experiments it can be said that no change in chlorophyll concentration and physical damage like necrosis, rotting etc. may take place in short term experiments. Terbutryne is a slow killing herbicide and it may take 2 to 3 weeks before any

physical damage appears on the treated plants (T.O. Robson, per. comm.).

III(iii)(c) CONCLUSIONS

The experiments have helped towards a closer understanding of the mode of action of terbutryne, and of triazine herbicides in general, over a wide range of temperatures in an aquatic system. The metabolic and physiological responses of submerged aquatic plants to temperatures as high as 30° and 35°C are now better known than before. Although much more has to be studied under field conditions, the information gained from these controlled experiments may help in formulating field trials in a tropical country like Bangladesh, where aquatic weeds are a serious problem and where chemical methods of aquatic weed control may be introduced.

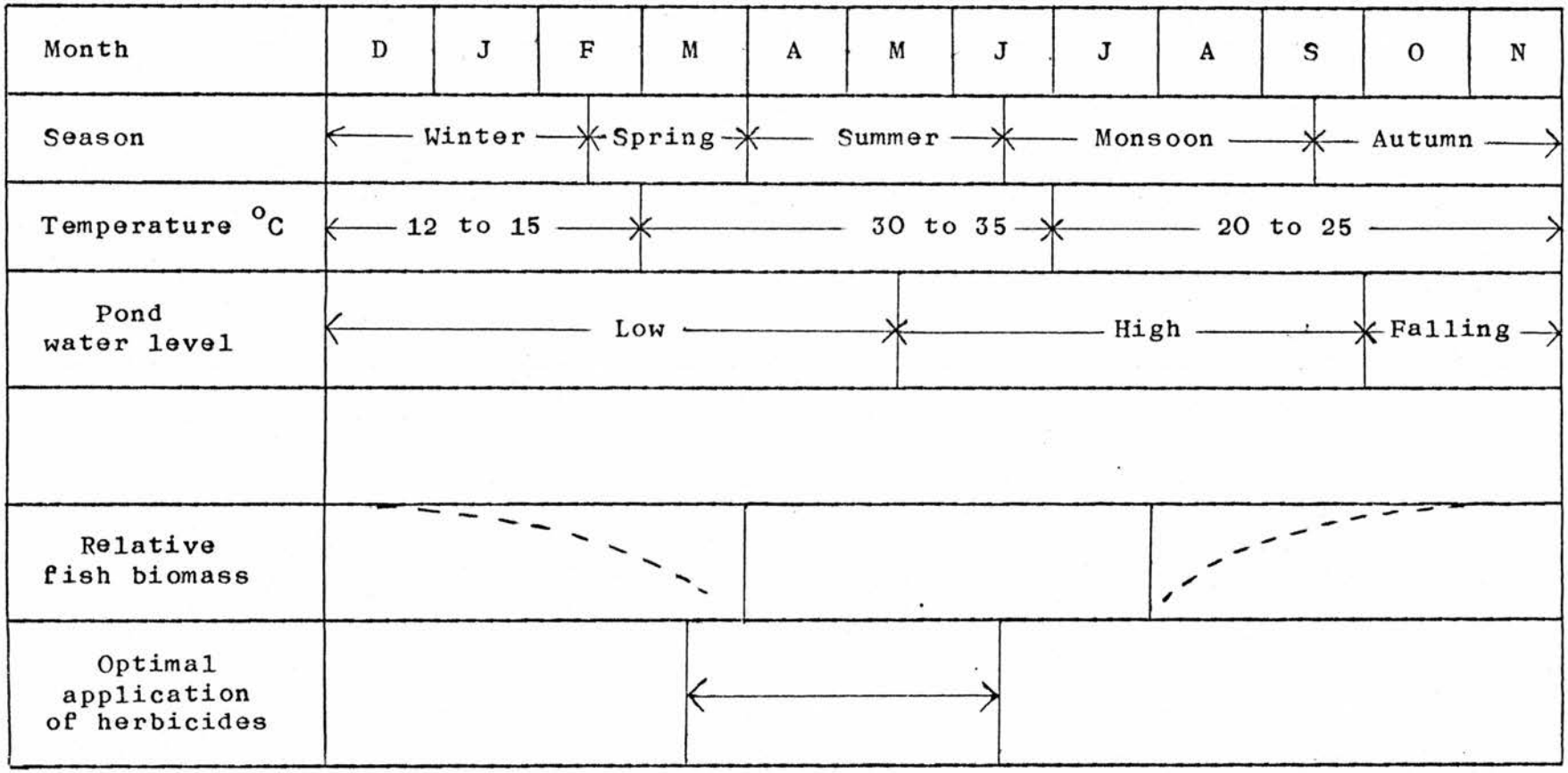
In Bangladesh water temperature varies from 12° to 15°C in winter, from December to February, and 30° to 35°C in summer, from March to May. Since triazine herbicides are active over this range of temperatures, they may be used in controlling aquatic weeds under tropical conditions all through the year.

As the present experiments have shown, however, the activity of these herbicides depends strongly on temperature, so summer is the best time for their use, even in the tropics. A second reason for applying herbicides in summer is the reduced fish population. Ponds are fished in winter and in spring but, by March, stocks are very low indeed. Water levels in the ponds also drop during this period due to seasonal changes and there is very little fluctuation in water volume during

summer. A combination of low water volume, high water temperature and PAR, with low fish stock, should make this the best period for the application of triazine herbicides (see Table 9.1 for summary).

Taking advantage of high water temperature, weeds can be controlled with a small quantity of herbicide probably at lower concentrations than the recommended dose. Because of the low stock of fish in the ponds during summer, the possibility of economic loss from fish kill due to deoxygenation following triazine application may be kept to a minimum. Furthermore, if herbicide is applied in summer, the long gap between the end of the spring fish crop and the beginning of the second fish crop in August and the increase in the volume of water during the monsoon from June to August will remove any risk of residual effects of the herbicides on fish. The long gap will also help to restore the food chain and unbalanced ecological conditions of the treated ponds.

Table 9.1. Summary of seasonal changes in water temperature, water level and fish population in ponds of Bangladesh and the possible time of herbicide application.



Data collected and summarised from the records of the Directorate of Fisheries, Bangladesh.

IV(i). SUMMARY

This thesis has attempted to describe how the action of terbutryne on the metabolic and physiological responses of E. canadensis is affected by variation in dose concentrations and temperatures, under fluctuating natural light, and constant saturating artificial light conditions. Efforts have been directed towards describing the possible interaction of the factors and to quantify their effects.

METABOLIC RESPONSE

It has been shown that,

Net photosynthesis:

(1) Terbutryne concentration - photosynthetic inhibition occurred at all concentrations tested but with varying degrees. At a given temperature an increase in dose concentration increased phytotoxicity of terbutryne due to increased absorption and accumulation of herbicide within the plants.

(2) Temperature - at a given concentration the photosynthetic inhibitory effect of terbutryne was increased by increasing temperature. The greater inhibition that occurred with rising temperature was due to an interaction between terbutryne concentration and temperature. Increasing temperature enhanced the absorption and accumulation of terbutryne within plants. At all concentrations the inhibitory effects increased steadily up to a temperature of 20^{o}C, and above that, the

effects slowed down.

(3) Light - Any effect of light quantity on the activity of terbutryne could not be separated from the effect of temperature in the present experiments.

The photosynthetic response of E. canadensis in relation to concentration varied from day to day. At all temperature levels, the inhibitory effects of different concentrations of terbutryne were only partially expressed on the first day of terbutryne treatment, possibly due to the short light period that followed application. As compared with control, the rates of photosynthesis were also higher in some treatments at some temperatures on the first day of treatment. The higher rates were possibly due to integration of rates of photosynthesis for four hours without terbutryne.

At all concentrations and temperatures the maximum inhibitory effects were expressed on the second day of treatment although, at temperatures $10^{o}C$ and below, inhibition continued on the third day.

In general plants started recovering from photosynthetic inhibitory effects from the third day of treatment. Recovery varied with concentration and temperature being higher at lower concentrations and temperatures.

Dark respiration:

The decrease in dark respiration was due to an indirect effect of low accumulation of photosynthetic assimilates in the plants as a consequence of photosynthetic inhibition caused by terbutryne.

Terbutryne concentration, temperature and light had

the same effect on dark respiration as they had on photosynthesis. At a given temperature dark respiration decreased more with increasing concentration, and at a given concentration it decreased more with rising temperature. Decrease in dark respiration was greatly influenced by the interaction between terbutryne concentration and temperature.

The respiratory response of E. canadensis in relation to concentration and temperature varied from day to day. Up to a temperature of 25°C, the rates of dark respiration, in the treatments as compared to controls, were higher on the first day of treatment. The increase in dark respiration was possibly due to stimulation by terbutryne; and was higher at lower concentrations than at higher concentration. At temperatures 30° and 35°C, dark respiration decreased on the first day, possibly because of limitation of dissolved oxygen concentration in the medium as a consequence of higher photosynthetic inhibition.

At most concentrations and temperatures dark respiration decreased from the second day of treatment and it continued to decrease with time, although it did not stop completely during the experimental period.

Net photosynthesis (nP): Dark respiration (dR):

Terbutryne is a photosynthetic inhibitor, but respiration has also been found to be affected. Decrease in dark respiration following terbutryne treatment was probably due to the limitations of photosynthetic assimilates and dissolved oxygen concentration in the medium as a consequence of photosynthetic inhibition. Over

the temperature range 5° or 7° to 20°C the decrease in dark respiration was due to limitation of photosynthetic assimilates, and over the temperature range the decrease was more due to the limitation of dissolved oxygen concentration in the medium.

PHYSIOLOGICAL RESPONSE

It has been shown that:

Dry weight:

Dry weight of E. canadensis decreased at all terbutryne concentrations and temperatures. Decrease in dry weight was firstly, due to lack of photosynthetic assimilates as a consequence of inhibition, and secondly, due to using up of reserve carbohydrate through respiration. Although any relationship between decrease in dry weight and concentration was not apparent, the trend was to decrease more in weight with increasing concentration.

Chlorophyll concentration:

No significant change in chlorophyll concentration in the plants at any dose concentration and temperature occurred and no symptom of physical damage like chlorosis, necrosis or rotting etc. of the plants was visible during the short period of the experiments.

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APPENDIX I

Mean oxygen concentration (mg l^{-1}) at 09.00 and 17.00 hours in 0.00 (Control), 0.0125, 0.025 and 0.05 mg l^{-1} terbutryne treated plants at different temperatures, under natural light conditions.

Day	Concentration of terbutryne mg l^{-1}	Dissolved oxygen concentration mg l^{-1}															
		1979 Experiments								1980 Experiments							
		Temperature °C															
		5		10		15		20		20		25		30		35	
		Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour	
		09.00	17.00	09.00	17.00	09.00	17.00	09.00	17.00	09.00	17.00	09.00	17.00	09.00	17.00	09.00	17.00
-1	0.00 (Control)	7.37	10.53	5.07	12.88	3.69	10.81	9.67	16.24	1.75	9.41	4.40	16.24	1.90	14.66	2.30	13.86
	0.0125	7.55	10.74	5.75	12.99	3.22	10.37	9.76	16.55	1.67	9.50	3.61	16.08	1.33	14.17	1.04	13.86
	0.025	7.51	10.48	6.16	13.34	3.13	10.32	9.80	16.64	1.85	9.50	3.67	15.95	1.14	14.31	0.81	13.86
	0.05	7.49	10.54	6.03	13.15	3.34	10.42	9.49	16.29	2.04	9.52	3.69	16.24	1.14	14.32	0.88	13.86
1	0.00 (Control)	10.00	13.44	9.87	15.14	8.26	14.97	11.31	18.29	4.88	11.64	7.27	16.24	1.29	14.00	2.01	7.41
	0.0125	10.22	13.69	9.96	14.49	7.80	13.79	11.54	18.99	4.90	10.39	6.25	15.77	1.33	10.11	1.76	5.21
	0.025	9.99	12.95	10.18	14.32	7.60	13.21	11.54	17.31	4.95	9.14	6.07	15.22	1.64	8.43	1.63	3.95
	0.05	9.91	12.83	9.98	13.94	7.77	12.57	11.31	16.33	5.10	8.83	6.33	14.98	2.02	7.03	1.75	3.86
2	0.00 (Control)	10.91	13.78	11.79	14.41	11.20	16.34	12.33	19.53	6.59	16.38	6.68	16.24	1.20	11.67	1.16	12.23
	0.0125	11.25	13.01	11.16	12.19	10.22	11.30	12.25	13.49	5.53	8.52	4.58	7.37	0.75	2.69	0.74	3.52
	0.025	10.79	12.12	10.99	11.71	9.46	10.17	11.18	11.54	4.95	6.64	3.56	4.54	0.67	1.45	0.78	1.94
	0.05	10.53	11.93	10.42	11.27	8.92	9.29	11.05	10.78	4.59	5.46	3.19	2.70	0.69	1.05	0.92	1.54
3	0.00 (Control)	11.65	13.50	11.95	14.57	12.18	16.05	13.13	17.66	9.94	18.40	7.28	15.53	0.90	9.21	1,30	7.80
	0.0125	11.37	12.03	10.34	11.23	8.48	9.57	8.69	9.49	4.53	7.86	1.56	4.16	0.65	2.20	0.83	1.82
	0.025	10.68	11.00	10.01	10.60	7.50	8.24	7.45	7.89	3.66	5.93	1.01	2.09	0.63	1.35	0.90	1.45
	0.05	10.27	10.42	9.57	9.96	6.75	7.24	7.14	6.96	2.97	4.24	0.79	1.24	0.69	1.05	0.79	1.23
4	0.00 (Control)	11.65	14.58	11.57	13.42	11.89	15.51	11.09	18.89	12.43	20.00	4.54	14.37	1.03	12.27	1.13	6.62
	0.0125	10.82	12.36	9.02	10.05	7.15	8.46	5.76	8.78	4.28	7.37	1.01	3.63	0.82	3.35	0.86	1.83
	0.025	9.91	11.13	8.57	9.44	6.09	6.84	5.05	7.01	3.41	5.31	0.95	2.21	0.82	1.87	0.90	1.42
	0.05	9.32	10.36	7.96	8.75	5.28	5.84	4.48	5.72	2.59	3.59	0.90	1.46	0.82	1.33	0.85	1.20

APPENDIX I (contd.)

Mean oxygen concentration (mg l^{-1}) at 0.900 and 17.00 hour in 0.00 (Control), 0.0125, 0.025 and 0.05 mg l^{-1} terbutryne treated plants at different temperatures, under artificial light conditions.

Day	Concentration of terbutryne mg l^{-1}	Dissolved oxygen mg l^{-1}							
		Temperature °C 7		10		15		20	
		Hour 09.00	17.00	09.00	17.00	09.00	17.00	09.00	17.00
1	2	3	4	5	6	7	8	9	10
-1	0.00 (Control)	7.09	10.52	4.21	9.71	2.85	8.62	2.89	8.50
	0.0125	6.40	9.99	5.36	10.38	2.23	8.96	2.64	9.20
	0.025	6.71	10.24	4.51	9.60	2.65	9.48	2.99	8.80
	0.05	6.78	10.21	4.91	9.75	2.67	8.74	3.11	8.85
1	0.00 (Control)	8.97	11.19	7.72	11.16	6.11	10.39	5.44	10.05
	0.0125	8.39	10.76	8.60	11.87	6.06	10.34	5.72	10.34
	0.025	8.67	11.02	7.76	11.03	6.54	11.03	5.48	9.27
	0.05	8.57	10.49	8.32	10.73	6.13	9.51	5.56	8.69
2	0.00 (Control)	9.67	12.22	9.03	12.28	7.91	11.43	6.58	10.56
	0.0125	9.12	10.93	9.66	10.79	8.22	9.82	6.59	7.72
	0.025	9.42	10.85	8.75	9.60	8.21	9.23	5.80	6.60
	0.05	8.94	10.34	8.72	9.23	7.07	7.87	5.56	5.83
3	0.00 (Control)	9.92	11.93	9.93	13.12	8.63	11.53	6.76	10.27
	0.0125	8.77	10.12	9.00	10.39	7.06	8.17	4.81	6.11
	0.025	8.88	9.95	7.80	8.78	6.73	7.57	4.11	5.09
	0.05	8.39	9.54	7.65	8.36	5.64	6.28	3.72	4.25
4	0.00 (Control)	11.12	13.04	11.24	13.89	7.46	11.42	6.54	10.16
	0.0125	9.30	10.59	9.11	10.68	5.03	6.94	3.99	5.20
	0.025	9.26	10.24	7.51	8.88	4.96	6.43	3.46	4.43
	0.05	8.67	9.61	7.44	8.46	4.15	5.27	3.18	3.71

APPENDIX II

Dissolved oxygen conversion constants for BIL oxygen meter, Model No. 1520.

Temp. °C	C	Temp. °C	C	Temp. °C	C
0.0	0.1418	14.0	0.1000	28.0	0.0772
0.5	0.1397	14.5	0.0990	25.5	0.0766
1.0	0.1377	15.0	0.0979	29.0	0.0760
1.5	0.1357	15.5	0.0969	29.5	0.0754
2.0	0.1338	16.0	0.0960	30.0	0.0748
2.5	0.1319	16.5	0.0950	30.5	0.0742
3.0	0.1301	17.0	0.0941	31.0	0.0737
3.5	0.1284	17.5	0.0931	31.5	0.0731
4.0	0.1267	18.0	0.0922	32.0	0.0725
4.5	0.1250	18.5	0.0913	32.5	0.0720
5.0	0.1234	19.0	0.0905	33.0	0.0714
5.5	0.1218	19.5	0.0896	33.5	0.0709
6.0	0.1203	20.0	0.0388	34.0	0.0704
6.5	0.1188	20.5	0.0880	34.5	0.0699
7.0	0.1173	21.0	0.0872	35.0	0.0693
7.5	0.1159	21.5	0.0864		
8.0	0.1145	23.0	0.0856		
8.5	0.1131	22.5	0.0848		
9.0	0.1118	23.0	0.0841		
9.5	0.1105	23.5	0.0833		
10.0	0.1092	24.0	0.0826		
10.5	0.1080	24.5	0.0819		
11.0	0.1067	25.0	0.0812		
11.5	0.1056	25.5	0.0805		
12.0	0.1044	26.0	0.0798		
12.5	0.1033	26.5	0.0792		
13.0	0.1022	27.0	0.0785		
13.5	0.1011	27.5	0.0779		

APPENDIX III

Calculation of the rates of net photosynthesis and dark respiration of E. canadensis.

Net photosynthesis:

$$\frac{\Delta DO_2 \text{mg } l^{-1} \text{ (17.00-09.00) x 3 (volume of water in sweet jar)}}{\text{(Length of light period) 8 x Dry weight of plant.}}$$

worked example:-

(1) Dissolved oxygen concentration (mg l^{-1}) at

17.00h	13.44
09.00h	10.56

(2) Dry weight of plant 0.8929g

$$\therefore \text{ Rate of net photosynthesis} = \frac{(13.44-10.56) \times 3}{8 \times 0.8929}$$

$$= 1.21 \text{ mg}O_2/\text{gdw/h.}$$

Dark respiration:

$$\frac{\Delta DO_2 \text{mg } l^{-1} \text{ (17.00-09.00) x 3 (Volume of water in sweet jar)}}{\text{(Length of dark period) 16 x Dry weight of plant}}$$

worked example:-

(1) Dissolved oxygen concentration (mg l^{-1}) at

17.00h	13.44
09.00h	10.90

(2) Dry wight of plant 0.8929g

$$\therefore \text{ Rate of dark respiration} = \frac{(13.44-10.90) \times 3}{16 \times 0.8929}$$

$$= 0.53 \text{ mg}O_2/\text{gdw/h.}$$

APPENDIX IV

Calculation of coefficient of correlation (r) and the regression values. Calculated values are presented in Tables A, B and C.

Coefficient of correlation

r is given by the equation,

$$r = \frac{\sum dxdy}{\sqrt{(\sum d^2x . \sum d^2y)}}$$

where dx stands for $(x-\bar{x})$ and dy for $(y-\bar{y})$ i.e. deviation from the means.

worked example:

Temperature ^oC	Rates of net photosynthesis $mgO_2/gdw/h$			
x	y	x^2	y^2	xy
5	1.17	25	1.37	5.85
10	0.99	100	0.98	9.90
15	1.79	225	3.20	26.85
20	2.41	400	5.81	48.20
$\sum x$ 50	$\sum y$ 6.36	$\sum x^2$ 750	$\sum y^2$ 11.36	$\sum xy$ 90.80

n = 4 (number of observations)

$$\sum d^2x = 750 - \frac{(50)^2}{4} = 125$$

$$\sum d^2y = 11.36 - \frac{(6.36)^2}{4} = 1.25$$

$$\sum dxdy = 90.80 - \frac{(50 \times 6.36)}{4} = 11.30$$

$$\therefore r = \frac{11.30}{\sqrt{125 \times 1.25}} = \frac{11.30}{12.50} = 0.90$$

In the example, (n-1) = 3 degrees of freedom and when p = 0.05, r = 0.878. The calculated value of r exceeds this, so there is positive correlation between the

APPENDIX IV (contd.)

variables, significant at 5% level. Increase in net photosynthesis is correlated with the increase in temperature.

Regression value

For the regression of y on x is given by the equation

$y = \bar{y} + b\,(x-\bar{x})$ where b stands for the gradient of the line and is given by the equation, $b = \frac{\sum dxdy}{\sum d^2x}$.

worked example:-

Using data from pre page,

$\sum d^2x = 125$

$\sum d^2y = 1.25$

$\sum dxdy = 11.30$

$\bar{x} = 12.50$

$\bar{y} = 1.59$

$$b = \frac{\sum dxdy}{\sum d^2x} = \frac{11.30}{125} = 0.09$$

substituting the value of x, $\bar{x}$, y, $\bar{y}$ and b in the equation for the regression of y on x,

$\therefore \quad y = 1.59 + 0.09\,(5-12.5)$

$= 1.59 + 0.45 - 1.12$

$\therefore \quad y = 0.92.$

Table A. The regression values and the coefficient of correlation (r) in net photosynthesis of *E. canadensis* treated at different concentrations of terbutryne and temperatures, under natural and artificial light conditions.

Concentration of terbutryne mg l^{-1}	Temp. °C	Natural light conditions			Artificial light conditions		
		Actual values	Regression values	Coefficient of correlation (r)	Actual values	Regression values	Coefficient of correlation (r)
0.00 (Control)	5/7	1.17	0.92	0.90 S*	0.77	0.82	1.00 S
	10	0.99	1.37		0.97	0.94	
	15	1.79	1.82		1.16	1.14	
	20	2.41	2.27		1.33	1.34	
	20	2.01	2.10				
	25	1.91	1.95				
	30	2.22	1.80				
	35	1.38	1.65	-0.59 NS**			
0.0125	5/7	0.79	0.68	0.85 NS	0.65	0.67	0.81 NS
	10	0.68	0.83		0.62	0.70	
	15	0.90	0.98		0.88	0.75	
	20	1.24	1.13		0.77	0.80	
	20	1.25	1.28				
	25	1.08	1.03				
	30	0.87	0.78				
	35	0.47	0.53	-0.97 S			
0.025	5/7	0.69	0.66	0.70 NS	0.58	0.55	0.97 S
	10	0.58	0.71		0.54	0.58	
	15	0.84	0.76		0.65	0.62	
	20	0.86	0.81		0.68	0.67	
	20	0.90	0.90				
	25	0.73	0.70				
	30	0.53	0.50				
	35	0.26	0.30	-0.98 S			
0.05	5/7	0.64	0.62	0.07 NS	0.57	0.50	-0.47 NS
	10	0.58	0.62		0.37	0.48	
	15	0.67	0.63		0.48	0.45	
	20	0.62	0.63		0.42	0.42	
	20	0.60	0.57				
	25	0.53	0.47				
	30	0.37	0.37				
	35	0.21	0.27	-0.99 S			

* Significant $p < 0.05$

Table B. The regression values and the coefficients of correlation (r) in dark respiration of E. canadensis treated at different concentrations of terbutryne and temperatures, under natural and artificial light conditions.

Concentration of terbutryne mg l^{-1}	Temp. °C	Natural light conditions			Artificial light conditions		
		Actual values	Regression values	Coefficient of correlation (r)	Actual values	Regression values	Coefficient of correlation (r)
0.00 (Control)	5/7	0.46	0.33	0.95 S	0.27	0.25	1.00 S
	10	0.47	0.58		0.33	0.34	
	15	0.75	0.83		0.50	0.49	
	20	1.14	1.08		0.63	0.64	
	20	0.72	0.87				
	25	1.02	0.89				
	30	1.10	0.92				
	35	0.78	0.94	0.18 NS			
0.0125	5/7	0.38	0.30	0.93 S	0.29	0.29	1.00 S
	10	0.45	0.50		0.29	0.35	
	15	0.56	0.70		0.53	0.45	
	20	1.01	0.90		0.54	0.55	
	20	0.70	0.80				
	25	0.83	0.65				
	30	0.50	0.50				
	35	0.31	0.35	-0.83 NS			
0.025	5/7	0.38	0.34	0.99 S	0.29	0.27	1.14 S
	10	0.43	0.49		0.30	0.33	
	15	0.61	0.64		0.44	0.43	
	20	0.87	0.79		0.53	0.53	
	20	0.58	0.58				
	25	0.63	0.48				
	30	0.34	0.38				
	35	0.18	0.28	-0.88 S			
0.05	5/7	0.40	0.34	0.94 S	0.31	0.27	0.71 NS
	10	0.48	0.49		0.25	0.30	
	15	0.58	0.64		0.37	0.35	
	20	0.81	0.79		0.41	0.40	
	20	0.49	0.52				
	25	0.54	0.42				
	30	0.28	0.32				
	35	0.16	0.22	-0.93 S			

Table C. The regression values and the coefficient of correlation (r) in net photosynthesis (nP): dark respiration (dR) of E. canadensis treated at di fferent concentrations of terbutryne and temperatures, under natural and artificial light conditions.

Concentration of terbutryne mg l^{-1}	Temp. °C	Natural light conditions			Artificial light conditions		
		Actual values	Regression values	Coefficient of correlation (r)	Actu al valu es	Regression values	Coefficient of correlation (r)
0.00 (Control)	5/7	2.54	2.44	-0.62 NS	2.8 5	2.97	-0.95 S
	10	2.11	2.34		2.7 4	2.76	
	15	2.39	2.24		2.4 2	2.41	
	20	2.11	2.14		2.0 1	2.06	
	20	2.79	2.48				
	25	1.87	2.23				
	30	2.02	1.98				
	35	1.77	1.73	-0.81 NS			
0.0125	5/7	2.08	1.98	-0.90 S	2.2 4	2.28	-0.98 S
	10	1.51	1.73		2.1 4	2.07	
	15	1.61	1.48		1.6 6	1.72	
	20	1.23	1.23		1.4 2	1.37	
	20	1.78	1.62				
	25	1.30	1.59				
	30	1.74	1.56				
	35	1.52	1.53	-0.18 NS			
0.025	5/7	1.81	1.75	-0.93 S	2.0 0	1.94	-0.99 S
	10	1.35	1.75		1.8 0	1.79	
	15	1.38	1.25		1.4 8	1.54	
	20	0.99	1.00		1.2 8	1.29	
	20	1.55	1.46				
	25	1.16	1.43				
	30	1.56	1.40				
	35	1.44	1.37	-0.23 NS			
0.05	5/7	1.60	1.55	-0.97 S	1.8 4	1.79	-0.99 S
	10	1.21	1.30		1.5 2	1.52	
	15	1.15	1.05		1.3 0	1.31	
	20	0.76	0.80		1.0 5	1.01	
	20	1.22	1.13				
	25	0.98	1.18				
	30	1.32	1.23				
	35	1.31	1.28	0.51 NS			

APPENDIX V

Results of the analysis of variance (*significant at $p \geqslant 0.05$).

Table A

Natural light conditions

(1) Net photosynthesis.

DAY 1

Source of variance *Units *stratum	Degrees of Freedom	Sum of squares	Sum of squares %	Mean squares	Variance ratio
Dose	3	3.33435	7.30	1.11145	35.347 S*
Temperature	6	34.88501	76.42	5.81417	184.904 S
Dose x temperature	18	4:78611	10.49	0.26589	8.456

DAY 2

Dose	3	50.28456	79.54	16.76152	1409.519 S
Temperature	6	1.50030	2.37	0.25005	21.027 S
Dose x Temperature	18	10.43250	16.50	0.57958	48.739 S

DAY 3

Dose	3	27.58427	83.72	9.19475	1006.170 S
Temperature	6	1.57482	4.78	0.26247	28.722 S
Dose x temperature	18	3.02112	9.17	0.16784	18.367 S

DAY 4

Dose	3	33.00621	61.22	11.00207	623.148 S
Temperature	6	10.17636	18.88	1.69606	96.063 S
Dose x temperature	18	9.24721	17.15	0.51373	29.097 S

APPENDIX V (contd.)

Table B

Natural light conditions

(2) Dark respiration.

Source of variable *Units *stratum	Degrees of freedom	Sum of squares	Sum of squares %	Mean square	Variable ratio
DAY 1					
Dose	3	0.08542	0.67	0.02847	3.973 S
Temperature	6	10.45521	82.17	1.74253	243.155 S
Dose x Temperature	18	1.58096	12.43	0.08783	12.256 S
DAY 2					
Dose	3	4.34962	36.44	1.44987	337.508 S
Temperature	6	4.22682	35.41	0.70447	163.989 S
Dose x Temperature	18	2.99829	25.12	0.16657	38.775 S
DAY 3					
Dose	3	5.26328	48.69	1.75443	918.778 S
Temperature	6	3.28028	30.34	0.54671	286.309 S
Dose x Temperature	18	2.10660	19.49	0.11703	61.289 S

APPENDIX V (contd.)

Table C

Artificial light conditions

(1) Net photosynthesis.

Source of variance *Units *stratum	Degrees of freedom	Sum of squares	Sum of squares %	Mean squares	Variance ratio
DAY 1					
Dose	3	1.47730	18.75	0.49243	24.885 S
Temperature	3	4.92137	62.45	1.64046	82.902 S
Dose x Temperature	9	0.53224	6.75	0.05914	2.989 S
DAY 2					
Dose	3	6.30916	78.13	2.10305	360.299 S
Temperature	3	0.43009	5.33	0.14336	24.561 S
Dose x Temperature	9	1.05618	13.08	0.11735	20.105 S
DAY 3					
Dose	3	4.40690	80.77	1.46896	243.707 S
Temperature	3	0.12175	2.23	0.04058	6.733 S
Dose x Temperature	9	0.63801	11.69	0.07089	11.761 S
DAY 4					
Dose	3	4.17715	73.67	1.39238	346.383 S
Temperature	3	0.39481	6.96	0.13160	32.739 S
Dose x Temperature	9	0.90544	15.97	0.10060	25.027 S

APPENDIX V (contd.)

Table D

Artificial light conditions

(2) Dark respiration.

Source of variance *Units *stratum	Degree of Freedom	Sum of squares	Sum of squares %	Mean squares	Variance ratio
DAY 1					
Dose	3	0.08288	6.35	0.02762	21.442 S
Temperature	3	1.12681	86.27	0.37560	291.495 S
Dose x Temperature	9	0.03462	2.65	0.00384	2.986 S
DAY 2					
Dose	3	0.10105	14.91	0.03368	15.954 S
Temperature	3	0.38796	57.23	0.12932	61.248 S
Dose x Temperature	9	0.08756	12.92	0.00973	4.608 S
DAY 3					
Dose	3	0.38946	19.54	0.12982	56.089 S
Temperature	3	1.19778	60.10	0.39926	172.498 S
Dose x Temperature	9	0.29479	14.79	0.03275	14.152 S

APPENDIX VI

Mean light intensity (w/m^2) between 09.00 and 17.00 hours.

DAY	YEAR 1979				1980		
	MONTH July	August	September	October	April	May	June
1	-	635.22	263.11	-	-	479.00	-
2	-	306.55	434.77	-	-	-	-
3	-	388.11	230.44	435.11	-	-	-
4	-	480.88	432.44	309.00	-	-	-
5	-	555.66	476.88	135.11	-	-	511.55
6	-	-	-	280.22	-	361.88	488.66
7	-	-	-	248.77	-	204.33	429.44
8	-	-	-	279.33	-	344.88	365.11
9	-	-	-	-	-	663.88	503.55
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-
13	-	-	-	-	-	682.00	-
14	-	-	-	-	-	707.00	-
15	-	-	-	-	-	725.00	-
16	418.33	-	-	-	-	724.00	-
17	528.22	-	-	-	-	-	-
18	281.77	-	-	-	-	659.11	-
19	258.11	-	-	-	-	-	-
20	477.77	-	-	-	-	127.00	-
21	412.00	-	-	-	-	464.44	-
22	-	-	355.77	-	-	242.66	-
23	-	-	-	-	-	215.66	-
24	-	367.33	-	-	-	-	-
25	-	297.33	323.00	-	-	-	-
26	514.14	470.55	155.77	-	-	-	-
27	566.00	382.11	474.00	-	-	-	-
28	331.22	587.44	316.22	-	200.22	-	-
29	310.77	483.22	-	-	159.55	-	-
30	-	529.44	-	-	390.88	-	-
31	552.11	187.11	-	-	-	-	-