Plant Growth Regulators for Agricultural and Amenity Use

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Preface

In a practical sense, a plant growth regulator may be defined as any treatment which imposes a beneficial change upon plant growth or development. Thus the scope of the subject is extremely broad, even broader if, as some workers suggest, the science also encompasses compounds used as desiccants and defoliants, or includes some aspects of treatments more conventionally regarded as fertilizers.

This diversity poses a problem for the organisers of a conference on plant growth regulators; if the meeting sets out to represent the whole field, it may seem to lack any theme. Conscious of this problem, we chose not to be fully representative for the present meeting. Instead, we focused on areas which appear to be commanding particular attention at the moment, on new ideas which may have application in the future, and on the possible interactions between growth regulators and the emerging sciences of biotechnology. To set the meeting in context, we invited leading authorities from the public sector and from industry to give their personal views on the state of the science and its likely future.

This conference was a joint venture between the British Plant Growth Regulator Group, and the British Crop Protection Council. On behalf of the programme committee we would like to extend sincere thanks to the BCPC for all their work towards this meeting and indeed, for producing these proceedings.

July 1987

A. F. Hawkins
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ABBREVIATIONS

acid equivalent	a.e.
active ingredient	a.i.
aqueous concentrate	a.c.
boiling point	b.p.
British Standards Institution	BSI
centimetre(s)	cm
concentration	concn
concentration × time product	ct
concentration required to kill 50% of test organisms	LC50
correlation coefficient	*r*
cultivar	cv.
cultivars	cvs.
day(s)	d
days after treatment	DAT
degrees Celsius (centigrade)	°C
dose required to kill 50% of test organisms	LD50
dry matter	d.m.
Edition	Edn
Editor	Ed.
Editors	Eds
emulsifiable concentrate	e.c.
freezing point	f.p.
gas chromatography–mass spectrometry	g.c.m.s.
gas–liquid chromatography	g.l.c.
gram(s)	g
growth stage	GS
hectare(s)	ha
high performance (or pressure) liquid chromatography	h.p.l.c.
hour	h
infrared	i.r.
International Standardisation Organisation	ISO
Kelvin	K
kilogram(s)	kg
least significant difference	L.S.D.
litre(s)	litre
litres per hectare	l/ha
mass	*m*
mass per mass	*m/m*
mass per volume	*m/V*
mass spectrometry	m.s.
maximum	max.
melting point	m.p
metre(s)	m
milligram(s)	mg
millilitre(s)	ml
millimetre(s)	mm
minimum	min.
minute (time unit)	min
molar concentration	M

nuclear magnetic resonance	nmr
number average diameter	n.a.d.
number median diameter	n.m.d.
organic matter	o.m.
page	p.
pages	pp.
parts per million by volume	mg/l
parts per million by weight	mg/kg
pascal	Pa
percentage	%
post-emergence	post-em.
power take off	p.t.o.
pre-emergence	pre-em.
probability (statistical)	*P*
relative humidity	r.h.
revolutions per minute	rev/min
second (time unit)	s
standard error	S.E.
standard error of means	S.E.M.
soluble powder	s.p.
species (singular)	sp.
species (plural)	spp.
square metre	m^2
subspecies	ssp.
surface mean diameter	s.m.d.
suspension concentrate	s.c.
temperature	temp.
thin-layer chromatography	t.l.c.
tonne(s)	t
ultraviolet	u.v.
vapour pressure	v.p.
variety (wild plant use)	var.
volume	*V*
volume median diameter of drop spray	v.a.d.
weight	wt
weight by volume (mass by volume is more correct)	wt/*V* (*m/V*)
weight by weight (mass by mass is more correct)	wt/wt *(m/m)*
wettable powder	w.p.
approximately	≃
less than	<
more than	>
not less than	≮
not more than	≯
Multiplying symbols—	Prefixes
mega (× 10^6)	M
kilo (× 10^3)	k
milli (× 10^{-3})	m
micro (× 10^{-6})	μ
nano (× 10^{-9})	n
pico (× 10^{-12})	p

1.
Objectives and Perspectives

Chairman: Professor J. MOORBY

PLANT GROWTH REGULATOR RESEARCH OF THE AGRICULTURAL AND FOOD RESEARCH COUNCIL

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ABSTRACT

Understanding the regulatory role of plant growth substances (hormones) in growth and development, and notably in reproductive processes that underly yield formation in economically-important crops, has been a long-standing research goal of plant science. The 100 year history of the subject reveals much careful, but some careless, experimentation which has provided a bulk of descriptive knowledge with correlative evidence of function but a paucity of etablished causal information with respect to mechanism of action. Modern advances in analytical procedures and the development of biotechnologies that enable genes and gene products to be more precisely studied have led to the schools of research exploiting concepts of hormonal regulation of gene expression. The outlets for such knowledge are seen to be through genetic improvements that eventually may encompass 'engineering' or through development of chemicals that modulate hormone-related physiologicl/biochemical events in crops, both leading to improved production efficiency and regularity of yield of required quality.

The AFRC has in the past significantly supported plant hormone research in its Institutes and underpinned University Research in this area. Council recognised some 5 years ago the needs of this science for capital-expensive equipment and more co-ordinated activity than those previously isolated programmes, either relevant to some crop commodity interest or based on specialist skills in chemistry or plant physiology in various science departments. An inter-disciplinary approach was recognised as essential and a 'task-force' team effort envisaged as the way forward, especially if the considered interaction of function of the different hormone classes was to be effectively investigated. Such an AFRC hormone research centre was developed at Long Ashton but against a back-cloth of continuing erosion of funding. Bearing in mind the competition for reduced resources and conflicts of scientific interest, is advantage being taken of the new facilities provided, is co-ordination through improved communication amongst hormone research groups improving progress and is there an adequate critical mass of effort in the subject? Finally when might improved understanding of hormone function be expected to provide a scientific basis for selected gene transfer or indeed 'designed' chemical development? An attempt will be made to address these questions in terms of present AFRC policy and efforts both in house and in university groups.

INTRODUCTION

The potential for use of plant growth regulators has to be considered in the context of the present and anticipated climate for land use and agricultural production in the UK and Europe and in relation to world needs, developments and markets. The perspectives of UK and European agriculture have changed dramatically in the past decade. Contrary to all official predictions of the mid 1970s based on economic indicators and production statistics, by 1980 the UK had approached and even exceeded self-sufficiency in most of our major food commodities. The enormous costs of the Common Agricultural Policy with its production subsidies, guaranteed prices and intervention which provided increased production incentives have led to economically and socially unacceptable 'mountains' of grain, butter and meat in Europe. These have antagonised public opinion and fuelled the arguments against farming practices involving hedgerow removal, strawburning and environmental pollution through intensive use of agrochemicals necessary for high yields and efficient food production.

When the national priority was to increase agricultural production, the ability of public and private sector research and development agencies and the effectiveness of the farming industry to adopt new improved varieties and husbandry, significantly contributed to this objective. The achievement of the agricultural industry in eliminating food shortages and improving food quality, as well as the positive national benefits of employment and countryside management tend all-too easily to be ignored. Food surpluses are a result of the politico-economic framework in which the agricultural industry operates and are not caused by effective research and development.

The need to control agricultural production is obvious but there still is considerable research required to provide consistency of yield and higher nutritive value and its achievement more efficiently through reduced input costs and in ways that are environmentally more acceptable. Plant growth regulators have considerable potential to contribute to these aims.

STRATEGY AND FUNDING PROSPECTS OF THE AFRC IN PLANT GROWTH REGULATOR RESEARCH

Ahead of the present problems of excessive food surpluses, the Agricultural Research Council was already giving priority to work aimed at providing understanding of the causes of variation in crop yields between sites and from year to year having recognised that simply increasing production per unit area of land was a research target which successfully was being met.

Historically, all of the plant and soils Research Stations that comprised the Agricultural Research Service, had long-standing programmes on endogenous plant growth substances. Understandably, these growth regulation research activities, for the most part, related to the plant and crop commodity interests for which the respective institutes had primary responsibility. Research teams comprised mainly of plant physiologists tended to be small but they contributed significantly to knowledge of growth regulator function, occurrence in plant organs and response to application of extracted natural compounds and their synthetic analogues. Such was the state of the art that until some 10 years ago most research teams relied on bioassay-derived information with physico-chemical analysis confined to radiochemistry and gas-liquid chromatography. Much of the research was quantatively descriptive but, with few exceptions, was not mechanistic in its approach.

The rapid development of high performance liquid chromatography and need for combined gas chromatography-mass spectroscopy for unequivocal characterisation of the hormones and their metabolites was having a significant impact on research resources. Moreover, the dramatic technical advances in molecular biology provided new opportunities to study growth regulation and mechanisms of action of plant hormones at the gene level. To provide such multidisciplinary inputs to the study of plant growth regulators some consolidation of work porammes and scientific teams was required. These were the findings of a major review of AFRC-supported plant hormone work, conducted in early 1982, that led to the establishment at Long Ashton of a new Council-coordinated programme. To strengthen the chemical and biochemical expertise of the team, staff were transferred to LARS from Rothamsted and East Malling and new facilities and equipment were provided to the programme. These are available to other institute and university research groups requiring such resources but full advantage is not yet being taken. Alongside the basic research team a new study of the potential for growth regulator modulation of arable crop growth and yield formation was begun in 1982 with the prospect of forming a cohesive programme of basic and applied research with a view to providing a scientific basis for plant growth regulator use.

In a situation of budgets with strict cash limits, identification of resources further to strengthen the work into the necessary molecular biology and membrane biochemical skills was proving difficult. The changed Council strategy to increase research into improved food quality, increase its commitment to fund university research and create resources to enable new initiatives in basic biological sciences had already produced a scenario of changing funding emphasis affecting arable crop research. The scale of reduction intitially planned, of the order of £2.5M in annual expenditure, led to the decision to consolidate the highest priority research of Letcombe Laboratory and the Weed Research Organisation respectively to Rothamsted and Long Ashton. Thus additional skills in hormone physiology, molecular biology, regulation of ion flux and developmental botany were centralised at Long Ashton. Further retrenchment in government funding both in the science vote and in the Ministry of Agriculture, Fisheries and Food commissioned-research with the AFRC was announced in 1984 and are being implemented over the 1986 and 1987 budget periods.

The inevitable losses of research projects and scientific staff which resulted, proportionately have been considerably smaller in growth regulation activities than for the overall programme in plants and soils research. Indeed, through extending links with university research groups and developing collaborative work with industry and commerce there has been a small but progressive increase in AFRC institute-based plant growth regulator research. Funding of university research through AFRC grants progressively has been increased during the past 3 years of reducing institute budgets. Whilst some change in emphasis has occurred, new programme funds amounting to £560K for 3 years were added to existing project support in plant growth regulation research during 1986. Many of the new research grants emphasised collaboration with institute activities, contributing to scientific interaction and improving cost-effectiveness of the research effort.

OBJECTIVES OF PLANT GROWTH REGULATOR RESEARCH IN THE AFRC

The AFRC publishes annually the programmes of work of each of its institutes, identifying the current research objectives, the programme leader and main researcher activities. Reviews of progress are conducted by representatives of Council's Plants and Soils Research Committees. This Information System serves also to identify sponsorship of each Programme Unit, the subject area and main crop commodity relationship and are in part intended, as a summary of activities, to identify key points of reference within the AFRS to outside agencies. In providing this overview I have extracted only examples of research from the Institutes of Horticultural Research - East Malling Research Station (EMRS) and Arable Crops Research - Long Ashton Research Station to highlight major activities in basic work toward understanding endogenous plant hormone action that link with strategic and applied studies aimed at chemical manipulation of physiological processes underlying growth and yield attributes of annual arable crops and perennial fruit and ornamental subjects. The molecular biology expertise and objectives of programmes in growth regulation of the John Innes Institute and the Plant Breeding Institute are aimed at understanding the characteristics of hormonally regulated genes in plants and several collaborative projects exist with pgr programmes at LARS and other institutes.

Metabolism and mode of action of endogenous hormones

Combined genetic, chemical and molecular approaches are used to determine the function of plant hormones and to establish the relative importance of hormone concentration and tissue responsiveness in developmental processes. Whilst there still are gaps in knowledge of pathways both of bio-synthesis and metabolism of all classes of growth substances, effort at LARS, in collaboration with the Bristol University School of Chemistry, focuses on characterising the regulation of gibberellin (GA) turnover and the subcellular localisation of the metabolising enzymes (Hedden). Similar studies are conducted on auxins, cytokinins and ABA in cooperation with university groups.

Recent advances in immunocytochemical techniques should make possible the localisation of the key regulatory enzymes, if not the hormones themselves, and if successful with model systems in which high yields of GAs are obtainable will then be applied to receptor studies in systems such as wheat isogenic lines that are GA sensitive or insensitive. Studies by Hoad of structure-activity relations of GA analogues complement this approach by probing GA action in responsive tissues. Particular attention is given to photoaffinity probes for GA-binding proteins to isolate and characterise the primary receptor using oat aleurone protoplasts.

The properties of ethylene and auxin receptors are being characterised, with particular emphasis on flowering and fruit development in the IHR-EMRS and immunological procedures are being investigated for cytokinins in relation to root-stock effects. At LARS, developmental mutants and near-isogenic lines of plant species such as tomato and wheat are being characterised as either synthesis or sensitivity types. The dwarfing (Rht) wheat genes that reduce stem length, confer insensitivity to GAs, implying a lesion, possibly at a primary receptor site. In recent studies of Lenton (unpublished) marked differences in pool sizes of GA_1 in the upper internodes of Rht_3, compared with tall (rht), were observed but there was no difference in concentration of GA_{20}, the immediate precursor of GA_1. The Rht gene, associated with GA-insensitivity and increased GA_1 concentrations is, however, not expressed in the developing ear. Knowledge of these GA insensitivity genes has considerable economic importance since they have contributed to the dramatic increases in world wheat yield and have the potential for control of losses in yield and quality through preharvest sprouting. Attempts to examine the importance of hormone concentration by using specific biosynthesis inhibitors are referred to later.

In many systems hormones are used as modulators to study gene expression but the significance in relation to developmentally-regulated gene expression is not established. With the range of molecular techniques now available it is possible to determine whether or not hormones are affecting transcription, translation or post-translational events. Recent studies by Hooley (unpublished) of the expression of high and low pI -amylase genes of the wild oat aleurone reveal unusual differences between A. fatua in which only low pI -amylase proteins are synthesised and wheat and barley in which two related families of -amylase genes are coordinately expressed during germination. Complementary studies of genes controlling -amylase in wheat at the PBI use recombinant DNA techniques to characterise their structure and chromosomal organisation with effort on determining features of their regulatory sequences to elucidate hormonal control mechanisms. Isolation and characterisation of grain storage-protein genes of wheat and barley are related activities at the PBI and at Rothamsted.

Molecular and cellular approaches are also used at LARS to study pgr control of gene expression in developing and germinating legume seeds (Barratt) in collaboration with the JII. Production of mRNA at different stages in seed development is studied using in vitro translation, quantified by cDNA probes. Near-isogenic pea lines containing the r locus affecting starch production, protein quality and other attributes provide a system in which ABA increases legumin m RNA. With storage and secretory proteins it is conceivable that hormones may be influencing packaging and movement of the protein within the endomembrane system as well as having more direct effects on the genome. There is little doubt that hormones elicit rapid membrane responses and the involvement of non-hormonal secondary messengers such as calcium-calmodulin, in signal transduction following initial hormonal stimulus are being studied at LARS, EMRS and in several university groups. The roles of extracellular sequestered and cytosolic calcium are investigated as are the influences of applied pgrs on calcium fluxes into cells of roots and on cell membrane potentials.

Application of molecular cloning techniques are dependant upon efficient methods of plant transformation. Suitable vectors based on the Ti plasmid of Agrobacterium facilitate the insertion of new genes, these vectors containing various promoters enabling the expression of the new genes in particular ways. Insertions of genes involving infection of plant cells by A. tumefaciens and regeneration using callus culture techniques have profound effects on cytokinin and auxin metabolism and on plant development. Mild strains of specific viruses sometimes protect plants against infection by related virulent strains. Characterisation of viral genes and determination of their resultant effects on plants is examined in work at the PBI and virus-induced changes in pgr metabolism in control of growth and resistance is being characterised at the IHR-NVRS; similar relationships between fungal organisms and host plant pgr metabolism form part of the programme of study of cell recognition phenomena at LARS. These, together with gene micro-injection techniques, provide valuable tools for studies aimed at furthering understanding of hormone function, as well as being practical methods for genetic improvement programmes.

Mechanisms regulating growth and development

Plant development is an integration of genetically-determined events modified by environment. Changes in sensitivity of plant responses to environmental stimuli are exhibited during ontogeny and often involve endogenous hormone concentration differences which may reflect changes in metabolism or inter- and intra-cellular movement. Research approaches range from whole plants to isolated organs and tissue culture methods to examine hormonal control or modulation of differentiation, morphogenesis and key physiological processes such as carbon and nutrient assimilation, compartmentation and utilisation efficiency.

Sub-optimal water, nutrient, temperature and light environments slow growth and reduce the genetic-yield potential of crops. Mechanisms of injury or the ability to adapt physiologically are known to involve plant hormones and understanding these phenomena may provide opportunities for greater tolerance in particular species. Identification of regulatory steps in intermediary metabolism and morphogenesis of plants and notably those which are hormonally-regulated needs a greater resource availability particularly to target plant improvement either conventionally or through specific gene insertion techniques and to pinpoint opportunities for development of novel chemical regulators.

The research expertise in the AFRC institutes on intermediary metabolism together with the quality of equipment and facilities provide an ideal opportunity for club research with private industry to examine basic principles of growth regulation as an essential input to the rapidly-developing biotechnologies.

Chemical regulation of crop yield and quality

Exogenously-applied natural or synthetic analogues of plant hormones have for many years been examined with virtually all crop species in attempts to overcome physiological traits known, or thought likely, to be associated with non-realisation of genetic potential, irregularity of yield and quality characteristics such as size, colour and even flavour of the economic product. Such an approach has often been open to scientific criticism of 'spray and pray' but without this pragmatic evaluation the proven use of fruit-setting sprays of gibberellins and other mixtures, in top fruit and grapes and of hormone powders in rooting of vegetatively-propagated crops would not have been timely. In like fashion the pgr use essential to organ and tissue culture procedures would not now make possible the ability to regenerate plants transformed by chemical or genetic mutation as a critical stage in bioengineering. However, there is only a limited horizon for chemical application studies unless closely linked with biochemical and molecular genetic aspects.

Cereals, oil and protein crops

Plant growth retardant chemicals increasingly are being used in broad-acre crops to reduce lodging risk through strengthening and shortening the stem. Of the relatively few commercially available molecules, though in several formulations, chlormequat (CCC) is the most commonly applied and yield increases in the absence of lodging have been obtained with applications at early stem extension. These are related to increased tiller survival but associated with a greater number of smaller grains and thus the compound mimics the dwarfing Rht semi-dwarfing genes of wheat. Field-based studies of CCC response are linked with determination of chemical uptake and metabolism so often a missing element in pgr evaluation. At 3mM concentration, Lenton (unpublished) reports that CCC markedly reduced GA_1 concentration of Rht_3 (GA-insensitive) wheat without reducing shoot growth.

The newer growth retardants of the triazole type are considerably more effective than CCC at inhibiting growth of tall (rht) wheat but effects of both inhibitors are reversed by applied GA. Leaf GA concentration, manipulated by 2S,3S paclobutrazol, when increased by addition to the target GA-responsive meristematic tissue showed growth responses related linearly to $\log_{10}$ (GA_1), thus indicating the importance of hormone concentration in determining shoot height. Paclobutrazol applied to root and cell suspension cultures leads to accumulation of sterols and affects cell division and root growth that were not reversed by GA but were by e.g. stigmasterol. These pgr studies are closely associated at LARS with those of triazole fungicide mode of action. Triazole retardant treatments are being examined to improve winter survival of cereals (with seed treatment by tetcyclasis and on stem growth (experimental chemicals e.g. BAS 11100W) of spring barley, canopy and yield formation in grain legumes and oil-seed rape which will be discussed later in this symposium programme by Robin Child and Harry Anderson

Ethylene-releasing chemicals such as ethephon, applied to cereals prior to ear emergence, stiffen the peduncle and improve the proportion of large grains by an unknown mechanism. The retention of an upright posture of the ear may be an advantage in harvesting efficiency particularly with the new stripper-harvester development.

Responses of field crops to pgrs are disappointingly variable and close integration with basic research into hormone function is essential to place such use on a scientific basis. Such investigations on uptake, metabolism, transport of different formulations now form a cohesive programme at LARS linked with determination of responses in the endogenous hormone systems (Jackson, Butcher and Baker) and investigation of physiological status and environmental influences. Much of the core programme work is extended by collaboration with industry.

Fruit, ornamental crops, amenities and vegetation management

Pgrs play a significant role in several aspects of fruit plant culture. At EMRs physiological studies of vegetative propagation by cuttings of fruit and hardy ornamental nusery stock, which forms the basis of this industry, continue to be focused on hormonal rooting agents and understanding the mechanisms of response. Micropropagation of top and soft fruit cultivars is of practical importance but also explores the potential for producing improved somaclones, examining genetic transformation, protoplast regeneration and fusion techniques, all of which involve pgr study.

The distinctive pattern of flowering, fruit development and bud initiation, vegetative growth and cambial activity present a complexity of competition for resources in extent and time in perennial fruit trees. Together with environmental factors, particularly low temperature and water status, these intrinsic factors lead to much variability in fruit set and quality from year to year. With some cultivars good response to pgrs is obtained, whereas some of the commercially important UK cultivars such as Cox's Orange Pippin seem not as amenable to chemical control of cropping. The practical successes of paclobutrazol are described fully by Jim Quinlan in this Symposium. Whilst these studies are backed up by investigation with fruit subjects of hormone changes in tissues post-pgr application, there seems still a gap in the horticultural programme in studies of hormone turnover, localisation and in physiological processes such as regulation of nutrient and assimilate resources that are notoriously difficult in perennial tree species.

The controlled application of pgrs is an important aspect relevant to physiological efficiency and to economic and environmentally-acceptable practices both for orchard fruit and amenity tree plantings extending to agroforestry, the latter being an alternative land-use strategy being given serious consideration. This should encourage links between the AFRC and NERC and the Forestry Commission.

In the event of land being taken out of cereal production, there are few arable alternatives excepting protein crops, such as pea and field bean, and possibly linseed that are either already in European surplus or for which presently available cultivars are not suited to UK conditions. If production control is effected by a set-aside policy or even were the price structure such as to force particular land types out of cereal production, it is inconceivable that such land should not continue to be managed. Agroforestry, with low density planting, may be one option for paticular land with grass undersown or simply swards planted for 2 or 3 years in a rotation is yet another. Tree and sward management have numerous unsolved problems in the manipulation of productivity or for amenity purposes. Plant growth regulators, such as mefluidide, to retard growth and flowering whilst maintaining species diversity in amenity plantings, such as mefluidide that will be reported later in this Symposium, have shown promise. A greater number of pgrs is, however, necessary with a range of persistency to provide choice in management strategies.

CONCLUSION

A significant body of pgr research is conducted in institutes and also receives AFRC support through university funding. There are good opportunities to extend further partnership research with the agrochemical industry both in the more applied aspects of chemical growth regulation and in work at the pre-competitive level aimed at basic understanding of hormone action and gene expression in plants. Better cohesiveness in multidisciplinary inputs to provide a continuance of basic and applied work is now developing but the various work programmes of the AFRC still have inadequate effort in molecular sciences and membrane studies devoted to pgr action. There are opportunities to exploit these available skills in the AFRC through greater coordination of research effort between institutes.

PROSPECTS FOR CHEMICAL PLANT GROWTH REGULATION: AN INDUSTRIAL VIEW POINT

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ABSTRACT

Although the Plant Growth Regulator (PGR) market has fallen short of the optimistic forecasts made in the seventies, it is growing faster than the total chemical plant protection market. Predictions are that it will increase from the present 500 million $ at the producers level to about 800-1000 million $ in the early nineties. This volume would then represent about 7% of total pesticide sales. Whilst the present range of commercial PGRs covers a wide spectrum of physiological effects, the focus is on growth retardation. To make PGR research more productive, the following major impediments/constraints must be recognized and overcome: a) the inherent conceptual complexity of this area as compared to conventional pesticide research; b) the inadequacy of whole-plant screening systems caused by technical limitations and the historical perception of herbicide testing, c) conventional and simplistic thinking in the approach to demanding objectives such as overcoming stress or increasing yield, and d) the lack of physiological and biochemical information which is relevant to specific target mechanisms and/or agronomic traits. Measures to alleviate the above constraints are discussed.

INTRODUCTION

Though the plant growth regulator (PGR) market is constantly increasing in size and diversity, it has, so far, fallen short of widespread hopes, promises and expectations. In our evaluation we shall briefly analyze the development and composition of the market and attempt to formulate appropriate future objectives within the anticipated environmental contraints. The focus of our discussion will be on present impediments in research and on ways of overcoming them.

DEVELOPMENT AND COMPOSITION OF PRESENT MARKET

Though the different information sources we consulted were partially conflicting and rather imprecise, the order of magnitude of the market figures presented in Table I should be sufficiently correct to allow some interpretation. In 1975 the PGR-market (including defoliants and desiccants) was not much more than about 200 mio. U.S. $ at the producers level. By 1985, the figure had risen to somewhat more than 500 mio. $ and is optimistically predicted to reach approximately one billion $ in the early nineties. During the past ten years, the PGR-market average growth rate was slightly above that of the total chemical plant protection market. However, medium term it is likely to stay below the 10% threshold in terms of total plant protection expenditures. Exaggerated expectations, voiced in the early seventies, have thus not materialized and breakthroughs of significant economic importance are not yet seen on the horizon.

TABLE I

Actual and anticipated development of the plant growth regulator (PGR) market as compared to the total chemical plant protection (PP) market.
(Lürssen 1981, Hewin 1985, BMI 1986).

expressed in billion US$ at producers´ level

Year	PP	PGR	PGR %
1975	6	0.20	3.3
1980	10	0.35	3.5
1985	12.5	0.50	4.5
1990	14	0.90	6.5

In spite of reduced and more realistic expectations, industrial research in PGRs seems to continue in most major companies. However, the corresponding resources are carefully balanced against those allocated to defending or improving their respective positions in the more sizeable weed, insect and disease control markets.

A short-hand version of the present PGR-market in terms of major crops, physiological effects achieved, relative market size and chemicals employed is provided in Table II. Without going into undue detail, we believe that this table provides three conclusions, which will also be relevant in the future:

- only in large-acreage crops, such as small grain cereals, rice, cotton, or in high-value crops, such as tobacco and certain fruits and vegetables, is the existing and/or potential market size sufficient to justify above average R&D expenses.

- the emphasis of the present range of commercial and developmental PGRs is on growth inhibition or retardation. This is not surprising, as the search for them in terms of conceptual and technical approaches is quite close to that for conventional herbicides. Medium term, the focus on growth retardants is likely to continue.

- a limited number of compounds, the most prominent being the ethylene-releaser ethephon have been established in more than one crop. Whilst this is another way to achieve adequate market size, the particular case history of ethephon also reflects the importance of government and trade laboratories or universities in systematically picking-up candidate PGRs and testing them for their effects and usefulness in those crops in which these institutions are specialized.

TABLE II

Composition of present plant growth regulator (PGR) market in terms of major crops (crop groups), physiological effects, approximate market size (++++ = >150, +++ = 100-150, ++ = 50-100, + = <50 mio. U.S. $) and main chemical products.

Crop	main physiological effect	market size	main products
Small grain cereals & rice	growth inhibition lodging prevention	++++	Chlormequat, Ethephon, Mepiquat
Cotton	desiccation/defoliation growth inhibition fruit set	+++	Mepiquat, Ethephon, Thidiazuron, Dimethipin, Def Defoliant
Tobacco	growth inhibition, sucker control	++	Maleic hydrazide, Flumethralin, fatty alcohols
Sugarcane	metabolic effects, ripening	+	Glyphosine, Glyphosate
Turf	growth inhibition	+	Maleic hydrazide, Mefluidide, Amidochlor, Paclobutrazol
Deciduous trees	growth inhib. (pruning) fruit set, thinning	++	GA_3, Naphtylacet. acid, Daminozide, Ethephon
Rubber trees	latex flow, yield increase	+	Ethephon
Grapes	yield increase, metabolic effects	+	GA_3
Vegetables	storage, shoot prevention	++	Maleic hydrazide, Carbamates

FUTURE OBJECTIVES

Apart from the scientific/technical feasibility, which will be discussed later, the choice of ongoing or future objectives in PGR-research must take into account the following three criteria, which, basically, also apply to plant protection agents:

- the economic value of the envisaged PGR-treatment must be quantifiable and be perceived and recognized as such by the farmer.

- it must be ecologically sound in terms of rate of application and selectivity vis-à-vis non-target organisms; and it must, where possible, make a contribution to those crop production techniques which are meant to alleviate pressure on natural resources. PGRs used in vegetation management or in mixed cropping in order to reduce soil erosion would exemplify this approach.

- the potential market value of a candidate PGR in one or several crops must be sufficient to justify high R&D expenses, which are likely to be in excess of 50 mio $ per compound (excluding investments).

With these criteria in mind, we believe that the following five PGR-objectives continue to be economically and ecologically viable:

- growth inhibition/retardation
- yield enhancement (fruit, seed)
- stress tolerance (drought, coldness, etc.)
- plant composition; terminal metabolites
- flowering, sexual expression

IMPEDIMENTS TO BE OVERCOME

Except for the area of growth inhibition/retardation, it is fair to say that, in spite of considerable efforts made by the agrochemical industry, PGR-research has, so far, not been overly successful. What are the impediments or hurdles which have prevented success? We wish to offer four for discussion:

- the inherent conceptual and scientific complexity of PGR-research when compared to the activities involved in the search for conventional plant protection agents, such as herbicides.
- the inadequacy of empirical whole plant screening systems, imposed by a number of technical limitations and also by historical perception of screening criteria.
- the present over-reliance on conventional and simplistic thinking in approaching such demanding PGR-objectives as yield enhancement or overcoming plant stress.
- the continuing lack of physiological and especially biochemical and molecular information which is relevant to specific potential target mechanisms and/or agronomically important traits.

To cope with the first three of these impediments, we have systematically analyzed the scientific/technical differences between herbicide and PGR research approaches in the areas of whole plant screening, field testing and basic research support. A summary of this analysis is presented in Table III.

TABLE III

Differences between herbicide and PGR research and development in terms of empirical whole plant screening, field testing and basic research support activities.

		Herbicides	PGRs
Objective		to kill	to regulate
Whole Plant Screen	Effects	immediat. visible	only part. visible
	Dose response	simple	complex
	Plant spectrum	establ. indicators	question. indicat.
	Duration	short	long
	Facilities	easily controlled	complicated
Field Testing	Screen input	reliable	unreliable
	Quantification	effort low	effort high
	Climatic factors	not very critical	highly critical
Basic Research	Molecular targets	many identified simple inhibition	few identified modulation
	Second.metabolic effects	unimportant	important

Keeping in mind that the basic objective for herbicides is to kill weed plants, whereas that for PGRs is to regulate crop growth, the table demonstrates the following major difficulties in PGR-research when compared to the usual herbicide approaches:

- in whole plant screening systems, the envisaged morphological effects are only partially visible, the dose response curve is often bell-shaped rather than linear; the selection and use of suitable indicator plants is questionable; the duration of experiments until the anticipated effects are observed is long and the facilities required to secure acceptable reproducibility are complicated and costly.

- in field testing, the screening input is normally unreliable, the effort required to achieve adequate quantification of results in terms of number, size and geographical distribution of plots is high and the prevailing climatic or meteorological conditions during the testing period are very critical.

- at the basic research support level, where the PGR objective is not simply to inhibit, but to modulate molecular targets, we find that few such targets have so far been adequately identified. In addition we must recognize that whilst for herbicides, secondary metabolic effects induced by target modulation are unimportant, they are normally a key constraint in the attempted regulation of crop growth.

In looking at the series of difficulties encountered in whole plant screening systems, we conclude that the chances of overcoming all of them in a satisfactory way are practically nil. Therefore, and except for plant inhibition or retardation, this way of discovering and developing compounds for the above mentioned PGR objectives is of limited use. We believe that the alternative or biorational approach will be more productive and successful. (Geissbuehler *et al.* 1983). In this approach, primary screening and optimization of identified lead structures to achieve adequate intrinsic activity is conducted on physiological, biochemical or even molecular model systems, depending on the objective pursued and/or the scientific information available. This approach also allows for the exploitation of modern molecular modelling procedures. In addition, the subsequent phase of working back to the whole plant and eventually to field testing can be designed more rationally and systematically and in tune with the acquired technical information.

With this concept in mind, we now turn to the fourth impediment, i.e. the continuing lack of physiological and biochemical information which is relevant to PGR-research. In Table IV we have attempted to summarize the availability of biochemical information on a number of potential PGR-target sites in five major areas of plant growth and development: plant hormones, regulatory plant systems, assimilate transportation and distribution, storage products, and resistance to stress. The table demonstrates that, whilst in most areas, we have quite extensive knowledge on the identity of the molecules involved in these processes and on their biosynthetic pathways, our information on the mechanisms of action at the molecular level remains seriously deficient.

TABLE IV

Availability of biochemical information on potential targets for biorational approaches to plant growth regulation (+ = sufficient information; - = insufficient or no information). Last column lists commercial or developmental plant growth regulators which appear to interfere with corresponding target mechanisms. (Geissbuehler *et al.* 1987).

Potential Targets	Involved molecules	Biosyn- thesis	Molecular mechanism	Commercial/ exptl. PGRs
Hormones				
Gibberellins	+	+	-	+
Auxins	+	+	-	+
Abscisic acid	+	+	-	(+)
Ethylene	+	+	-	+
Cytokinins	+	(+)	-	(+)
Regulatory syst.				
Calmodulin	+	(+)	-	+
Phytochrome	+	+	-	-
Assimilate Trsp.				
source	+	+	+	-
transport	(+)		-	-
sink	(+)	(+)	-	(+)
Storage products	(+)	+	-	(+)
Stress resistance	(-)	-	-	-

This leaves us with three options for pursuing biorational approaches:

- to interfere more systematically and extensively with the biosynthetic pathway of any of the listed growth components.

- to investigate in much more detail than previously done, the mode of action of the current commercial and developmental PGRs in order to identify and describe suitable targets and their relevance in modifying agricultural traits. The last column in Table IV indicates the availability of such compounds when classified according to their apparent interference with the listed target mechanisms.

- to generate much more extensive information on the mechanism of action at the biochemical and molecular levels of the listed natural growth and development components. Molecular plant biology is indeed doing this by providing a continuous flow of new information in several areas, including the relationships between gene expression and hormonal regulation. However, more objective-oriented and extended contributions, both by academic institutions and industry, are required to translate such information into useful model systems.

Whichever option is chosen, we believe that, at the start, we should not unduly focus on any particular practical objective, but rather search for strong intrinsic activity in a key growth-regulating mechanism and then explore it further at the physiological and eventually whole plant level.

We recognize that biotechnology offers alternative genetic options for achieving the same basic objectives as have been listed before for chemical PGRs, especially if the envisaged target mechanisms involve the regulation and expression of specific genes. However, we believe that the availability of these options does not reduce the desirability nor the chances of chemical solutions. Indeed, we think that by selecting and investigating models of certain key mechanisms, and in tune with the information becoming available, the choice between chemical and/or genetic solutions can be rationalized.

CONCLUSIONS

Whilst we have briefly touched on the future size and composition of the PGR-market and on the selection of appropriate objectives, the emphasis of our discussion was on overcoming present impediments in research. We believe that by focusing on the biochemical and molecular aspects of potential target mechanisms, the search for PGRs can be made more productive and successful.

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2.
Field Crops

Chairman: Dr D. S. H. DRENNAN

CONTROL OF CANOPY STRUCTURE IN OILSEED RAPE WITH GROWTH RETARDANTS AND CONSEQUENCES FOR YIELD

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ABSTRACT

Canopy height and its structure in oilseed rape were altered by application of the experimental triazole growth retardants BAS 11100W and triapenthenol. Treatments at the beginning of stem extension resulted in shorter stems and increased lateral bud outgrowth which led to greater axillary shoot leaf area and more flowers. This treatment increased branching and canopy density during pod development.

Treatment at the end of the stem extension period shortened branches and reduced petal size and colour. The canopy was more open and the pod layer shallower. Measurement of vertical light profiles indicated increased light penetration to the lower parts in the crop.

When the incidence of light leaf spot (_Pyrenopeziza brassicae_) was prevalent, yields increased following all times of application of these chemicals as a consequence of their fungicidal properties. When disease incidence was small, yields were still increased by treatments given at the end of stem extension as a consequence of their growth regulating activity.

INTRODUCTION

The morphology of oilseed rape (_Brassica napus_ L.) plants derives from those characters of the primitive species which evolved as a result of successful competition and survival in mixed populations of varying density. Modern cultivars form a rosette of leaves which ensure early, efficient light interception and storage of assimilates prior to rapid upward growth of the stem. Continued leaf production during stem extension results in maximum leaf area index just before flowering. Several axillary buds at the top of the stem grow out to form long branches. Their extension is complete by the end of the three - four week flowering period when plant height may vary from 1 - 2 m depending on cultivar.

Canopy structure in oilseed rape is determined by the interaction of plant population with husbandry factors and varietal characteristics which influence the number and length of branches, size of leaves and length of the stem. Although light profiles may change with canopy structure, seed yields do not vary greatly because of compensation in pod and seed numbers (Scarisbrick _et al._, 1982b). For example, only small differences in seed yield per ha were obtained by Helps (1971) in crops, with 30-200 established plants per m^2. Daniels _et al._ (1982) have calculated from the relationship between solar radiation and total dry matter production that the potential yield of winter oilseed rape is over 7 t/ha. Occasionally, yields recorded from small hand-harvested plots approach this value but in field experiments the range is usually 2-4 t/ha.

Year to year fluctuations in the yield of winter oilseed rape have been related to variations in weather and to time of sowing both of which limit the amount of radiation intercepted. Early (September) sowings produce bigger plants prior to regrowth in the spring and small fluctuation in yield. Crops sown at this time produce dense canopies during flowering and pod development; much pod abortion occurs after set and mutual shading prevents development of pods low in the canopy. Later sowing provides less time for establishment and greater chance of crop failure but can give exceptionally heavy yields. For example, the highest yield during the period 1970-77 was obtained from the cv. 'Victor' sown at low density on 3 November, 1976. The features of this exceptional crop were prolonged photosynthetic activity of the leaves during flowering and high survival rate of pods and seeds (Mendham et al., 1981).

Adverse weather conditions, which damage over-wintered leaves and delay regrowth in the spring may shorten the period of photo-assimilation prior to flowering. The importance of assimilates produced at this time has been demonstrated by Evans (1984). Shading and defoliation during early stem extension reduced pod set, seed number and weight, suggesting that reserves of carbohydrates might have an important role in determining yield.

Chapman et al. (1984) found that at the time of early flowering, leaves are the main producers of assimilate exported to developing organs. Towards the end of flowering the demand for assimilates becomes acute and the mass of yellow flowers reduces radiation interception by the lower pods and leaves. Mendham et al. (1981) have shown that flowers reflect or absorb 60% of incident light at this time. This is probably an important cause of senescence in the lower part of the canopy and although leaves near the top of the stem remain green during pod development, they have been shown to contribute little to seed growth. Brar and Thies (1977) have estimated that 30% of assimilated ^{14}C delivered to the reproductive organs came from stems and branches. The remainder was believed to derive from the activity of the pods themselves. Canopy structure during seed development and its effect on light interception by pods therefore plays an important role in determining yield. There is much evidence that canopy density at this time determines which pods survive and contribute to seed yield.

For example, Mendham and Scott (1975) found that upper and middle portions of the branches in the winter oilseed rape cv. 'Victor' were the most productive in crops of approximately 50 plants per m^2. Clarke (1979) found that lower sections of the canopy were more productive in less dense crops.

The features of crops which may limit light interception and therefore yield, would appear to be (i) the early "lag phase" at the beginning of stem extension which may reduce leaf area, (ii) shading of the leaves by the dense inflorescence and (iii) inability of low-placed pods to intercept sufficient light for grain filling due to shading by the upper parts of the canopy.

Several growth retardants have been reported to shorten stems and crop height of oilseed rape. Application times seem to have been based on experience with cereals and little attention has been paid to the timing of treatments which might improve light interception.

Scarisbrick et al. (1982a) found a slight reduction in crop height following treatment with chlormequat at the beginning of stem extension in the spring had no effect on seed yield. Child (1984) investigated the duration of effect of retardants applied at differing times during stem extension and used pot-grown plants of cv. 'Jet Neuf' to identify effects on leaf areas, stem and branch length. Chlormequat applied at early stem extension reduced stem length temporarily but later, growth was more vigorous and final plant heights were similar to the unsprayed plants. Treatment at late stem extension restricted branch growth during flowering. Daniels et al. (1984) suggested that autumn applications of the experimental triazole retardants, paclobutrazol and R201, affected apical development and changed branch hierachy. Yield increased followed these treatments in 1981 and 1982 but subsequent trials failed to confirm these results. Child et al. (1985) reported yield increases in the cv. 'Jet Neuf' after treatment during stem extension with the experimental triazole retardants BAS 11100W (supplied by BASF) and triapenthenol (supplied by Bayer).

The objectives of the work discussed in this paper were to investigate the effects of retardants applied during stem extension on (i) plant and crop structure and final yield; and (ii) on light profiles in the crop canopy that might result from changes in the shoot system and affect yield beneficially.

MATERIALS AND METHODS

Experiments with pot-grown plants BAS11100W at 3.3 l/250 l water was applied to run-off to pot-grown plants of cvs. 'Bienvenu', 'Rafal' and 'Jet Neuf' when the plants were approximately 30 cm in high: on 9 April, 12 April and 16 April respectively.

Triapenthenol at 700 g/250 l water was applied to run-off to pot-grown plants of 'Jet Neuf' when the plants were approximately (i) 5-10 cm high on 28 March, or (ii) 30 cm high, on 16 April or (iii) when branches were beginning to extend, on 1 May.

Treatments were applied to six blocks of four rows of eight pots which were arranged so that plant density was equivalent to 35 plants/m^2. There was one plant in each pot (15 cm diam.) Records of the number of open flowers and plant height (to the end of the longest branch) were taken during flowering. The lengths of main stem, terminal raceme and all branches were recorded at the end of flowering; pod counts were made at harvest on 15 July. Measurements were made on plants in the middle of each treatment plot.

Experiments with crops in 1985

Winter oilseed rape cv. 'Jet Neuf' was sown at 7.5 kg seed/ha (row spacing of 114 mm) on 25 August, 1984 into a well prepared soil bed which had received five cultivations and basal fertiliser (0:24:24) at 260 kg/ha prior to sowing. A top dressing of nitrochalk equivalent to 127 kg N/ha was applied on 28 February, 1985 and a further dressing equivalent to 104 kg N/ha on 9 March.

We examined the effect of applying a single treatment of either triapenthenol or BAS1100W at three different stages of development. Triapenthenol at 700 g/250 l/ha (= 490 g ai) or BAS1100W at 3.3 l/250 l/ha (=585 g ai) was applied on (i) 27 March, 1985 when two-three internodes were visible and the stems were approximately 5-10 cm high (Code 2,02 on the Sylvester-Bradley and Makepeace (1984) growth stage key), (ii) 16 April, when the flower buds were visible and stems were approximately 30-35 cm high (Code 3,3) or (iii) 1 May, when branches were beginning to extend and stems were approximately 70 cm high (Code 3,6). Plots were 12 x 4 m. A strip 1.8 m x 9 m in the centre of each plot was combined between 6 and 8 August, when moisture content of the pods was approximately 27%. Seed yield data were calculated as tonnes/ha equivalent at 8% moisture. Treatments were randomised in each of six replicate blocks. Additional plots were used for observation and recording of development.

Records of leaf area at early regrowth, stem and branch length and pod counts at the end of the flowering period were made on plants selected for uniformity.

Light profiles in the crop were measured with seven tube solarimeters 0.9 m long (Delta-T Devices Ltd.) in each treatment (with and without triapenthenol), one above the crop and six equally spaced between the top of the crop and the ground. The solarimeters were placed with as little disturbance to the canopy and positioned to avoid mutual shading. Profiles were obtained from daily mean values when the radiation above the crop was greater than 50 W/m^2.

Plants were harvested from the area occupied by the solarimeters by making sequential cuts at the heights of the instruments. The material from each height interval was kept separate, weighed (fresh weight), and a sample of known weight removed for separation into component parts. The plan area of each component part of the samples was measured separately (stems, leaves and pods) using a leaf area meter (Delta-T Devices Ltd.). The total plan area was estimated on a fresh weight basis and used to calculate the plant area index for each height interval.

Field trials in 1986

The cvs 'Mikado' and 'Bienvenu', which have shorter stems, more uniform branch lengths and in general are less susceptible to disease compared with 'Jet Neuf', allow better assessment of the effect of changed crop architecture on yield.

The winter oilseed rape cvs 'Bienvenu' and 'Mikado' were sown at 2.5 kg seed/ha on 6 September, 1985 in beds prepared as in 1985. Top dressings of nitrochalk, equivalent to 150 kg N/ha, were applied to 'Mikado' in February/March, 1986. The effects on yield of single applications of BAS11104W at 3.0 l/250 l/ha (= 450 g/ai/ha) or triapenthenol, given at the same rate as in 1985, and at the same developmental stages as above, on yield were recorded at harvest.

RESULTS AND DISCUSSION

Effects on structure of the shoot system of pot-grown plants

Treatments with both triazole retardants significantly reduced stem and branch length (Tables 1,2). The growth responses were sustained until harvest in all treatments; terminal racemes were significantly shorter but in plants sprayed with BAS11100W, total branch lengths were similar to the control. All treatments resulted in increased numbers of primary and secondary branches.

TABLE 1

Effect of BAS11100W applied at mid-stem extension[*] on shoot system structure of pot-grown winter oilseed rape cultivars during pod-filling (25 June 1985)

	Bienvenu		Jet Neuf		SED
	Unsprayed	Sprayed 9.4.86	Unsprayed	Sprayed 16.4.86	(20 df)
Mainstem length (cm)	72.9	62.7*	86.0	62.3*	3.95
Terminal raceme length (cm)	44.4	29.1*	58.6	25.2***	6.44
Total branch length (cm)	387.9	358.3	337.5	347.3	38.9
Number of branches	7.1	9.2**	5.9	10.0*	0.66
Number of secondary branches	5.4	19.5**	0.6	19.5***	4.06

[*] approximately 30 cm high
*, **, *** indicates significant difference from unsprayed at 5, 1 or 0.1% level of probability.

TABLE 2

Effect of time of application of triapenthenol on plant structure of pot-grown winter oilseed rape cv. Jet Neuf during pod filling (18 June 1985)

	Date of application				SED
	0	28 March	16 April	1 May	(18 df)
Mainstem length (cm)	87.8	18.5***	65.5***	84.4	3.92
Terminal raceme length (cm)	51.7	23.4***	22.3***	29.2***	2.23
Total branch length (cm)	436.5	306.0***	215.7***	215.9***	28.89
Number of branches	9.0	11.8***	11.4***	9.4	0.53
Number of secondary branches	2.9	8.6***	8.0**	3.9	1.45

*,**,*** Indicate significant differences from unsprayed at 5, 1 or 0.1% level of probability

Smaller main stem leaves were observed on plants treated at early or mid-stem extension. Outgrowth of axillary shoots was noted as a response to the earlier treatment, which gave the plants a bushier and more compact appearance. Marked reductions in the size and yellowness of the petals were observed due to treatments at mid or late stem extension with both chemicals. Flowering commenced at the same time in treated and untreated plants but continued longer in treated plants as a result of increased branching (Figure 1). The numbers of flowers and pods were greater but the set did not increase similarly.

The differences in cultivar response were attributable to differences in growth habit and slight differences in stage of development at the time of spraying. These varied even though stem heights were similar at time of treatment.

These results suggested that it might be possible to change canopy structure in such a way by using these chemicals to reduce crop height and individual branch length, without reduction of total pod-bearing branch length or pod numbers. In addition, although there were fewer pods on individual branches in the pot experiments, seed production was concentrated in those pods which developed from the first-formed flowers located at the base of the branches. These are potentially higher yielding than pods formed from later flowers situated towards the ends of the branches.

Effects of crop structure during early stem extension

The crop had overwintered (1984/85) with stems approximately 10 - 15 cm high, but by early March ground cover was still incomplete because of mechanical damage due to wind and frost to the larger leaves. Light leaf spot (*Pyrenopeziza brassicae*) infection in the autumn was evenly distributed throughout the crop and reduced green-leaf area at regrowth. Ground cover was patchy and incomplete during late March and early April following the rapid upward growth of the stem. Treatments with either chemical at early regrowth inhibited extension growth and resulted in a lower and darker-green canopy within two weeks of treatment, compared with controls. Buds near the base of the stem grew out to give a denser canopy and more complete ground cover. Leaf area on these axillary shoots was significantly greater than in untreated plants. The number of healthy leaves on the main stem was significantly increased by the fungicidal properties of BAS111OOW. Although the retardants reduced the size of individual leaves, total area was not decreased because leaves abscinded more slowly.

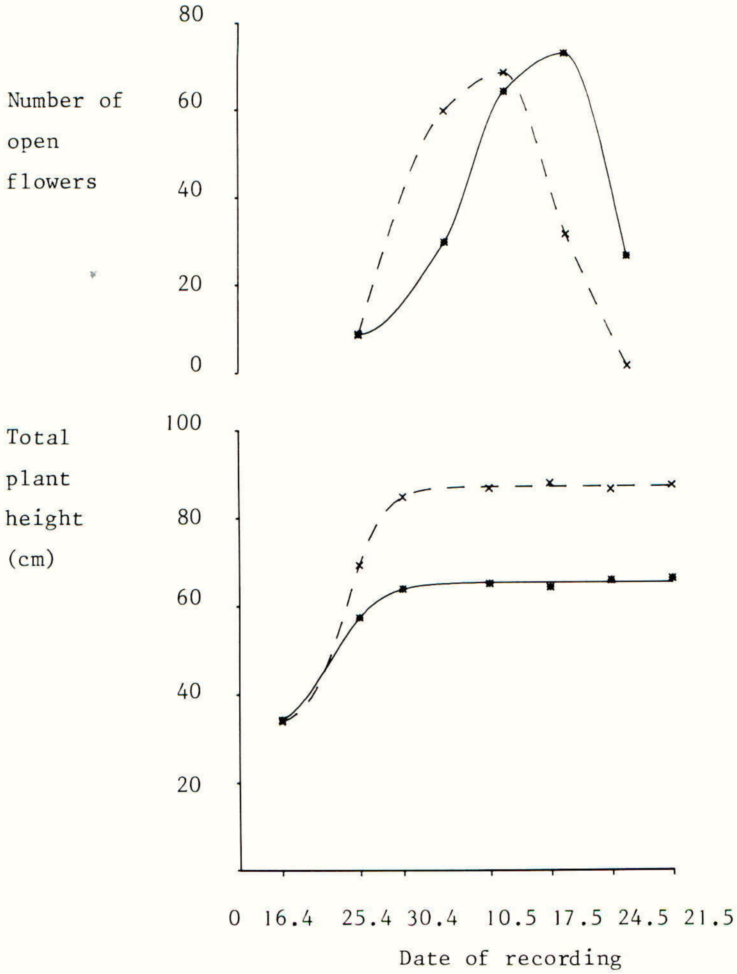

Figure 1. Effect of triapenthenol on duration of flowering and plant height. ----- unsprayed; ______ Triapenthenol.

TABLE 3

Effect of BAS11100W applied on 27 March 1985 on shoot (early stem extension) structure in a crop of winter oilseed rape cv. 'Jet Neuf' assessed on 25 April 1985)

	Length of mainstem (cm)	Number of leaves on mainstem	Leaf area (cm^2)/plant Main stem	Axillary shoots	Total
Unsprayed	53.2	10.2	323	16	340
Sprayed	31.8***	11.4*	321	63***	384
SED (10df)	3.57	0.37	36.9	10.1	41.0

*; *** indicates significant differences from unsprayed at 5 and 0.1% probability level.

Increased axillary bud outgrowth resulted in increased leaf area on these extra shoots. Consequently, total leaf area per plant was unaffected (Table 3); leaf area index (not measured) would not have been affected by this growth response and reduction of individual leaf size would not necessarily have resulted in reduced photosynthesis on a unit leaf area basis. It is known that anatomical changes resulting in thicker leaves with smaller area are associated with treatment with growth retardants. Hawkins & Hughes (1985) suggested that paclobutrazol increased rates of CO_2 fixation on a unit area basis and increased stomatal frequency in soy bean leaves but did not affect the amount of photosynthate produced by the plant as a whole. If this was the affect in our experiment with oilseed rape, then it is unlikely that carbohydrate production, or yield, would be affected by individual structural changes.

Treated plants produced leaves which were more vertically inclined. It has been suggested by Evans (1975), that such leaves have a higher photosynthetic rate than those with a horizontal pose because of reduced light saturation of the upper leaves and more uniform distribution of light throughout the canopy. This indicates greater productivity of photosynthates on a whole plant basis which might account for increased yield.

Effects on flowering and pod filling

(a) Canopy structure

Treatment with BAS11100W or triapenthenol at early regrowth was without effect on flower size or colour but later treatments resulted in smaller, paler petals. The period of flowering was extended by all treatments (Figure 1) but whereas early application resulted in a denser appearance due to increased branch numbers, later treatment produced a more open canopy due to reduced branch lengths.

By the end of the flowering period marked differences in canopy structure, due to time of application, were recorded. These were similar for both chemicals. However, although growth responses were less in the field than in the pot-grown plants, significant reduction in canopy height was recorded following treatment at early or mid-stem extension. These treatments also increased the number of branches, which caused mutual shading of the leaves. The number of pods was not significantly increased by any treatment. The extended period of set resulted in similar extension of the period for pod maturity. At harvest, treated plots were greener than untreated plots due to the greater proportion of younger unripe pods.
Treatment at early branch extension shortened branch length and the depth of the pod-bearing portion of the canopy (Figure 2).

(b) Light interception

The difference between profiles in the control and triapenthenol treatments reflect differences in heights of the two canopies; however, the total amount of light transmitted to the base of the canopy is similar in both plots. The light profiles were analysed in relation to the plant area at different height intervals between the solarimeters (Figure 3).

The plan area of all the components of the canopy (stems, leaves and pods) were used to calculate the plant area index of each plot, values of 3.5 and 2.2 were found for the control and triapenthenol treatment respectively. It is surprising that similar amounts of light were transmitted through each canopy, but this could be explained by a change in the pattern of light interception brought about by growth responses to the chemical.

The pattern of light interception in vegetation can be described by Beer's law:

$$I_z = I_o \exp(-kA)$$

where I_o and I_z are radiated intensities above the canopy and at a height, z, within the canopy, k is an extinction coefficient and A is the plant area (per unit ground area) between the top of the canopy) and z. Values of k found by plotting $\log_e (I_z/I_o)$ against A were different for the control and triapenthenol treatment (Figure 4). A larger value of k may result from a change in the relative areas of pods, leaves and stems in the treated plot. The chemical also causes leaves to be thicker and darker than the control; the treated leaves would absorb a greater proportion of the incident radiation and this would tend to increase the value of k. It should be pointed out, however, that these are preliminary results which require confirmation.

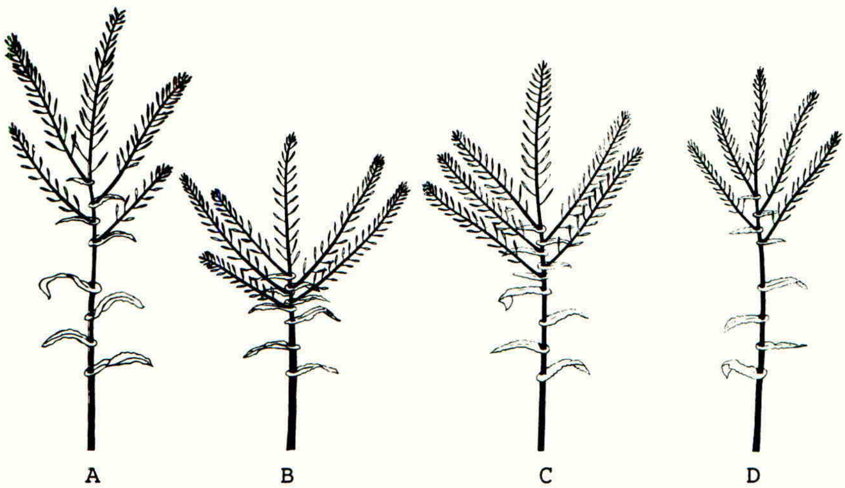

Figure 2. Effect of triapenthenol on plant structure of oilseed rape cv. 'Jet Neuf' depends on time of application. A = unsprayed (control); triapenthenol applied at early stem extension (B) or mid-stem extension (c) or early branch extension (D).

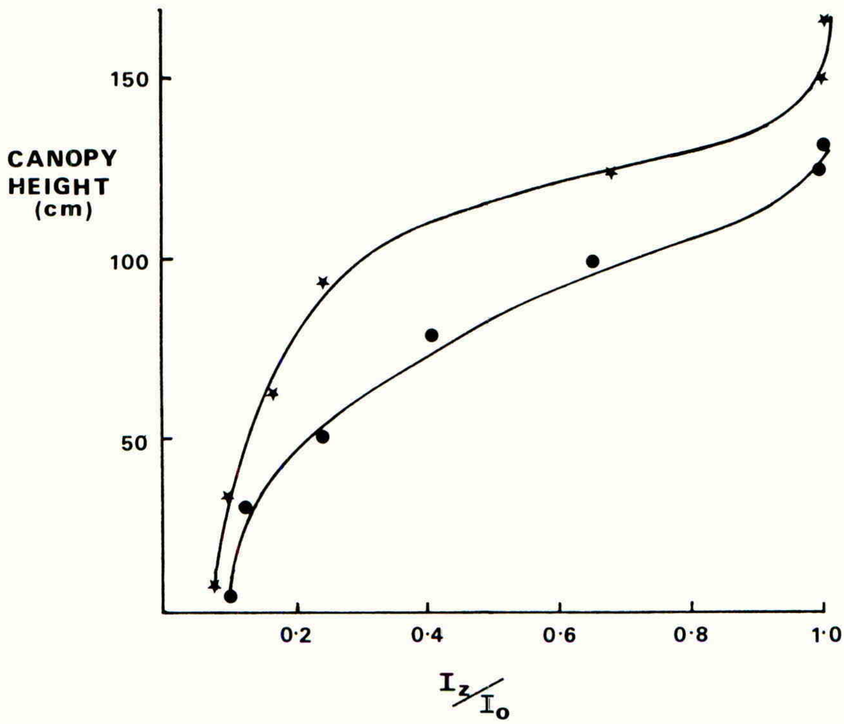

Figure 3. Light profiles measured in oilseed rape canopies (cv. 'Jet Neuf') soon after flowering. *Unsprayed (control) •Sprayed with triapenthenol at early stem extension. I_z/I_o = light values at given position in canopy (I_z) in relation to light values above canopy (I_o).

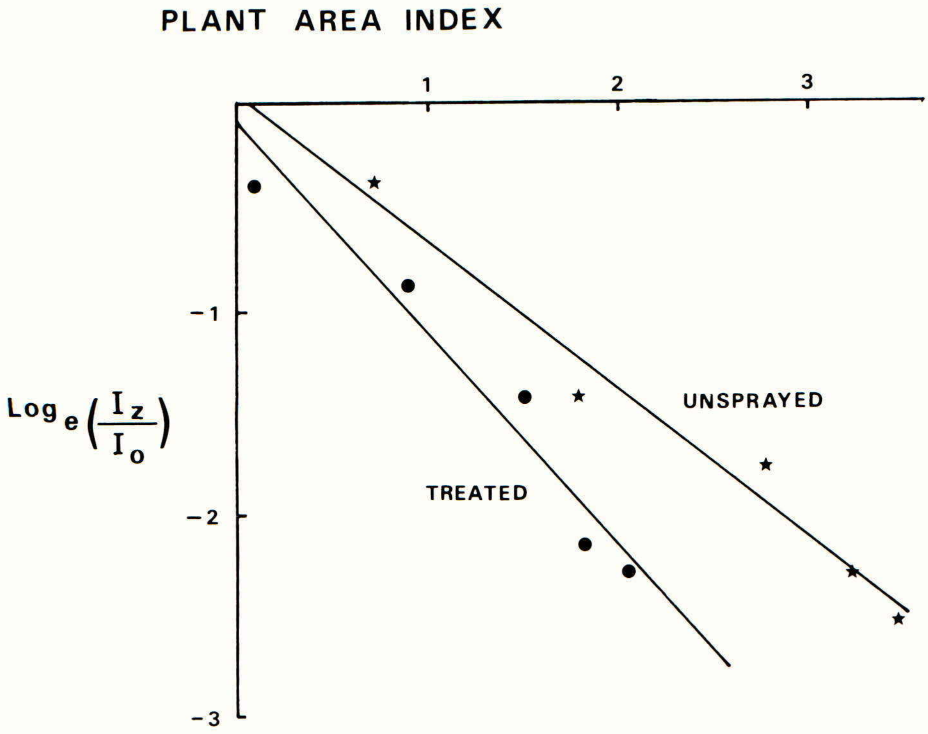

Figure 4. The relationship between light profiles and plant area index. The slope of the line is equal to the extinction coefficient (k).
*Unsprayed (control).
●Sprayed with triapenthenol at early stem extension.

Effect of treatment with BAS11100W and triapenthenol on yield

The adverse weather conditions in July and early August 1985 delayed combining and yields were low. In spite of this, and the large losses due to shatter, significant increases in yield were obtained in 'Jet Neuf' as a result of treatment with both retardants (Table 4).

TABLE 4

Yields (t/ha at 8% moisture) of oilseed rape cv. 'Jet Neuf' treated with growth retardants at Long Ashton in 1985.

Growth retardant branch	Stage of application			
	0	Early re-growth	Mid-stem extension	Early extension
	-	(27 March)	(16 April)	(1 May)
BAS11100W	2.54	3.00	3.27	3.03
SED 0.212 (df24)				
CV (%)	8.7			
Triapenthenol	2.23	3.12	2.85	3.12
SED 0.142 (df30)				
CV (%)	13.4			

Treatment with both BAS11100W and triapenthenol at the beginning of stem extension in 1986 increased yield in 'Bienvenu' (where disease incidence was relatively high during pod filling) but was without effect on 'Mikado', where disease incidence was lower. Treatment at late stem extension reduced petal size and colour and shortened branches during flowering and pod filling. Yield increased in both cultivars as a result of this treatment. (Tables 5, 6).

TABLE 5

Yield (t/ha at 80% moisture) of winter oilseed rape cv. 'Bienvenu' at Long Ashton in 1986

Growth retardant	Stage of application			
	0	Early regrowth	Mid-stem extension	Early branch extension
		(4.4.86)	-	(7.4.86)
BAS11104W	3.15	3.37	-	3.41
SED 0.053 (df 50)				
CV (%)	6.7			
Triapenthenol	3.20	3.51	-	3.44
SED 0.126 (df 52)				
CV (%)	4.0			

TABLE 6

Yield (t/ha at 8% moisture) of winter oilseed rape cv. 'Mikado' at Long Ashton in 1986

Growth retardant	0	Early regrowth	Mid-stem extension	Early branch extension
BAS111040	3.70	3.61	3.71	4.03
SED 0.085 (df30)				
CV (%)	5.6			

CONCLUSIONS

Canopy structure in oilseed rape is significantly altered by treatment with triazole growth retardants applied during stem extension. The consequence light interception at important stages of crop growth may be great enough to influence yield.

We identified three periods during growth when light interception may be limiting to set and yield. The first occurs at the beginning of regrowth in late winter/early spring. At this time, ground cover and light interception may be influenced by crop density and vigour. It may also be affected by leaf damage during the winter. Treatment at early regrowth reduces stem height and increases axillary bud growth and leaf area. It is possible that this leads to increased carbon assimilation which may be in excess of the requirement for growth at this time. The consequence for carbohydrates (sugars and starch) need to be identified. Also, the effects on leaf area index require to be quantified.

The second period of limited light interception in the crop occurs during flowering. The reduction of stem height and increases in branching resulted in a lower, denser canopy during flowering and pod development. Shading by the flower canopy may have been reduced and the ability of the leaves to intercept light and produce photo-assimilates during set may have been prolonged. In pot-grown plants this increased flowering and pod set, but in the crop, although more flowers were produced, pod set remained similar to the control. Effects of treatment on canopy structure and the pattern of light interception in oilseed rape during grain filling might be expected to increase assimilate production by the pods. The relative importance of pod and leaf photosynthesis after flowering may be changed for example; if the pod canopy permits greater penetration of radiation to leaves this may delay leaf senescence and provide increased assimilate during pod filling. Further work is planned to measure photosynthetic rates of pods and leaves in canopies treated with PGRs which aims to explain the significance of altering canopy structure and light interception patterns for the productivity of rape crops.

Yields of 'Jet Neuf' in 1985 may have been further increased as a result of the fungicidal properties of the two triazole retardants. Disease incidence was greatly reduced on the leaves and pods as a result of all treatments. The major disease was light leaf spot

(Pyrenopeziza brassicae). Stinchcombe et al., (1986) have shown that reduced disease incidence in 'Jet Neuf' follows the application of BAS11100W and triapenthenol. However, in contrast, the less disease-susceptible cv 'Mikado' failed to respond with increased yield following applications at early or mid-stem extension (Table 7) although growth and canopy structure was significantly altered. Increased yield was recorded due to treatment applied at early branch extension. This reduced branch length and may have changed light profiles in the crop. The significance of such effect on canopy structure during flowering and pod filling was indicated by the light profile measurements taken in 'Jet Neuf'. These showed higher values deeper in the canopy and may have enabled more pods to survive and contribute to yield. Final set at the end of flowering was increased but it is not known whether more pods survived in the crop of 'Jet Neuf'. Measurements in the crop are difficult and techniques based on the measurement of light profiles and photographic image analysis are being developed which may enable better assessment of canopy structure. These techniques now require calibration with growth analysis to establish relationships between light values and crop surface area and dry matter production. The development of such techniques would help greatly to define effects of growth regulators on the oilseed rape crop.

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FURTHER RESULTS WITH BAS 111 04 W, A NEW GROWTH REGULATOR FOR USE IN OILSEED RAPE

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ABSTRACT

BAS 111 04 W is a new triazole-type plant growth regulator currently developed for the use in oilseed rape and other plant species. In oilseed rape this compound reduces the crop height, thereby diminishing the risk of early lodging. In addition to this, yield increases are observed in most cases. In 53 individual trials with 20 different varieties of oilseed rape treatment with BAS 111 04 W led to an average reduction of shoot height by 12 % and to an average increase of seed yield of 9 %.

Under the influence of BAS 111 04 W the process of pod senescence was clearly delayed. In comparison to the controls, the translocation of assimilates into the growing seeds appeared to be enhanced. Furthermore, the susceptibility of the pods to infection with Alternaria brassicae and A. brassicicola was reduced in treated plants. - In addition to an improved canopy structure these factors are most likely part of the overall response of oilseed rape plants accounting for the observed yield increases.

INTRODUCTION

The acreage of oilseed rape has more than doubled in the countries of the European Community throughout the last ten years and now totals for more than 1.5 million hectares. In order to optimize the productivity of this crop, the use of plant growth regulators can be of relevance. The main objectives for such compounds have been defined for instance by Dawkins (1986). Among these are the reduction of stem length to make the plants less susceptible to lodging and to provide opportunities for easier crop management. Yield increases may be obtained by optimizing the canopy structure of the crop, by improving winter hardiness or by reducing pod abortion or pod shatter. Triazole-type plant growth regulators appear to offer advantages over the substances used so far such as chlormequat chloride, mepiquat chloride, ethephon or daminozide (Scarisbrick et al. 1982, Chapman et al. 1983, Child 1984, 1985).

A triazole compound currently under development for oilseed rape and other plant species is BAS 111 04 W. The basic properties of the active ingredient BAS 111 .. W have recently been dealt with in contributions by Rademacher et al. (1985) and Jung et al. (1987). The principle mode of action of BAS 111 .. W is that it blocks the oxidative steps that lead from kaurene to kaurenoic acid in the course of gibberellin biosynthesis. As a result plants with a more compact shoot are obtained. In oilseed rape

BAS 111 04 W can be successfully applied to reduce the risk of early lodging. In addition to this, yield increases of up to 30 % have been obtained even in the absence of early lodging (Child et al. 1985, Luib et al. 1986 a, b).

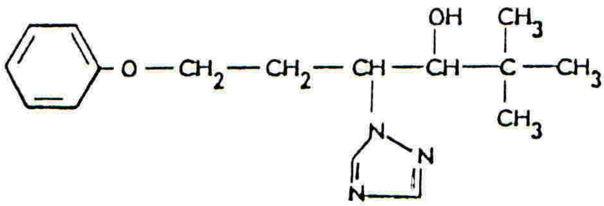

Fig. 1 Structure of BAS 111 .. W, active ingredient of BAS 111 04 W [1-Phenoxy-3-(1H-1,2,4-triazol-1-yl)-4-hydroxy-5,5-dimethylhexane]

According to Luib et al. (1986 a) the most favourable time to apply BAS 111 04 W in oilseed rape is at the onset of stem elongation, which is observed in winter rape shortly after growth recommences in spring. This is between growth stages 31 and 33 on the scale of Schütte et al. (1982). Normally a rate of 450 g active ingredient is required per hectare. The active ingredient is taken up both by the leaves and by the roots bringing about a rapid reduction of shoot growth which prevents or reduces early lodging of the crop. When applied in autumn, BAS 111 04 W prevents overgrowth and increases the winter hardiness of the young rape plants. Less fungal infection is very often observed under the influence of the compound (Child et al. 1985, Luib et al. 1986 b, Stinchcombe et al. 1986, Wissmüller 1986).

In this contribution more evidence is presented for true yield increases caused by BAS 111 04 W in oilseed rape. In addition to this, further facts about the underlying actions of the compound are dealt with.

RESULTS

a) Response of different varieties of oilseed rape towards BAS 111 04 W

20 different cultivars of oilseed rape were chosen to investigate the performance of BAS 111 04 W. The experiments were carried out in 1985 and 1986 under field conditions at different locations in the Federal Republic of Germany. Applications were made at a growth stage between 31 and 33 with rates of 450 g active ingredient per hectare. In all of the 53 trials lodging was no significant problem. The results in relation to the respective controls are shown in Fig. 2.

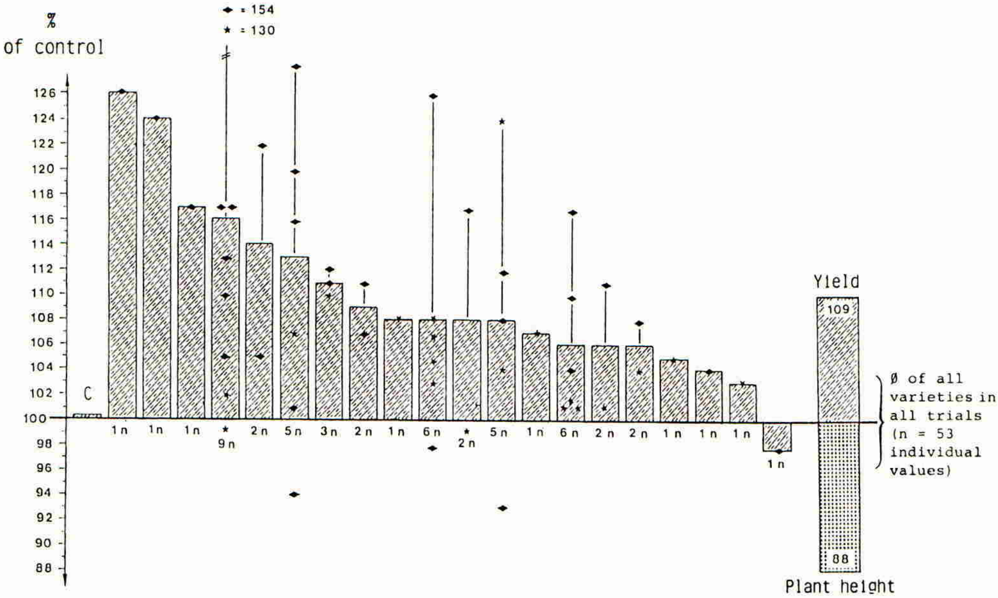

Fig. 2 Influence of BAS 111 04 W on average shoot length and yield of 20 different varieties of oilseed rape. (Individual results are marked by dots ◆= 1985, *= 1986. The bars represent the respective mean values. For details see text.)

On average over all trials, plant height was reduced by 12 % while the overall yield was increased by 9 %. Reductions in yield of more than 5 % were observed in only two cases. In contrast, yield increases of 5 % or more were found in 35 instances out of which 9 gave + 20 % or more.

b) Influence of BAS 111 04 W on the senescence of pod walls

Winter oilseed rape (cv. Mirander) grown under field conditions was treated (450 g a.i./ha) at growth stage 33. Starting shortly after flowering, pods were sampled at weekly intervals. As a measure of pod senescence, the specific DNase activity of pod wall homogenates was determined using the methods described by Grossmann and Jung (1982). In order to enable a better comparison, all results are expressed as a percentage of fully senescent pods (growth stage 89).

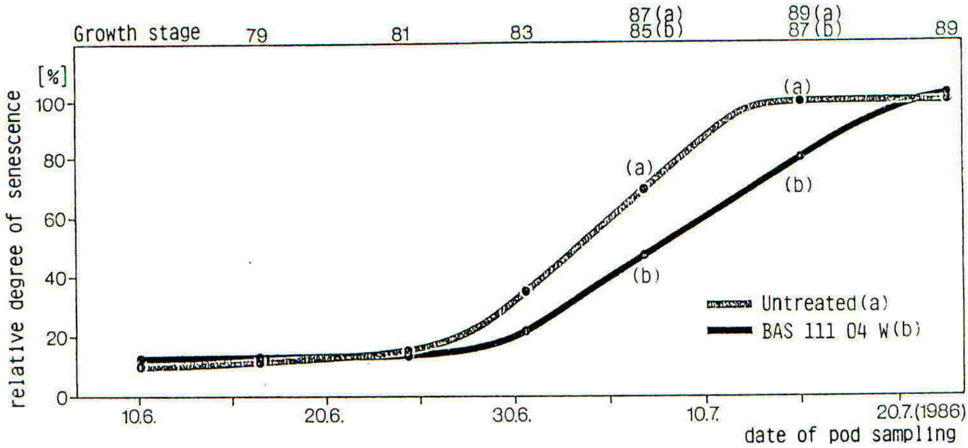

Fig. 3 Progress of senescence in pod walls of oilseed rape. (See text for details).

As can be seen from Fig. 3, treatment with BAS 111 04 W clearly slows down the process of ageing. Over a period of time of more than three weeks the pods of treated plants are less senescent than those of the untreated plants. This phenomenon can be perceived visually as a delay in development of approximately two growth stages.

c) Susceptibility of pods of oilseed rape towards infection by Alternaria spp.(black spot disease) under the influence of BAS 111 04 W

The plant material used in this experiment was cultivated and treated as described under b). Pods were sampled at different stages of development. In order to determine their resistance to *A. brassicicola*, the pods were treated with a high level of inoculum of this fungus and incubated under optimal conditions for disease development. After 70 h the extent of necrosis was determined. If no necrosis occurred the pods were described as 100 % resistant, whereas 0 % resistance was assessed when the tissue was totally destroyed. Parallel to the work with detached pods, the degree of infection of pods by *A. brassicae* developing on intact plants was monitored (field attack).

It can be seen from Fig. 4 that the rape pods gradually loose their natural resistance to *Alteraria spp.* at the onset of ripening (June 24, approximately growth stage 81). Resistance was lost more slowly in the pods of plants that had been treated with BAS 111 04 W (a). Accordingly, the field attack by *A. brassicae* started later on pods of treated plants (b).

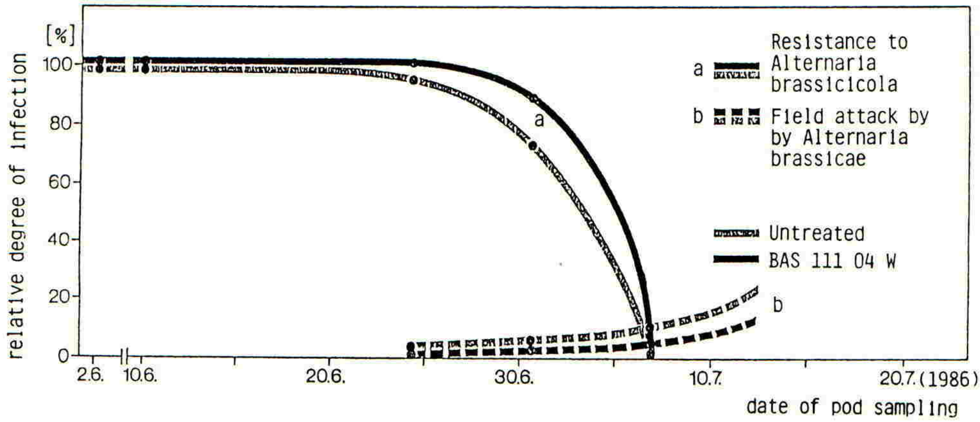

Fig. 4 Susceptibility of rape pods towards infection by <u>A. brassicola</u> and <u>A. brassicae</u> (See text for details).

d) <u>Uptake and partitioning of sucrose in pods of oilseed rape under the influence of BAS 111 04 W</u>

The cultivation and treatment of the plant material with BAS 111 04 W was carried out as described under b). ^{14}C-Sucrose was fed to detached pods of different age via the pedicels for 24 h. Pods were then separated into the walls (the carpels and the false septum) and the seeds. From these samples radioactivity was determined on a fresh weight basis. In order to enable a better comparison, the data are expressed as a percentage of the highest value of the untreated pods. The results are given in Fig. 5.

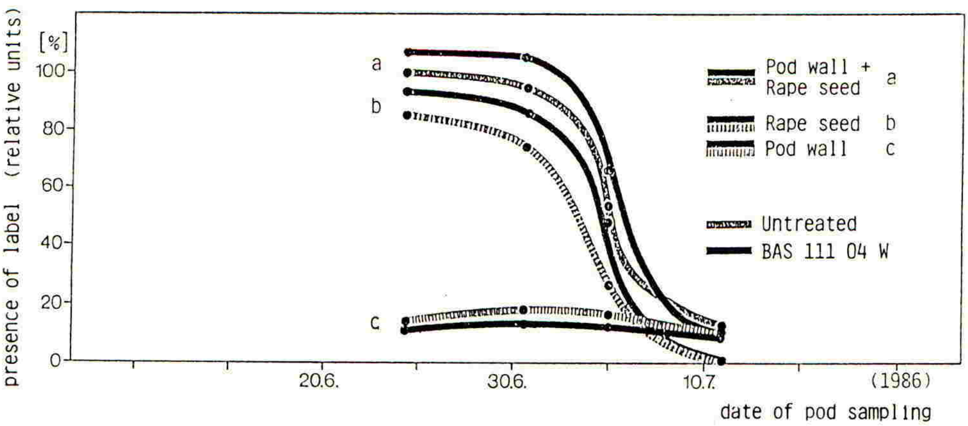

Fig. 5 Uptake and partitioning of ^{14}C-labeled sucrose in rape pods. (Details are given in the text).

Throughout the experimental period both the total uptake of labeled sucrose by the whole pods and the sink strength of the seeds were higher in the treated plants than in the controls. In contrast, the pod walls of the untreated plants appear to retain more of the labeled compound than those of the treated ones.

DISCUSSION

From the results presented here as well as from data published by Child et al. (1985 and 1987) it is evident that clear yield increases - even in the absence of early lodging - can be obtained in oilseed rape after treatment with BAS 111 04 W. Part of the observed yield enhancements may be explained by the influence of BAS 111 04 W on the canopy structure of the oilseed rape plants. This results in a better penetration of light to the racemes and other plant parts (Child et al. 1987).

Under the influence of BAS 111 04 W the senescence of pods is delayed. Since almost 50 % of the assimilates that are stored in the seeds originate from the pod walls (Scarisbrick and Daniels 1984), the seed-filling period could thus be prolonged. Parallel to the delayed senescence the susceptibility of the pod walls towards infection by *A. brassicae* and *A. brassicicola* is reduced. Similarly, the occurrence of light leaf spot caused by *Pyrenopeziza brassicae* was found to be greatly reduced by BAS 111 04 W (Child et al. 1985, Stinchcombe et al. 1986). In addition to, or as a result of delayed senescence and reduced susceptibility towards fungal infection the sink strength of the seeds is increased. All of these factors would lead to higher yields.

In conclusion it may be said that it is still difficult to precisely identify the factors that are causing the increase of yield in oilseed rape under the influence of BAS 111 04 W. More basic work is required to shed light onto the complex correlations that determine yield formation in oilseed rape and how this system is affected by a growth regulator like BAS 111 04 W.

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IMPROVEMENT OF WINTER HARDINESS AND SEEDLING GROWTH OF OATS WITH SEED DRESSINGS OF TETCYCLACIS

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ABSTRACT

Winter hardiness is one of the main factors affecting harvestable yields in winter oats. The experimental plant growth regulator tetcyclacis, applied as a seed treatment, improved winter hardiness of two cultivars of winter oats sown at four depths. The treatments caused a reduction in the length of subcrown internodes resulting in the shoot apex of plants overwintering deeper in the soil. Soil temperature measurements showed the number of hours to which the shoot apices of plants were subjected to sub-zero temperatures decreased with increasing soil depth. Thus the added protection against winter damage afforded by tetcyclacis may, at least in part, be attributable to the depth at which the shoot apex overwinters.

INTRODUCTION

Winter hardiness is one of the main factors affecting harvestable yields in winter oats (Lawes et al., 1982). Whilst the main breeding objective is to produce cultivars having potential for high yields combined with good standing ability and stress tolerance, the combination of these characters has proved difficult to achieve in practice. Until plant breeders overcome this problem, the application of plant growth regulators might provide a means of improving winter hardiness in winter oats through the inhibition of subcrown internode extension thus decreasing exposure of susceptible tissues to damaging temperatures.

The plant growth regulator used in this investigation was the experimental norbornenodiazetine derivative tetcyclacis which inhibits gibberellin biosynthesis by blocking the oxidative reactions from ent-kaurene to ent-kaurenoic acid (Rademacher et al., 1984). In addition to its effects on gibberellin biosynthesis, tetcyclacis may also inhibit sterol biosynthesis (Nitsche et al., 1985). The possible improvement of winter hardiness was investigated by applying tetcyclacis as a seed treatment to two cultivars of winter oats sown at four different depths.

MATERIALS AND METHODS

Graded seed (maximum 2.2 mm) of the winter oat cultivars Peniarth and the higher yielding, but less winter hardy, Bulwark were soaked for 16 h in solutions containing 0, 0.2x and 1.0x the recommended concentration (0.1 g experimental sample (not a.i.)/kg seed/1800 ml water) of tetcyclacis. The wetter 'Agral' was added at 0.1% v/v in all solutions. After soaking, the seeds were surface dried before sowing in 27 cm plastic pots containing John Innes Potting Compost Number 3 on 5 October 1984. Six replicates of each seed treatment were sown at 2,4,6 and 8 cm depth with twenty seeds per pot. Following sowing the pots were plunged in an ash bed outdoors so that the level of the soil in the pots was the same as that of the ash. The layout within each cultivar was a randomised split plot design.

Soil temperatures in the pots were measured with thermocouple probes during December 1984 and January 1985. Four probes were used, each having copper-constantan junctions at the soil surface and at depths of 2,5,11 and 21 cm. The thermocouples were used to measure temperature differences within a single pot which, in turn, were referenced to an ice bath. Readings were recorded every 15 minutes using a data logger and subsequently averaged over each hour.

Counts of emerged seedlings were on a per pot basis at two to three day intervals from the first sign of emergence (15 October 1984) until no further seedlings emerged (2 November 1984). Two replicates were destructively sampled on 19 November 1984 to determine the position of the shoot apex by measuring the length of the subcrown internodes from the seed to the shoot apex. A third replicate was destructively sampled on 5 March 1985 to determine the extent of winter damage by visually assessing the percentage necrotic tissue on individual plants. In addition, the main stems of six randomly selected plants from each pot were dissected to determine the viability of the shoot apices.

RESULTS

Germination and emergence

Neither sowing depth nor tetcyclacis concentration affected germination, 95% being the average attained by all treatments in both cultivars. Seedlings started emerging ten days after sowing. Increasing both sowing depth and tetcyclacis concentration slowed emergence, Bulwark being the slower of the two cultivars to emerge (Table 1). Maximum emergence was reached by shallow sown, low concentration treatments within a few days but it took some of the deep sown, high concentration treatments up to 28 days from sowing to reach maximum emergence. However, by that time in only one case (1.0x tetcyclacis at 8 cm in Bulwark) was final emergence appreciably below 90% of sowing density.

TABLE 1

Effect of tetcyclacis seed treatment and sowing depth on percentage emergence in winter oats

Cultivar	Concn of tetcyclacis	10 days after sowing Sowing depth (cm)				28 days after sowing Sowing depth (cm)			
		2	4	6	8	2	4	6	8
Bulwark	0	90	91	83	76	96	98	94	94
	0.2x	66	64	9	0	97	94	92	91
	1.0x	13	0	0	0	92	91	89	59
Peniarth	0	93	89	81	73	94	94	99	95
	0.2x	97	89	78	47	97	97	95	94
	1.0x	79	13	0	0	95	94	95	88

Subcrown internode lengths

In both cultivars, tetcyclacis concentration and sowing depth had significant effects on subcrown internode lengths which were reduced at all sowing depths with increasing tetcyclacis concentration (Table 2). Within tetcyclacis treatments, subcrown internode lengths were increased with deeper sowing. However, at all sowing depths, tetcyclacis seed treatment resulted in shoot apices of plants arising from treated seed overwintering deeper in the soil compared with plants arising from untreated seed thus affording them some additional thermal insulation against winter damage.

TABLE 2

Effect of tetcyclacis seed treatment and sowing depth on subcrown internode lengths (cm) in winter oats

Cultivar	Concn of tetcyclacis	Sowing depth (cm)			
		2	4	6	8
Bulwark	0	1.73	2.22	4.02	5.82
	0.2x	0.89	0.74	2.53	5.01
	1.0x	0.20	0.48	2.02	3.91
			SED ±	0.19	
Peniarth	0	0.97	3.16	4.50	5.53
	0.2x	0.80	2.02	4.02	5.09
	1.0x	0.04	0.77	2.99	4.94
			SED ±	0.18	

Variation of soil temperature with depth

On two occasions when the soil in the pots was frozen, a significant gradient of temperature with depth persisted in the frozen layer. Over these periods, the temperature gradient was steeper near the soil surface than in deeper layers.

When measurements of soil temperature were made over a freeze-thaw cycle lasting seven days (14 - 20 January 1985), significant changes with depth occurred both in the total number of hours that soil temperature was below -2°C and in the cumulated degree-hours below -2°C (Fig 1).

The number of hours during which the shoot apices of plants were subjected to temperatures less than -2°C during the freeze-thaw cycle are presented in Table 3. When considered along with data from Table 2, the deeper the shoot apices overwintered in the soil, the shorter was the length of time they were subjected to potentially damaging temperatures.

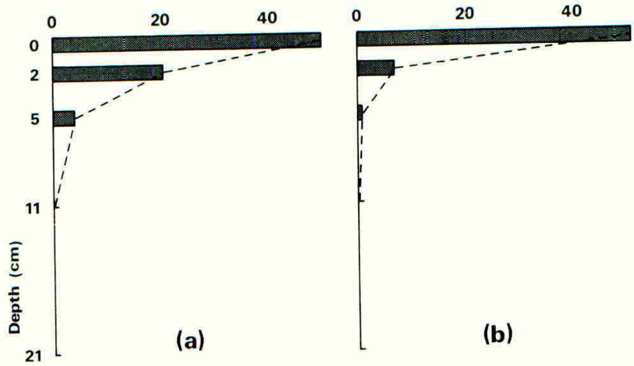

Fig. 1. Variation with depth of (a) total hours with soil temperature < $-2^{o}C$, (b) cumulative degree hours < $-2^{o}C$ over a seven day freeze-thaw cycle in January 1985.

TABLE 3

Number of hours to which shoot apices of winter oat plants were subjected to temperatures of below $-2^{o}C$ over a 7 day freeze-thaw cycle

Cultivar	Concn of tetcyclacis	Sowing depth (cm)			
		2	4	6	8
Bulwark	0	45	22	20	19
	0.2x	33	16	11	14
	1.0x	22	11	8	7
Peniarth	0	34	36	27	17
	0.2x	31	20	20	15
	1.0x	20	13	14	14

Winter damage

The first visible sign of winter damage was necrosis of the leaves in early spring. Bulwark suffered more damage than Peniarth but the pattern was similar for both cultivars (Table 4). The plants could be divided into two distinct groups according to the amount of damage sustained. Within each group there was little, if any, significant difference between treatments. In the group which suffered least damage (Group 1) were plants arising from seed treated with 1.0x tetcyclacis at all sowing depths and plants arising from seed treated with 0.2x tetcyclacis sown either 6 cm or 8 cm deep. In the other group which suffered extensive damage (Group 2) were plants arising from untreated seed at all sowing depths and plants arising from seed treated with 0.2x tetcyclacis sown either 2 cm or 4 cm deep.

TABLE 4

Effect of tetcyclacis seed treatment and sowing depth on percentage necrotic tissue in winter oats recorded 5 March 1985

Cultivar	Concn of tetcyclacis	Sowing depth (cm)			
		2	4	6	8
Bulwark	0	52.8	54.4	56.1	37.8
	0.2x	57.8	54.4	23.9	23.1
	1.0x	16.7	14.7	13.9	14.4
			SED ± 4.9		
Peniarth	0	37.2	28.3	35.6	24.4
	0.2x	31.1	35.6	15.6	12.8
	1.0x	12.9	14.4	11.7	13.1
			SED ± 4.4		

Damage to leaves need not in itself cause the death of the plant, the viability of the shoot apex is the determining factor, so a sample of main stem crowns from both groups of plants were dissected. For Group 2 plants, 72% and 47% of main stem apices in Bulwark and Peniarth respectively, had been killed. The corresponding figures for Group 1 plants were 6% and 0%. No tillers were dissected.

DISCUSSION

Soaking seeds of winter oats in solutions of the experimental plant growth regulator tetcyclacis before sowing, at all four sowing depths resulted in a reduction of subcrown internode lengths. In the shallow sowings (2 cm and 4 cm) the mesocotyl was the only subcrown internode to extend, irrespective of tetcyclacis concentration. This also occurred in plants arising from untreated seed from the deep sowings (6 cm and 8 cm). However, with the addition of tetcyclacis, extension of the coleoptile internode, and of the main stem internode between leaves one and two, compensated to some extent for the reduction in mesocotyl length. Even with this added extension, tetcyclacis seed treatment resulted in the apex of plants over-wintering at a greater depth in the soil.

Temperature measurements confirmed that soil layers near the surface experienced a larger diurnal variation in temperature, freezing sooner and more often, than those at greater depth, although the deeper layers often took longer to thaw. At all sowing depths, the shoot apices of plants arising from untreated seed over-wintered within the top 2.5 cm of soil, in the region in which these larger variations in soil temperature occurred. By contrast the shoot apices of plants arising from tetcyclacis treated seed (except those sown at 2 cm, which over-wintered within the top 2 cm of soil) generally over-wintered in the soil below 2.5 cm and were thus subjected to less variation in soil temperature. Therefore, small increases in the depth at which shoot apices over-wintered appeared to decrease the risk of frost damage.

The beneficial effects of tetcyclacis on increasing winter hardiness apparently due, at least in part, to its ability to shorten subcrown internode lengths, and thus affecting shoot apex depth, were reflected in the amount of damage sustained by the plants during winter. Generally, less than 20% of the total leaf area in Group 1 plants was necrotic and very few main stem apices were killed. By contrast, within Group 2 plants, over 50% of the total leaf area in Bulwark was necrotic and 72% of main stem apices killed; the corresponding figures for Peniarth were over 30% and 47% respectively. Death of the main stem apex need not result in the non-productivity of the whole plant, as tillers may survive the winter and proceed to ear. In this experiment no tillers were dissected so the extent of tiller death is not known.

The effect of tetcyclacis seed dressing on plant growth and yield is an important consideration if the treatment is to be accepted in commercial practice. In a separate seed dressing experiment using the same two cultivars and concentrations of tetcyclacis, early plant growth was most effected by treatment (Anderson, unpublished). Throughout autumn and winter, plants arising from seed treated with 1.0x tetcyclacis had lower dry weights and fewer tillers compared with plants from either of the other two treatments (0 and 0.2x tetcyclacis); differences between the latter two treatments rarely being significant. Once plants started growing away in spring, significant differences in growth due to seed treatments largely disappeared. Although tetcyclacis caused an increase in yield, due to an increase both in grain numbers and grain size, differences between treatments were rarely significant.

As neither growth nor yield of winter oats seem to be adversely affected following tetcyclacis seed treatment, and winter hardiness was improved, the treatment may prove useful in agronomic practice.

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3.
Plant Growth Regulators for New Target Effects

Chairman: Dr G. V. HOAD

WATER CONSUMPTION AND YIELD FORMATION IN CROP PLANTS UNDER THE INFLUENCE OF SYNTHETIC ANALOGUES OF ABSCISIC ACID

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ABSTRACT

The influence of LAB 144 143 and LAB 173 711 - synthetic analogues of abscisic acid - on the transpiration and productivity of different plant species has been investigated. Both compounds as well as abscisic acid can be applied via the roots. This would be of relevance under conditions that impose the risk of photodecomposition after leaf application. It was found that a rapid opening and closing of sunflower stomates can be induced by applying the antitranspirants via the substrate. Most probably this phenomenon indicates an increase of the hydraulic conductance of the roots which increases the hydrostatic pressure in the xylem vessels. C_4 species are generally less sensitive to abscisic acid and its analogues than C_3 species. In plants that were grown over longer periods of time clear reductions of water consumption could be induced by administering the antitranspirants with the irrigation water. Reductions of transpiration up to 30% were widely tolerated which resulted in clearly improved rates of water use efficiency for the formation of shoot biomass, cereal seeds and tomato fruits.

INTRODUCTION

In many areas of the world water is a minimum factor that severely restricts crop productivity. Artificial watering is used in many cases to overcome this problem. It is estimated that 200 million hectares - this is approximately 10% of the agronomically used area - are subjected to irrigation worldwide (Tekinel 1979; Fenton et al. 1982). In the USA approximately 80% of the sugar beet, 70% of the fruits and vegetables, 40% of the soybeans and cotton, 30% of the alfalfa, 25% of the barley, and 10% of the maize and wheat are harvested from irrigated land (Wittwer 1981).

In most cases artificial irrigation requires a relatively high level of expenditure: surface waters have to be diverted over long distances or ground water has to be supplied at a high input of energy. The depletion of water resources can be forecasted for the coming decades for instance in large areas of southwestern USA and in the Middle East (Fenton et al. 1982). A further problem often connected with the artificial supply of water is caused by salinization (McWilliam 1986). Casey (1972) indicates that there are salinity problems in at least one third of the area under irrigation. Against this background, solutions are intensively being sought to use the available water in arid and semiarid areas as economically as possible.

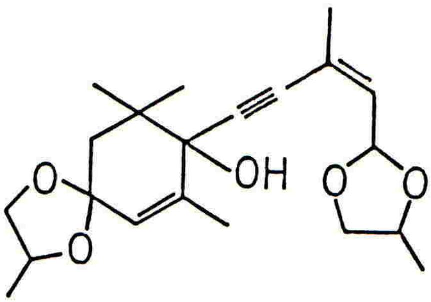
The following processes are the main causes of losses of water from natural precipitation or artificial irrigation (Davenport and Hagan 1981):

- surface runoff
- deep percolation into soil layers which cannot be reached by roots
- evaporation
- transpiration

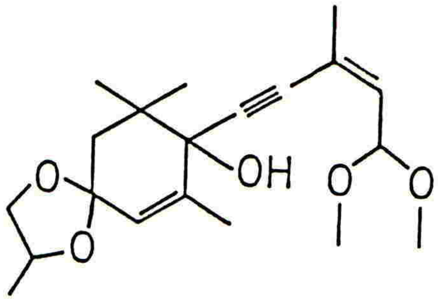
Several measures in terms of water economy and agronomical practice can be employed relatively efficiently to keep losses of water from runoff, percolation and evaporation from the soil surface within limits (Davenport and Hagan 1981). In addition to this, attempts are being made to reduce the stomatal and cuticular transpiration of the plants or otherwise improve the plants' efficiency in utilizing water (Karamanos 1979), although none of these approaches has found a major practical application up to now:

- reduction of transpiration
  - physical measures (e.g. covering leaf surfaces with films, increasing leaf reflection, reducing air convection)
  - modification of the plant's behaviour by breeding or by the use of bioregulators (e.g. lowered number of stomates per unit of leaf area, diminished leaf surface, increased surface albedo of leaves, increased cuticular resistance, generally reduced degree of stomatal aperture, increased sensitivity of stomates towards an onset of drought)
- increase of water absorption via breeding or by the use of bioregulators
  - formation of a more extensive root system
  - enhancement of the hydraulic conductance of the root
- induction of drought tolerance
  - hardening of plants
  - improving the plant's properties via breeding or the use of bioregulators (e.g. optimized osmoregulation)

In general, physiologically acting antitranspirants are regarded as being relatively promising for further development (for reviews see Gale and Hagan 1966, Bergmann 1977, Das and Raghavendra 1979, Jones 1981, Fenton et al. 1982, Jung and Rademacher 1983, Davies et al. 1986). Abscisic acid (ABA), the natural regulator of stomatal aperture, has found no major application yet. Its relatively rapid metabolism in the plant, its instability to UV light, which largely excludes any leaf applications, and its relatively high price can be regarded as the decisive reasons. The situation is similar with ABA derivatives (e.g. ABA methyl ester) and other structures closely related to ABA (e.g. xanthoxin and vomifoliol). Stomatal closure can likewise be induced with farnesol, certain derivatives of fatty acids, phenylmercuric acetate, alkenylsuccinic acid, salicylaldoxim, and certain inhibitors of photosynthesis. However, due to phytotoxicity and other adverse effects none of these compounds is of particular practical relevance. Synthetic compounds related to ABA proved to be more promising. Certain aromatic analogues (Bittner et al. 1977), pentadienoic acids (Carbonnier et al. 1981, Oritani and Yamashita 1983) and acetyleneacetate-type compounds (Bliesener et al. 1985, Grossmann and Jung 1984, Jung and Grossmann 1985) should be mentioned here. For the time being, the acetyleneacetates LAB 144 143 and LAB 173 711 (Fig. 1) are most likely the most active synthetic analogues of ABA.



LAB 144 143



LAB 173 711

Fig. 1
Structures of
LAB 144 143: 5-[2,6,6-trimethyl-1-hydroxy-4-(propylene-1,2-dioxy)-cyclohex-2-en-1-yl]-3-methylpent-2-en-4-in-1-al-(1,2-propylenacetal)
and
LAB 173 711: 5-[2,6,6-trimethyl-1-hydroxy-4-(propylene-1,2-dioxy)-cyclohex-2-en-1-yl]-3-methylpent-2-en-4-in-1-al-dimethylacetal
(isomers not considered)

It has been reported (Grossmann and Jung 1984) that LAB 144 143 after leaf application reduces transpiration for at least four days under glasshouse conditions. The duration of its effects is, however, reduced when it is used under high light intensities in the field (Rademacher and Millhouse, unpublished). Most probably this loss of activity is primarily due to photochemical isomerization (Roeser, unpublished). Therefore, leaf applications of LAB 144 143 and related compounds will not necessarily be suitable for all types of potential uses.

It was obvious to study the activity of LAB 144 143 and LAB 173 711 after application via the soil as an alternative to leaf application. This technique would widely eliminate the risk of photochemical decomposition and would be relatively close to certain irrigation systems such as drip irrigation. Results obtained by Markhard (1982) and Arteca et al. (1985) indicate that at least ABA can be applied via the substrate to induce stomatal closure in soybeans or geraniums. In addition to this technique, the responsiveness of various plant species had to be investigated. Most importantly, however, the extent to which plant productivity is affected by reduced water consumption had to be evaluated. The overall aim of the work presented here was to obtain in model trials further data as prerequisites for experiments under practical conditions.

ACTIVITY OF ABA AND ITS ANALOGUES AFTER APPLICATION VIA THE SUBSTRATE

Affinity of different types of soils for ABA, LAB 144 143 and LAB 173 711

2 ml of 10^{-4} M aqueous solutions of ABA, LAB 144 143 and LAB 173 711 were applied to columns (15 x 1.5 cm) of sand, loamy sand, sandy loam and peat. Upon elution with water, 5 ml fractions were collected, the biological activity of which was tested with a transpiration assay.

The different types of soils used in this investigation did not show a significant affinity for ABA and its analogues: in all cases the bulk of activity was observed in the first 10 ml of eluate and recovery was complete (data not shown).

Uptake of antitranspirants via the roots

The uptake of ABA and its analogues via the roots and its subsequent translocation were investigated using plants that were hydroponically cultivated in a growth chamber [temperature: 26°C (day), 18°C (night); 45% relative humidity; PAR about 240 μE x m^{-2} x sec^{-1}]. The leaf temperature - as a measure of transpiration intensity - was constantly recorded with an infrared thermometer (Everest Interscience, Tustin, Ca., USA).

It was found that ABA, LAB 144 143 and LAB 173 711 readily enter plants via the roots and lead to stomatal closure. Approximately 60 min after administration of the compounds to the nutrient solution (final concentration: 10^{-5} M), the temperature of the leaves of all species hitherto tested (*Helianthus annuus*, *Phaseolus vulgaris*, *Gossypium hirsutum*, *Cucurbita maxima*) was significantly increased within a few hours.

Long-lasting oscillations of the leaf temperature, which were paralleled by changes in stomatal resistance, were constantly observed with preanthesis sunflower plants. At a frequency of 10 to 20 min an amplitude of up to 9°C was obtained. A typical example is shown in Fig. 2. These changes were paralleled by variations of stomatal conductance between approximately 20 and 90 sec x cm^{-1}. Similar oscillations but mostly at a far lower amplitude are known (e.g. Hopmans 1969, Barrs 1971). Under the influence of the antitranspirants all parts of a leaf and all intact leaves of a plant oscillate almost synchronously. Rapid changes in the degree of stomatal aperture could only be observed in plants with a functioning root system. Detached shoots of sunflowers that were fed with nutrient solution containing the antitranspirants showed an increased leaf temperature but no oscillations. Likewise, cooling of the roots stopped the oscillating.

ABA is known to increase the hydraulic conductance of the roots as an integrated part of the plant's response towards drought (Fiscus 1981). Therefore, the observed oscillations in sunflowers could have been induced by the antitranspirants by making the roots pump more water into the shoot parts than was actually transpired via the more or less closed stomates. Once a threshold of hydrostatic pressure had thus been uniformly reached in the xylem vessels, the stomates had to open to reduce this pressure. (Details of the findings mentioned in this part will be published elsewhere).

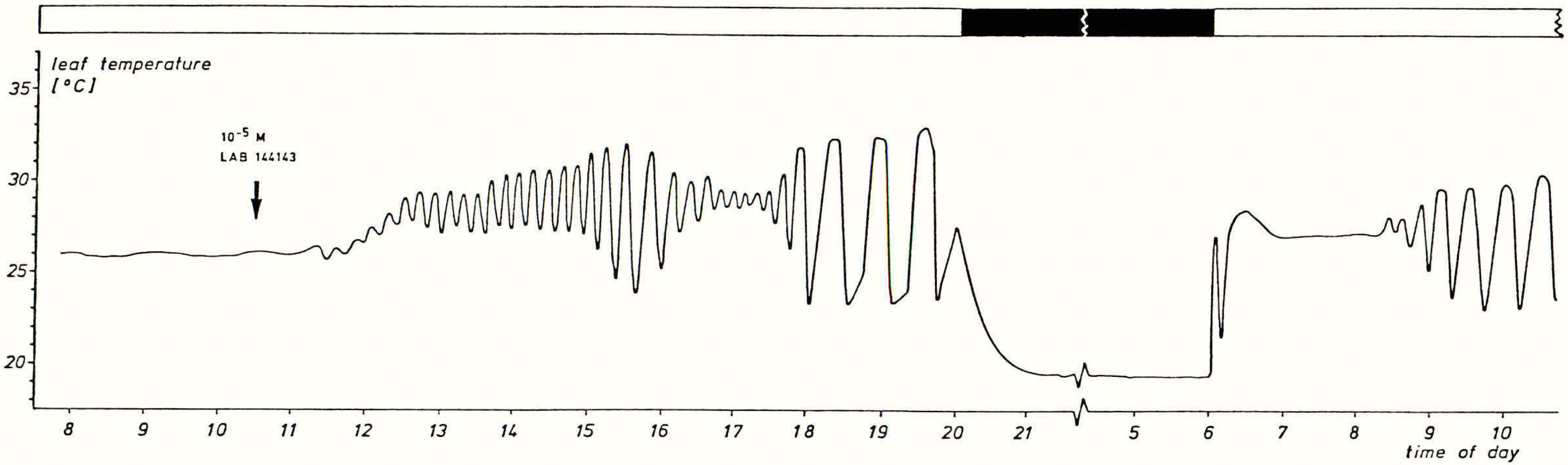


Fig. 2 Increase and oscillation of leaf temperature after the application of LAB 144 143

No information is presently available whether the rapid opening and closing of stomates is of ecological relevance. It may be speculated, however, that the plant manages to obtain a good supply of CO_2 for assimilation at a minimum expense of water by quickly opening and closing its stomates.

EFFICIENCY OF THE ANTITRANSPIRANTS IN DIFFERENT PLANT SPECIES

The responsiveness of different plant species was tested by applying aqueous solutions of ABA, LAB 144 143 and LAB 173 711 to detached leaves via their cut ends. Of the different plant species hitherto tested, C_3 plants generally responded relatively sensitively to the compounds under investigation. Higher doses had to be applied to achieve an equivalent effect in C_4 species. Details are given in Table 1.

Due to their physiological and anatomical properties, C_4 plants in general utilize water at a higher level of efficiency than do C_3 species (Kramer 1983). Therefore, the addition of antitranspirants may bring about only marginal effects.

PRODUCTIVITY OF PLANTS AT REDUCED LEVELS OF WATER CONSUMPTION

It has been claimed by many authors that a reduction in stomatal transpiration is directly connected to a lowered rate of photoassimilation since the degree of stomatal aperture would equally or even more intensively affect the uptake of CO_2 as compared with the diffusion of water vapour (e.g. O'Toole et al. 1977, Vaadia 1985). On the other hand evidence has been presented that the closing of stomates has more impact on the diffusion of water vapour than on the permeation of CO_2 and that factors other than stomatal conductance would primarily limit photoassimilation (Farquhar and Sharkey 1982, Fischer et al. 1986). To investigate the actual situation with the synthetic analogues, LAB 144 143, LAB 173 711 as well as ABA were applied to different C_3 plants and the resulting efficiency of water use was determined.

Production of vegetative biomass under the influence of antitranspirants

Plants of sunflowers, peas, soybeans, rye grass, and carrots were grown in Mitscherlich pots in a loamy sand-type soil under substantially outdoor conditions. Starting approximately 20 days after seeding, aqueous solutions of LAB 144 143 and ABA were constantly used for irrigation, maintaining the water saturation of the soil at the favourable level of 60%. The water consumption of the plants was recorded daily and corrected for evaporation from the soil surface. Approximately 10 weeks after seeding the dry matter of the shoots was determined. These data were brought into correlation with the amount of transpired water per unit of biomass as water use efficiency (WUE).

It was generally found that concentrations of LAB 144 143 and ABA of 10^{-5} M gave the best results: transpiration was clearly reduced while the production of dry matter and the morphological structure of the shoots were hardly affected. Consequently, the WUE could clearly be improved. These

TABLE 1

Transpiration by detached leaves of different C_3 and C_4 species under the influence of ABA, LAB 144 143 and LAB 173 711 (values in % of the respective control)

Plant species	Concentration applied (M)	Water consumption in the presence of ABA	LAB 144 143	LAB 173 711
Barley (C_3)	10^{-4}	18	23	22
	10^{-5}	22	35	24
	10^{-6}	38	67	62
	10^{-7}	57	94	92
Wheat (C_3)	10^{-4}	14	36	26
	10^{-5}	20	47	41
	10^{-6}	34	72	69
	10^{-7}	54	96	89
Rye (C_3)	10^{-4}	22	34	31
	10^{-5}	27	38	43
	10^{-6}	41	68	76
	10^{-7}	63	95	95
Maize (C_4)	10^{-4}	59	81	56
	10^{-5}	58	86	66
	10^{-6}	69	90	75
	10^{-7}	91	103	91
Sorghum (C_4)	10^{-4}	26	67	79
	10^{-5}	87	88	88
	10^{-6}	85	86	97
	10^{-7}	96	104	101
Sugar cane (C_4)	10^{-4}	-	89	-
	10^{-5}	-	95	-
	10^{-6}	-	108	-
Amaranthus retroflexus (C_4)	10^{-4}	36	-	41
	10^{-5}	61	-	90
	10^{-6}	83	-	91
	10^{-7}	100	-	104

TABLE 2

Influence of ABA and LAB 144 143 on different parameters of sunflower (cv. Spanners Allzweck) growth [a]

	Control	ABA 10^{-5} M	LAB 144 143 10^{-5} M
Transpiration per plant (ml)[b]	2 706 ± 83	2 487 ± 325	2 120 ± 706
Fresh weight per shoot (g)[c]	87.3 ± 8.9	95.8 ± 8.4	98.9 ± 9.6
Dry weight per shoot (g)	12.0	12.3	12.2
% dw of shoot	13.7	12.8	12.3
WUE $\left(\frac{\text{ml water}}{\text{g dw shoot}}\right)$	226	194	172
Length of shoot (cm)[c]	91.3 ± 3.6	94.4 ± 2.8	89.2 ± 2.9
Number of leaves per plant[c]	11.5 ± 0.6	11.2 ± 0.5	11.6 ± 0.4
Leaf area per plant (cm^2)[c]	889 ± 67	858 ± 76	895 ± 78
Chlorophyll a (mg/kg fw)	42.2	44.2	40.1
Chlorophyll b (mg/kg fw)	14.2	14.5	13.5
Total carotinoids (mg/kg fw)	7.7	8.6	7.5

a all values ± standard errors
b 3 repetitions with each 5 plants
c 15 parallels

findings confirm older results obtained with ABA (e.g. Jones and Mansfield 1972, Mizrahi et al. 1974). Concentrations of 10^{-6} M only slightly diminished water consumption whereas 10^{-4} M induced phytotoxicity. In all cases LAB 144 143 was more active than ABA. This is in contrast to short term experiments (cf. Table 1) in which ABA is the more active compound. This finding may indicate that LAB 144 143 is more slowly inactivated in the plant or in the soil than ABA.- Being representative of this series of experiments, the results with sunflowers are given in Table 2.

Grain production in wheat and barley under the influence of antitranspirants

Plants of spring wheat and spring barley were grown in Mitscherlich pots on a loamy sand-type soil substantially under outdoor conditions. LAB 144 143 and ABA were constantly applied with the irrigation water at concentrations of 10^{-5} M and 10^{-6} M. The water saturation of the soil was maintained either at 60% (good water supply) or at 40% (moderate drought stress). Anthesis was chosen to start with the application of compounds and with the drought stress in order to have all plants in identical vegetative states. The results obtained at maturation are shown in Tables 3 and 4.

TABLE 3

Yield formation in spring wheat (cv. Kolibri) under the influence of LAB 144 143 [a]

Treatment	Water transpiration per pot (ml)	Straw yield per pot (g)	Grain yield per pot (g)	WUE ($\frac{\text{ml water}}{\text{g grain}}$)
Control	11 680 ± 150	91.8 ± 2.0	49.3 ± 1.9	237
10^{-5} M	8 473 ± 353	89.9 ± 3.1	46.9 ± 2.7	181
10^{-6} M	11 007 ± 255	89.5 ± 2.3	52.9 ± 0.7	208

[a] 4 repetitions per treatment; values ± standard deviation

Under the influence of 10^{-5} LAB 144 143 transpiration in the wheat plants was reduced by 29%, while 10^{-6} M was only marginally effective. Since neither grain nor straw yield were significantly affected, WUE was improved considerably (with 10^{-5} M LAB 144 143 from 237 ml x g^{-1} to 181 ml x g^{-1}).

TABLE 4

Yield formation in spring barley (cv. Aramir) under the influence of ABA and LAB 144 143 at different levels of water supply [a]

Treatment	Water content of substrate (% of maximum)	Water transpired per pot (ml)	Straw yield per pot (g)	Grain yield per pot (g)	WUE ($\frac{\text{ml water}}{\text{g grain}}$)
Control	60	8 980 ± 495	67.9 ± 0.3	62.8 ± 3.0	143
ABA 10^{-5} M	60	6 265 ± 135	71.6 ± 2.0	59.6 ± 1.1	105
ABA 10^{-6}	60	8 645 ± 841	67.9 ± 7.4	60.3 ± 4.2	143
LAB 144 143 10^{-5} M	60	5 965 ± 21	67.8 ± 2.3	57.2 ± 2.0	104
LAB 144 143 10^{-6} M	60	8 035 ± 191	70.7 ± 1.4	62.3 ± 2.5	129
Control	40	5 035 ± 445	68.5 ± 1.8	61.0 ± 1.0	83
LAB 144 143 10^{-5} M	40	3 380 ± 467	68.0 ± 4.0	53.5 ± 6.4	63

[a] 2 repetitions per treatment; values ± standard deviation

The experiment with barley plants again demonstrates that LAB 144 143 reduces transpiration more actively than ABA. WUE was positively influenced by both compounds. However, it is noteworthy that transpiration is appreciably reduced - while the yield is still constant - just by offering less water to the plants. Accordingly WUE is improved in those plants that were cultivated at 40% water saturation of the soil as compared with the well-watered plants. This value could further be improved by administering 10^{-5} M LAB 144 143. These data clearly demonstrate that water is consumed relatively luxuriously and thus inefficiently when sufficient supplies are offered to the plants. Under the chosen conditions transpiration could be reduced by more than 60% by diminishing water supplies and simultaneously applying LAB 144 143. The concominant reduction of grain yield was in the range of only 9%.

PRODUCTION OF TOMATO FRUITS UNDER THE INFLUENCE OF LAB 173 711

Tomato plants were cultivated in a polythene tunnel. Three plants each were raised in a grow bag. Water, fertilizer and antitranspirant were applied to each plant via drip irrigation, maintaining a constant water content of the substrate.

LAB 173 711 in the concentration of 5 x 10^{-6} M and 10^{-5} M clearly reduced water consumption. While the vegetative shoot biomass was only slightly reduced at both rates, the higher concentration of LAB 173 711 proved to diminish and delay fruit formation too vigorously. Although fruit yield was reduced by approximately 9% at the lower concentration, WUE was improved from 97.1 l x kg $fruit^{-1}$ to 76.1 l x kg $fruit^{-1}$. The detailed results are given in Table 5.

TABLE 5

Yield formation in tomatoes (cv. Sonatine) under the influence of LAB 173 711 [a]

Treatment	Water consumption (l)	Fresh weight of shoots (kg)	Fresh weight of fruits (kg)	WUE $\left(\frac{\text{l water}}{\text{kg fw fruit}}\right)$
Control	2 380	33.0	24.5	97.1
1.0 x 10^{-5} M	1 130	27.0	12.5	90.4
5.0 x 10^{-6} M	1 690	30.0	22.2	76.1

[a] 33 plants per treatment; start of treatment: flowering of first cluster; end of experiment: flowering of 5th cluster; all fruits (ripe and unripe) were collected

This experiment, employing a cultivation system which by itself gives a high degree of economical water use, shows that practical applications of analogues of ABA are possible. Even a higher fruit yield might have been obtained if the concentration of nutrients had been raised in the irrigation water so that equal amounts of fertilizer would have reached both the control and the antitranspirant-treated plants.

CONCLUSIONS

The experiments dealt with in this contribution were carried out to evaluate the potential of compounds like LAB 144 143 and LAB 173 711 for practical uses. From the results obtained it becomes evident that such potentials do exist: especially C_3 plants respond relatively sensitively to these compounds. The antitranspirants may be applied via the soil if high light intensities impose the risk of photodecomposition after leaf application. Most importantly, however, reductions of water consumption by about 30% can be induced and are widely tolerated by the plants, thus clearly improving WUE.

It is obvious, however, that much more work is required before such compounds can be used in practice. Thus, it will be of special importance to investigate the performance of the synthetic analogues of ABA under relevant conditions in arid and semiarid areas.

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LIGNIN SYNTHESIS INHIBITORS : POTENTIAL TOOLS FOR IMPROVING NUTRITIONAL VALUE OF PLANT CROPS

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ABSTRACT

Lignins are the second most abundant polymer in the biosphere after cellulose. It would be particularly worthwile to reduce, even to a limited extent, the lignin content of forage crops in order to increase their digestibility and their nutritional value. In this way, we have designed potential inhibitors of the cinnamyl alcohol dehydrogenase, a specific enzyme of lignin synthesis, on the basis of structural analogies with the substrate and ability to bind Zn^{++}. The synthesized molecules were screened according to three different approaches :

- effects "in vitro" on the isolated enzyme,
- semi "in vivo" test, i.e. measurement of ^{14}C cinnamic acid incorporation into lignins of isolated xylem tissues,
- "in vivo" test on the lignin content of growth chamber cultivated plants sprayed or watered by solutions of the studied compound.

As an example, we present results obtained with two active compounds, N(O-hydroxyphenyl) sulfinamoyl tertiobutyl acetate (OHPAS) and N(O-aminophenyl) sulfinamoyl tertiobutyl acetate (NH_2PAS) which appear to inhibit very specifically the target enzyme.
Applied to young plantlets of maize, alfafa or <u>Bryona dioica</u> these compounds can, at a concentration of 80 µM, reduce the lignification without apparent damage for the plant. The interest of such inhibitors for basic research and for applied purpose will be discussed.

INTRODUCTION

Lignins are the second most abundant compounds in the biosphere after cellulose; they are supposed to represent around 20 % of the organic carbon. It is now clear that these molecules consist of aromatic nuclei. However, if the complete enzyme sequence leading from phenylalanine to the three monomeric units of lignins and finally to the polymer itself has been identified (Grisebach 1981) no final structure can be assigned to lignins. They are indeed very complex, non repetitive phenolic polymers and their structure is usually represented by models which emphasize the various types of potential linkages between the three different monomer units, the coumaryl, coniferyl and sinapyl alcohols. Moreover, the selective proportion of these mononers greatly vary from one plant to another one (Grand <u>et al.</u> 1982).

Lignification occurs in all vascular plants and plays important roles in the adaptative strategies of higher plants. Thus lignins are associated with water and solute conduction through xylem elements and bring mechanical resistance to cells and rigidity to plants. In addition, lignified cell walls are considered to be effective barriers to the progression of plant pathogens (Vance et al. 1980). For these different reasons it is not desirable to produce completely unlignified plants. Yet, the quite large variation in lignin content encountered in nature, even within a particular species (depending on the cultivar, the developmental stage or the environmental conditions) suggests that the lignin level of plants can be reduced to a certain extent without adversely affecting their development.

An induced reduction of the lignin content would be particularly adequate to improve the nutritional value of useful forage crops. It has been long known that lignins are the prime factor factor of cell wall indigestibility by cattle. The indigestible fraction of forage cell wall essentially comprises lignins and a part of cell wall polysaccharides that they protect from degradation. Thus, the apparent digestibility of plant materials decreases with advancing maturity and increased lignification, from up to 90% in young leafy herbage down to less than 40% in straws (Jarrige 1980). Secondarily, other cultivated crops would benefit from treatments likely to reduce lignification. This is the case of yam (Dioscorea dumetorum) in which lignification increases after harvesting the tubers making the resulting product uneatable after two or three weeks of storage (Sealy et al. 1985).

Different strategies can be used in order to obtain hypolignified plants. They include conventional genetic approaches, regulation of gene expression through molecular biology techniques or chemical inhibition of lignification key enzymes .In this article we will focus our attention on the latter aspect, i.e. the design of specific inhibitors of lignification which could be useful both for research and agricultural uses.

MATERIALS AND METHODS

The culture conditions of Populus x euramericana (Dode "cv I 214") have been described by Grand and Ranjeva, 1979. Maize (Zea mays cv INRA 400) and alfafa (cv "Milles Feuilles") were obtained in a growth chamber (22°C, photo period 15 hours, 10 000 lux, relative humidity 70%). The plants were grown on sand containing containers punctured at their basis (10 X 22 X 22cm). Every day the containers were partially immersed in a nutritive solution containing or not the inhibitors during a period of 4 hours.

Details on labelling experiments with ^{14}C cinnamic acid and on enzyme extraction, purification and assays have already been presented by Grand et al. (1985).

Lignin contents were measured in parallel by two different techniques : the gravimetric method of Klason (Effland 1977) and a modified spectrophotometric method of Johnson (Alibert and Boudet 1979). In all cases, the values obtained by the both methods were comparable on a relative basis.

Methods for the estimation of proteins, nucleic acids, chlorophylls, simple phenols and flavonoids have been described by Grand (1984).

Procedures for the synthesis of inhibitors and the determination of their chemical characteristics have already been described (De Blic et al. 1982, Duran 1985).

CINNAMYL ALCOHOL DEHYDROGENASE : A TARGET ENZYME FOR LIGNIN SYNTHESIS INHIBITORS

Molecules potentially able to reduce lignification have already been obtained. They inhibit phenylalanine ammonia lyase (PAL) (Amrhein 1981) or cinnamate 4-hydroxylase (Reichhart et al. 1982). However these two enzymes are involved in general phenylpropanoid metabolism rather than specifically related to lignification. In this way, α-aminooxyacetic acid and α-aminooxy-β-phenyl-propionic acid, potent competitive inhibitors of PAL, not only block lignin synthesis when applied to plants but also the production of different phenolic compounds (Massala et al. 1980, Amrhein et al. 1983) which can play important physiological functions. In addition, PAL inhibitors are also effective on pyridoxal containing enzymes and aminotransferases (see Grand et al. 1985).

Due to this lack of specificity, such inhibitors may induce many unexpected secondary effects and it is more appropriate to focus the attention on target enzymes specifically involved in lignification. These are cinnamoyl coenzyme A reductase (CCR), cinnamyl alcohol dehydrogenase (CAD), involved in lignin monomer synthesis and cell wall peroxidase involved in polymerization. As the particular properties of this last step are not yet well known peroxidase did not appear convenient for our purpose. The other two potential target enzymes, CCR and CAD, have been purified to homogeneity from soybean cell suspension cultures (Wyrambik and Grisebach 1979) spruce (Luderitz and Grisebach 1981) and poplar (Sarni et al. 1984). Despite the different taxonomic situation of the starting plant materials, the physico-chemical properties of these enzymes exhibit important homologies suggesting that the CCR and CAD have been highly conserved during evolution.

Concerning CAD, our results (Sarni et al. 1984) in agreement with the previous data of Wyrambik and Grisebach (1979) indicate the presence of zinc in the active site and the strict specificity towards C_6-C_3 substrates.

Having in mind that in brown midrib mutants of Sorghum, the lowered lignin content was associated with a reduction of the CAD activity (Bucholtz et al. 1980) we have selected this enzyme as a target for the design of lignin synthesis inhibitors.

DESIGN OF CAD INHIBITORS AND EFFECTS "IN VITRO" ON ENZYME ACTIVITY

It is obvious that a good knowledge of the active site of the enzyme and (or) of the catalysis mechanisms are prerequisites for an optimal definition of potentially active molecules. In our case, such complete data were not available. So we defined the putative CAD inhibitors on the following basis: ability to bind Zn++, the cation involved in the active center of the enzyme, and/or structural analogies with the substrates.

Among the various synthesized compounds, (more than two hundred) produced in collaboration with a group of chemists of our University[1], preliminary experiments led to the selection of five structures able to inhibit the CAD "in vitro" (TABLE 1).

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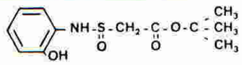
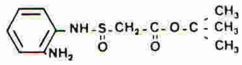
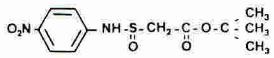
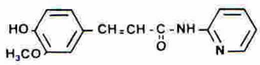
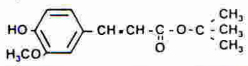
TABLE 1

Relative efficiency of inhibitors on CAD activity. Inhibitors were preincubated with the enzyme during 5 mins before starting the reaction. Column I : inhibitors tested on the conversion of cinnamyl alcohol to cinnamyl aldehyde. Column II : inhibitors tested on the opposite reaction

Abbreviated name	Chemical structure	I 50 (mM)	
		I	II
(1) OHPAS		0,5	2
(2) NH_2PAS		1	-
(3) NitroPAS		-	0.3
(4) FAP_2		-	0.14
(5) FTB		-	2

(1) N(o-hydroxyphenyl) sulfinamoyltertiobutyl acetate
(2) N(o-aminophenyl) sulfinamoyltertiobutyl acetate
(3) N(p-nitrophenyl) sulfinamoyltertiobutyl acetate
(4) N-(2 pyridyl)3-(4-hydroxy 3-methoxyphenyl)2-propene amide
(5) tertiobutyl ferulic acid

In order to determine if the inhibition of the CAD was reversible or not, the enzyme was preincubated for 5 mins with the inhibitors at the concentration of 2 mM then the mixture was dialysed against 0.1M Tris HCL buffer pH 7.5 for 14h at 4° C. This treatment did not cause a marked decrease of the inhibition level caused by OHPAS or NH_2PAS indicating that these inhibitors were bound extremely tightly to the enzyme. With the other compounds, the inhibition was completely (FTB, NitroPAS) or partially (FAP_2) restored after dialysis.

In addition, the kinetic studies have shown for OHPAS and NH_2PAS a dose dependent effect and a proportional increase of the inhibition with the preincubation time between the enzyme and these compounds before the addition of the substrate indicating that covalent modification has taken place. These observations, the irreversibility of the inhibition and the demonstration in a parallel work by Baltas (1984) that these two compounds are able to chelate zinc ions and are hydrolysed in alkaline medium, suggest that OH PAS and NH_2 PAS could act as suicide inhibitors. They could inhibit the target enzyme after hydrolysis at the enzyme active site and further covalent binding to reactive groups of the protein.

In the case of FTB and NitroPAS, two reversible inhibitors, the kinetic parameters estimated by the LINEWEAVER and BURK graphic method shown that these compounds act as competitive inhibitors of the substrate.

In order to test the "in vitro" specificity of the inhibitors, their effects were examined either on enzymes involved in phenolic metabolism and which act on C_6-C_3 compounds structurally very similar to CAD substrates or on metalloenzymes containing zinc ions in their structure such as ethanol dehydrogenase (Sekiya et al. 1977), superoxide dismutase (Asada et al. 1973) and carbonic anhydrase (Kandel et al. 1978). The enzymes were extracted from poplar stems, as shown in TABLE 2 all the inhibitors exhibited no affinity when tested against zinc metalloenzymes. However, most of the compounds acted, in addition to the CAD, on cinnamoyl COA reductase (CCR) and in the case of FTB, on phenylalanine ammonia lyase (PAL). As CCR is specifically involved in lignin synthesis the studied inhibitors, with the exception of FTB, can be considered as very specific.

TABLE 2

Effects of CAD inhibitors on different enzymes (preincubation time between inhibitors and enzyme : 5 mins)

Inhibitor	% of enzyme inhibition						
	PAL	OMT	COAL	CCR	EDH	SOD	CA
OHPAS (2 mM)	0	0	0	0	0	4	2
NH_2PAS (2 mM)	0	0	0	60	0	0	0
NitroPAS(0.3 mM)	0	0	-	0	0	-	-
FTB (2 mM)	60	0	0	28	0	0	0
FAP_2 (0.15 mM)	7	0	0	47	0	0	0

More detailed studies were then performed so as to estimate the "in vivo" effect of these inhibitors.

EFFECTS OF INHIBITORS ON "IN VIVO" LIGNIN LABELLING IN POPLAR PLANTS

A quantitative change of lignification is difficult to assess by the study of the net weight accumulation of the biopolymer during short-term experiments since the quantity actually synthesized is low with respect to the product already accumulated. In order to obtain information on the rate of lignin synthesis in presence of the inhibitors we measured the incorporation of ^{14}C cinnamic acid into lignins by sectioned shoots of 3 month old poplar plants.

The data of Fig. 1 outline that the incorporation of ^{14}C cinnamic acid into lignins significantly decreased (up to 60%) in treated plants depending on the nature and the concentration of the inhibitor used . Additional experiments performed on OH PAS and NH_2 PAS treated plants revealed that these inhibitors have no effects on the esterification of cinnamic acids with the cell wall components (soluble alkaline hydrolysis fraction).

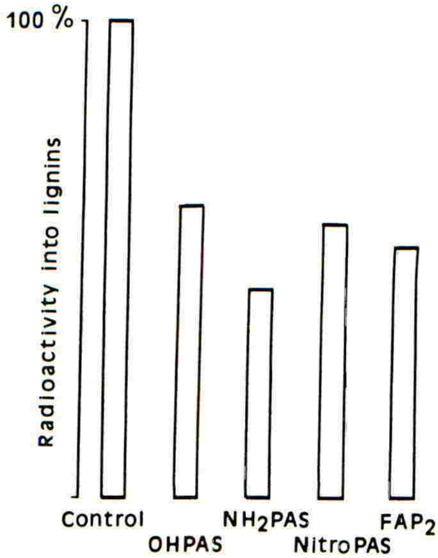


Fig. 1. Effects of different inhibitors (80 µM) on the incorporation of ^{14}C cinnamic acid into lignins synthetized by poplar stems during a 96 hours metabolisation period. The results are the means of three determinations with 7% confidence boundary.

EFFECTS OF CAD INHIBITORS ON THE LIGNIN CONTENT OF INTACT PLANTS

Besides the previous data demonstrating the efficiency of the inhibitors on the CAD activity "in vitro" or on lignin labelling when the molecules were provided to plants through sectionated stems, it was necessary to test their effects on intact plants. Indeed one important problem with plant growth regulators lies in their bioavailability which includes their capacity to cross biological membranes, their translocation (movement from one part of the plant to another) and their metabolism to provide inactive or more active molecules. Our assays were performed with the irreversible inhibitors, OH PAS and NH_2 PAS, by watering the plants or spraying their foliage with the nutritive solution enriched in inhibitors at different concentrations. A first set of experiments was performed on maize plants. Starting from the fifth day after germination the plants were treated every other day with a nutritive solution containing the inhibitor at a concentration of 80 µM. The lignin content of the treated "maize" shoots was greatly reduced (up to 45% with NH_2PAS) both on 20 day or 30 day old-plants. (TABLE 3)
For such a high lignin reduction, the plants loose their rigidity and the shoots bend. However the dry weight matter of the plants was not affected (TABLE 3). These results show that the tested molecules are able to depress lignification without reducing plant growth. In addition, they demonstrate the systemic effect of the studied compounds which once applied through the roots were active on the shoot system.

TABLE 3

Effects of OHPAS and NH_2PAS on lignin content and growth of corn plants (average values obtained from 20 plants samples)

Age of plants (days)	Lignin content % of the control		Dry weight matter g/plant		
	OHPAS	NH_2PAS	Control	OHPAS	NH_2PAS
20	62	60	0.30	0.31	0.29
30	60	55	0.41	0.42	0.40

Additional data were obtained using the same treatment procedure but lower concentrations of inhibitors on maize and alfafa (TABLE 4).

TABLE 4

Effects of OHPAS and NH_2PAS at different concentrations on lignin content of 30 day old maize and alfafa plants.(Average values obtained from 20 plant samples)

Inhibitor concentration (µM)	Lignin content (percent of control)			
	Maize		Alfafa	
	OHPAS	NH_2PAS	OHPAS	NH_2PAS
25	100	100	87	80
50	88	80	69	83

The two inhibitors were also active on alfafa plants and for a reduction of the lignin content ranging at around 20 %, no significant changes in the morphology of the plant were observed.
Similar results were obtained by spraying the foliage of the plants every day with a 50 µM solution of the inhibitors.
In order to check if the changes in lignin concentration observed on treated plants were associated with undesirable modifications of other biochemical constituents we have estimated comparatively different classes of metabolites in control and treated 30 days old maize plants (TABLE 5).

As shown on TABLE 5 no significant changes were observed for the different classes of compounds tested apart from a reduction of the flavonoid amounts and a slight increase in the polysaccharides content of treated plants. This last modification which was confirmed in additional experiments might in fact contribute to improve the nutritional value of forage plants.

TABLE 5

Effects of CAD inhibitors on concentration of representative classes of metabolites. Results expressed in mg/g dry matter (mg glucose equivalent for cell wall polysaccharides).

	Soluble proteins	Chloro-phylls	RNA	DNA	Cell wall polysaccha-rides	Flavo-noids
Control	49.4	7.4	19.5	1.4	225	48
OHPAS	49.5	7.8	21.1	1.3	235	28
NH_2PAS	49.3	7.7	19.8	1.4	245	25

Altogether the different data presented demonstrate that :
- the two inhibitors OHPAS and NH_2PAS are readily absorbed by the root or the shoot systems and are efficient lignin synthesis inhibitors when applied at concentrations around 50 µM,
- as expected from the great homology of CAD between different plant species, the inhibitors which are active on poplar CAD can reduce lignification in monocots and dicots,
- apart from their effects on lignification and in relation with their great specificity, the tested inhibitors do not induce marked changes of the biochemical composition of the treated plants.

DISCUSSION AND PROSPECTS

From man's stand point lignins are mainly an obstacle for the optimal transformation of the plant biomass. They decrease the digestibility and the quality of plant nutrients and they represent waste products of paper mills. As lignification seems to be a very flexible process both from the qualitative and quantitative points view, the lignin content can be to a certain extent manipulated in plants. A controlled reduction of the lignin synthesis does represent indeed an important challenge to improve the nutritional value of forage plants.

Three main approaches can be proposed to that end: the obtention of hypolignified cultivars by mutation or breeding, the repression of lignin synthesis genes in transgenic plants and the utilisation of chemical inhibitors.

Mutants of sorghum (Bucholtz et al. 1980) and corn (Kuc and Nelson 1964) with a lowered lignin content have already been obtained and as expected they are more digestible by ruminants than the normal cultivars (Kuc et al. 1968, Porter et al. 1978). However, they have also exhibited different agronomical limitations, particularly a low yield (Gallais et al. 1980). Moreover no easy methods are available for a routine screening of the required hypolignified cultivars at young stages of plant development.

The antisense RNA strategy seems very promising in this direction. Basically it consists in transforming plants with a polynucleotide corresponding to the inverse sequence of the gene under study. The expression of this "antisense gene" leads to "antisense mRNA" which hybridizes with normal mRNA and reduces the overall expression of the endogenous gene. Among the numerous potential applications of this technique in agriculture lignification represents a very convenient target and we are testing its feasibility for the repression of the CAD gene. However, this approach which has been recently demonstrated to be efficient in plant systems (Ecker and Davis 1986) is so far limited to easily transformable plants.

So, at the present time the use of chemical inhibitors remains the most general and flexible method to reduce the lignin content in plants .The results presented in this paper emphasize that specific molecules can be designed in this way. Due to their high specifity they can depress lignification without undesirable secondary effects.

At this stage the defined CAD inhibitors may be useful in fundamental research to assess the effects of lignification on differents aspects of plant development.

As an example De Jaegher _et al._ (1985) have shown that rubbing young internodes of _Bryona dioica_ resulted in a reduced elongation and in increased diameter of the internodes. Rubbing also caused an increase in lignin content and in the number of lignifying vessels. Using our inhibitors (OHPAS and NH_2PAS) De Jaegher _et al._ (unpublished) have shown that this induced lignification is responsible for the reduced elongation since the suppression of this lignin overproduction maintains the normal growth of the plant. These data are in agreement with the postulated role of lignins as a limiting factor of plant growth (Marigo and Boudet 1980).

Concerning the applied side and the use of such molecules in agriculture, it is clear that at present the required amounts of inhibitors and the frequencies of treatments are too excessive to provide a realistic means of reducing lignification on a large scale. The efficiency of the inhibitors must be further improved and all the aspects concerning the bioavailability of the compounds which are common to other crop chemical treatments must be deeply investigated. However the results already obtained show that the overall approach may potentially reach the defined goal i.e. reduce lignification without affecting the major functions of cultivated plants. In a further perspective and as lignin synthesis is particularly intense at certain stages of development, a special attention should be paid to the optimal period of treatment in the fields. Finally it is clear that such a type of treatment would be particularly adapted to specific production schemes as for example those associated to the industrial processing of forage plants into dehydrated nutrients for animals.

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THE ROLE OF ACETYLCHOLINE IN WHEAT GROWTH REGULATION.

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ABSTRACT

Field and laboratory investigations with the organophosphorus insecticide chlorfenvinphos show effects on the germination of wheat seeds and the later growth of the crop, leading to effects on yield. Chlorfenvinphos seed-treatments inhibited germination, causing delays in emergence of more than two weeks in the field. All methods of application to field-grown crops resulted in increased yield compared with untreated controls. The laboratory investigations suggest a mode of action for chlorfenvinphos in the delay of germination. The inhibition of acetylcholinesterase by the insecticide is linked with the delayed emergence of treated wheat seeds. The mode of action of acetylcholine in wheat germination is discussed. Apart from germination, acetylcholine may be involved in the regulation of other physiological processes in the wheat plant.

INTRODUCTION

McKinlay (1981, 1982, 1984) suggested that insecticides are able to exert a direct effect on the growth and development of cereal plants. Analysing the data from several years' field experiments on the use of insecticides to control wheat bulb fly (*Delia coarctata*) McKinlay (1981, 1982, 1984) concluded that wheat plants treated with organophosphorus insecticides were presented from expressing their full potential for grain yield. This phytotoxicity was symptomless in field-grown plants. This paper reports the results of field experiments to test the hypothesis that organophosphorus insecticides exert a direct effect on the growth and development of wheat plants. The reported occurence of acetylcholine and acetylcholinesterase in plants (Fluck and Jaffe, 1976) suggested to us that a mode of action of chlorfenvinphos in plants is as an inhibitor of acetylcholinesterase. The presence of this enzyme, and its inhibition by chlorfenvinphos, was investigated. The experiments were carried out in south-east Scotland during 1983-86.

LABORATORY EXPERIMENTS

Laboratory experiments have provided evidence of acetylcholinesterase activity in wheat seedlings; inhibition of cholinesterase by the organophosphorus insecticide chlorfenvinphos; and delayed germination linked to anti-cholinesterase activity of chlorfenvinphos. Cholinesterase activity in wheat seed extracts was determined using polyacrylamide gel electrophoresis (Andrews, 1986) followed by staining for acetylcholinesterase (Bunyan and Taylor, 1966). The observed activity and the extent of inhibition by chlorfenvinphos were quantified using the method of Ellman *et al* (1961) modified by UK (1979). Wheat seeds exhibited cholinesterase activity which was significantly inhibited by chlorfenvinphos. The insecticide inhibited wheat seed germination in laboratory tests carried out according to Perry (1978). Acetylcholine chloride solutions also significantly inhibited wheat seed germination (Molyneux and McKinlay, Manuscript in Preparation).

FIELD EXPERIMENTS

During 1983-84, 1984-85 and 1985-86, sites were chosen in south-east Scotland which had previously been free from wheat bulb fly attack so that the direct effect of the insecticide on the crop could be assessed. No other pest problems were found associated with the experimental areas or plants.

Chlorfenvinphos was applied to plots of 28m x 2m as the following treatments, 5 plots per treatment, 10 of untreated control.

CON	=	control			
SEEDT	=	chlorfenvinphos as liquid seed treatment			
SO 1	=	chlorfenvinphos as soil treatment	X1	recommended	rate
SO 3	=	" " "	X3	"	"
FS 1	=	" as foliar spray	X1	"	"
FS 3	=	" " "	X3	"	"
SO + FS	=	" as soil treatment plus foliar spray			

recommended rates: X1 = 5.6 l/ha Birlane
X3 = 16.8 l/ha Birlane

Germination

Germination was counted every week from sowing to full emergence, counting numbers of seedlings emerged per m^2 in three marked areas per plot. The commercially applied liquid seed treatment significantly delayed seedling emergence (Table 1). The soil treatments, SO 1 and SO 3, appeared to stimulate emergence in the first few weeks after sowing in the first two years field trials, although this effect was lost after a couple of months. We cannot account for the stimulated germination exhibited in the field. Possibly a temperature effect was involved, since the effect was only present in the first two years when the sowing date was very cold, whereas the third year was much wetter and milder.

TABLE 1 Seedling emergence with chlorfenvinphos treatments (21 days after sowing no. of seedlings m^{-2}).

	Year 1 (1983-84)	Year 2 (1984-85)	Year 3 (1985-86)
CON	72.8	33.1	191.4
SEEDT	19.9*	5.1*	109.4*
SO 1	100.7*	48.4*	177.3
SO 3	84.6	52.3*	192.7
SED	6.97	7.44	14.60

The delay of germination by the liquid seed treatment was evident throughout the winter, a delay of more than two weeks to reach a specified emergence being noted. Despite this by the beginning of the spring these plots exhibited the same total establishment as the control plots. All treated seedlings had more or less established to the same degree at the start of spring.

Plant growth and yield

At harvest, various parameters contributing to final yield were assessed. Final yield, or weight of grain per unit area, depends on a number of interrelated parameters including the size of the individual grains (assessed as thousand grain weight), the number of grains per spikelet, the number of spikelets per ear, and the number of ear-bearing shoots per unit area. These parameters were assessed on 10 half-metre rows per plot at harvest. The plant growth and harvest results for the 1983-84 season only are presented. The other two years' results are not available due to an unprecedented wheat bulb fly attack at the experimental site (wheat after oilseed rape) in 1984-85 and an appallingly bad season of weather in 1985-86.

All insecticidal treatments, regardless of method of application, resulted in a yield which was higher than the control (Table 2). The lower rate of foliar spray (FS 1), the liquid seed treatment (SEEDT), the higher rate of soil treatment (SO 3) and the combined treatment (SO + FS) resulted in yields which were significantly ($p<0.05$) higher than the untreated control (Table 2). It can be seen that those treatments which might be expected to have a high residue of chlorfenvinphos in contact with the plants in spring resulted in a high yield. An exception to this is the higher rate of foliar spray (FS 3). It was observed, however, that application of this treatment caused severe scorching and death of many leaves and tillers. It is, therefore, surprising that such a high yield was achieved.

TABLE 2 Harvest Yield, 1983-84

Weight of grain/metre row of crop	
CON	58.7
SEEDT	69.1*
SO 1	59.2
SO 3	68.4*
FS 1	70.4*
FS 3	59.5
SO + FS	66.8*
SED	1.94

There was no significant ($p>0.05$) effect on thousand grain weight (Table 3), indicating that seed size was not affected, but the three highest yielding treatments, FS 1, SO 3 and SEEDT exhibited the highest number of grains/ear (Table 3). The weight of grains per ear (Table 4) was therefore high in the same three treatments. The number of ears/m length of row was affected by the foliar spray treatments (Table 4). Although there were no significant ($p>0.05$) differences, the treatments which received a foliar spray had a higher number of ears/m row of crop (Table 4). Consequently, the high yield of the foliar spray treatment FS 1 is a high result of the combination of a high number of ears/m and the highest grain weight per ear.

TABLE 3 Harvest parameters 1983-84 I. Thousand grain weight and number of grains per ear

	Thousand grain weight (g)	Number of grain/ear
CON	50.2	31.6
SEEDT	50.6	33.7
SO 1	51.3	31.6
SO 3	50.9	35.3
FS 1	51.0	34.6
FS 3	49.8	30.5
SO + FS	49.5	30.3
SED	1.00	1.17

TABLE 4 Harvest parameters 1983-84 II. Weight of grains/ear and number of ears/m

	Weight of grains/ear (g)	No of ears/m
CON	1.27	46.8
SEEDT	1.47*	44.8
SO 1	1.22	45.1
SO 3	1.42*	46.7
FS 1	1.47	47.2
FS 3	1.20*	47.5
SO + FS	1.31	49.6
SED	0.06	2.01

The causes of the increased yield could be traced back through the 1983-84 season. There was no significant ($p>0.05$) effect of application of foliar sprays on the number of leaves or tillers per plant, tiller number being only slightly reduced by foliar spray, but overall size of plants was affected. Total leaf length, which can be used as an indication of photosynthetic area, was increased by foliar sprays during the late spring and summer (Table 5). When the leaves began to die back, the foliar spray-treated plants were also taller than control plants in June (Table 6). This increase in size of foliar spray-treated plants was evident as early as May, when FS 1 had the highest above ground dry weight (Table 7).

TABLE 5 Total leaf length per plant, 1983-84 (in cms)

	14 May	9 July
CON	36.0	29.2
SEEDT	35.2	31.9
SO 1	35.4	30.7
SO 3	34.6	33.5
FS 1	36.3	34.6*
FS 3	36.1	34.7*
SO + FS	37.0	33.9*
SED	2.08	2.27

TABLE 6 Mean plant height 1983-84

	7 May	11 June	19 June
CON	13.4	44.0	54.4
SEEDT	13.4	45.1	55.0
SO 1	13.2	44.1	54.9
SO 3	13.1	45.2	56.8
FS 1	13.5	47.5	58.2*
FS 3	12.7	44.7	56.3
SO + FS	13.9	47.5	55.9
SED	0.76	1.71	1.69

TABLE 7 Aerial parts, dry weight/m row (g), 1983-84

	27 May	28 June
CON	16.3	20.9
SEEDT	15.5	23.7
SO 1	14.9	19.5
SO 3	15.7	24.0
FS 1	17.3	24.3
FS 3	15.2	21.9
SO + FS	16.6	24.8*
SED	1.86	1.83

The highest yielding treatments exhibited higher harvest index values than the lower yielding treatments, (Table 8) indicating that a greater proportion of assimilate was directed to the growth of the grain than the vegetative parts.

TABLE 8 Harvest Index (Proportion of the total dry matter of the crop in the grain)

	%
CON	62.9
SEEDT	64.8
SO 1	63.1
SO 3	63.8
FS 1	64.7
FS 3	63.2
SO + FS	63.8
SED	2.9

DISCUSSION

There appear to be two effects of the insecticide chlorfenvinphos on the growth of winter wheat. The first is an inhibition of germination resulting in delayed emergence of seedlings in the field. The second effect involves the growth and development of the crop, leading to increased yields.

We believe delayed germination results from insecticidal inhibition of acetylcholinesterase activity. Our interpretation is that acetylcholine is a native growth regulator, and the inhibition by exogenous retardants of the plant acetylcholinesterase results in increased levels of acetylcholine.

The mode of action of acetylcholine as a growth regulator has yet to be elucidated. Jaffe (1972) supports the view that acetylcholine in the plant substitutes for red light and is able to regulate phytochrome-medicated events. However, germination responses to light regime, though common in weeds, have been demonstrated in few cultivated species, small-seeded vegetables such as lettuce being a notable exception (Borthwick et al, 1954). We consider a phytochrome response in germination to be unlikely, due to selection by plant breeders for homogenous germination independent of light regime in cereals.

We believe that a more likely mode of action for acetylcholine as a germination inhibitor is in interference with gibberellin biosynthesis. The growth retardant, AMO-1618, which is similar to acetylcholine in that it possesses a quarternary ammonium group, acts by interrupting gibberellin biosynthesis, but not with the action of gibberellin once formed (Dicks, 1979). Gibberellin is essential for mobilization of amylase in germinating seeds and for stem elongation. It is possible that acetylcholine also acts by regulating gibberellin biosynthesis.

The mode of action of chlorfenvinphos which results in increased yield has yet to be elucidated. Observed effects with other plant growth regulators include a stimulation of photosynthetic activity, an extension of leaf area duration and an alteration in the pattern of assimilate distribution (Hill, 1973). Our investigations did not involve a measurement of photosynthetic rate, but our field data have shown both an extension of leaf area duration and an increase in the harvest index of those treatments achieving the highest yields. This suggests that plant physiology had in some way been affected.

The possibliity that chlorfenvinphos exerts its effect via inhibition of acetylcholinesterase activity during later growth of the plant, remains to be investigated. Since acetylcholine interacts specifically with a receptor site in animal nerve cells, a search for a similar receptor site for acetylcholine in plants would greatly improve our knowledge of the mode of action of acetylcholine in plant growth and development.

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TRIACONTANOL RESEARCH IN PERSPECTIVE

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ABSTRACT

Triacontanol (TRIA) is a C_{30} primary alcohol and a natural constituent of plants. It increases the growth and sometimes the yield of many crop species. To date TRIA has not been sufficiently consistent in improving yields to warrant recommendation for field use in the U.S.A. With our present knowledge, the most effective results in the field may be obtained by applying a colloidal dispersion formulation, at concentrations of 0.1 to 10.0 μg/l in 10 to 1000 l of water per hectare. For optimum yield enhancement the spray should be applied between 1300 and 1700 HR, and on a day having a favorable temperature and soil moisture conditions for the growth of the crop being treated. TRIA applied to the shoots of rice seedlings elicits the formation of a messenger(s), which we have named TRIM (TRIA messenger). The TRIM or a signal elicited by TRIA moved 5 to 25 cm from the shoots to the roots within 1.0 min.

INTRODUCTION

After 10 years (1977 to 1986) of research with Triacontanol (TRIA), it is apparent that TRIA typifies many of the problems we have with both naturally occurring and synthetic plant growth regulators (PGR's). Although growth and physiological responses to TRIA can be clearly measured by individual researchers, often they cannot be repeated by others. In the case of yield enhancement, even those researchers who can show this effect fail to reproduce yield increases consistently. Although much has been learned about how hormones or PGR's affect growth, development and physiological systems, we still do not know the precise mode of action of any of these hormones and PGR's. TRIA is no exception. We have learned much about the effect of the physical and biological environment on the response of plants to TRIA, and understand much about how it affects the physiology of plants, but we still cannot obtain consistent yield enhancement in field trials.

The objective of this report is to briefly review what we know about TRIA and its effect on yield, plant growth and metabolism.

Crop yield

There have been more failures than successes in attempts to increase the yield of crops with field applications of TRIA in the United States. Success in our program has increased with improved formulations, knowledge about inhibitors of TRIA activity and an understanding of environmental factors that affect the activity of TRIA. However, the percentage of successful field tests is still less than 50%.

Our initial field research showed a significant increase in yield of several crops (Ries, et al., 1978). For example, the yields of dry beans, sweet corn and cucumbers were significantly increased 9%, 24% and 15%, respectively over the controls. Although Bhalla (1981) reported that TRIA increased the growth of maize and tomato seedlings in the greenhouse, the results from 46 field experiments generally showed no significant increases in crop yield. The only exception was a test in Japan where the yield of rice was increased 17% to 21% by soil applications of 0.057 to 4.0 g/ha of TRIA. Mamat, et al. (1983) found that TRIA applied as a soil drench promoted the growth and development of tabasco pepper both in the greenhouse and field. The increased yield of peppers in this research was due to an increase in the number of fruit per plant. Averaging the 3 harvests over both years of the study indicated that the average increase in yield for TRIA applied at rates of 0.01, 0.10, 1.0, 1.25 and 2.50 mg/l was 31, 36, 31, 51 and 23%, respectively.

Subbiah, et al. (1980) in India obtained a significant increase in tomato yield with both alfalfa and TRIA applications. The best TRIA treatment not only increased the yield 165% but also the ascorbic acid concentration in the tomatoes by 23%.

Lim (1982, 1983a,b) in South Korea reported on the effectiveness of foliar applications of TRIA-acetone formulations containing $CaCl_2$. He obtained significant increases in the yield of rice, cucumber, pepper, tomato, cabbage and Chinese radish. For example, the yield increases in rice, cabbage and radish were 16, 80 and 105%, respectively over the controls. The weight of sugarbeet roots at harvest was increased by TRIA applied to young seedlings without decreasing concentration of sugar in the roots. In Korea the growth and yield of both tomatoes and peppers were increased consistently with seed treatment of TRIA at 10 μg/l (Lim, 1982). The increase was due to more fruits, not higher fruit weight.

The most extensive use of TRIA on vegetable and agronomic crops has been in the Peoples Republic of China (PRC). Researchers at the Institute of Petroleum Chemistry at the Heilongjiang Academy of Sciences in Heilongjiang Province sprayed over 200 hectares of 26 crops in 1981. They reported statistically significant (6 to 18%) increases in yield of cereal crops and a 20% increase in yield of sugarbeets. The beets also yielded 10 kg more sugar per metric ton. Rice yielded more and matured 8 days earlier when treated with TRIA.

Scientists at the Jiangxi Engineering College conducted research on TRIA for 5 years and studied crop yield and physiological responses. In addition, over 100 different formulations were tested, as well as methods for improving the extraction of TRIA from beeswax. They now produce an average of 1.0 to 1.5 kg of pure Triacontanol per day. Sufficient TRIA was sold by this laboratory as a 0.1% emulsion for application to 67,000 ha in 1983. It is recommended for many crops at a concentration of 50 to 100 μl/l.

Near Harbin in the PRC large replicated plots were treated by aerial application in 1981 and 1982. For the 2-year period the average increase in yield for all rates of TRIA over the controls for the 1700 ha sprayed was 13%. In 1981 and 1982 lengthy reports of TRIA research in the PRC were published by the Jiangxi Agricultural College and Jiangxi Organic Chemistry Institute. Data selected from these reports show that the yield obtained from an application of 0.1 mg/l of TRIA averaged 11.5% higher than the controls for all crops (Table 1). The highest rate of 1.0 mg/l increased only the yield of wheat.

TABLE 1

Yield of several crops in Tian Ching, PRC after foliar applications of TRIA formulated as an emulsion

TRIA (mg/1)	Crop yield (kg/ha) Wheat	Rice	Maize	Sunflower	Peanut	Avg. change from control %
Control	5000	5370	5034	2795	2567	--
0.1	5166	5985	6049[a]	2884	3069[a]	+11.5
0.5	5168	5610	5593	3184	2640	+ 7.0
1.0	5662[a]	4448	4880	2584	2854	- 1.3

[a]LSD for difference from controls significant at 5% level.

In our most recent field tests (1981-1984) the yield response of crops to TRIA applied as a colloidal dispersion was tested with 13 different crop species in 45 field experiments over a 3-year period. Foliar application of TRIA resulted in treatment effects with 11 of the 13 crops and in 30 of the 45 experiments. The average yield increase was 14% with the optimum TRIA concentration in tests where yield was significantly increased and was 5% over all 45 experiments. In 7 experiments significant yield decreases averaging 10% were measured with TRIA concentrations that increased crop yield of the same crops in other tests.

The results of selected tests where TRIA increased the yield are shown in Table 2. The most effective TRIA concentrations with the colloidal dispersions generally were 0.1 to 1.0 μg/liter. These data clearly emphasize previous observations on seedling growth and crop yield that when TRIA does increase growth or yield in dose-response tests the increase is quadratic. The higher application rates usually do not increase growth or yield.

TABLE 2

Yield (MT/ha) of crops treated with several concentrations of colloidally dispersed TRIA

TRIA μg/1	Dry bean	Field corn	Potato	Melon	Onion
0	1.60	10.09	31.5	13.5	14.5
0.01	--	10.23	--	15.4	17.1[a]
0.1	1.65	10.64[a]	34.3	--	--
1.0	1.62	10.31	37.3[a]	18.8a	15.3
10.0	1.84[a]	10.17	35.0	--	--
100	1.66	10.01	34.0	18.7[a]	--

[a]F value for comparison with control significant at 5% level.

After several years and the involvement in hundreds of field tests, it is clear that TRIA sometimes increases yield, but we do not know how to use TRIA to obtain sufficiently consistent results to recommend its use.

One of the unusual and potentially important observations concerning TRIA is that most important agronomic and horticultural crop species have been shown to respond favorably, if not consistently, to small quantities of exogenously applied TRIA. The effect of TRIA is not specific for the plant part harvested. Vegetative, grain, fruit, bulb and tuber yields have been increased by TRIA.

Growth responses

The first observed effect of TRIA applied to the foliage of maize and rice seedlings is a rapid increase in leaf area and dry weight. The youngest leaf is visibly larger and/or other leaves are wider within 10 to 20 min after treatment (Ries and Wert, 1982). An apparent increase in total nitrogen of whole plants can also be measured within 1 h.
The consistency of this rapid response may be illustrated best by a summary of all short-term tests conducted in our laboratory over a 4-year period (1978 to 1981), except those used in a paper on the subject (Ries and Wert, 1982). All of the tests not included in this study were summarized, and the variance analyzed using the individual tests as replicates, regardless of whether there were statistically significant differences for the individual tests (Table 3).

TABLE 3

Summary of the dry weight average for all *in vivo* and *in vitro* tests conducted (1978-1981)

	Type of test		
	In vivo		In vitro
Treatments	Corn (mg/shoot)	Rice (mg/plant)	Corn (mg/ml)
Controls	76.4	76.5	3.50
TRIA	87.9[a]	86.0[a]	3.83[a]
Coefficient of variation	7.7%	5.6%	5.5%
Average time of treatment (min)	58	50	120
No. of tests	11	7	29

[a]F value for comparison of control with TRIA significant at 1% level.

The average time for determining significant increases in dry weight was less than 10 min for tests with whole maize and rice seedlings and 2 h for *in vitro* tests with maize.

The initial response of seedlings to TRIA is independent of light (Ries and Wert, 1977). Several studies showed that rice seedlings treated with 10 μg/l of TRIA in nutrient cultures increased in leaf area and dry weight whether grown in the light or dark. The response in the dark occurred within 3 h of treatment, but was eliminated by removing CO_2 from the atmosphere (Bittenbender, et al., 1978). After 6 h in the dark TRIA had increased the Kjeldahl nitrogen concentrations resulting in 30% more N per plant than controls. This activity of TRIA in the dark, particularly if it could be explained, should be helpful in determining the mode of TRIA action. For example, it indicates that the initial effect of TRIA is not involved directly with photosynthesis.

Early research with TRIA established that the 28-C primary alcohol, octacosanol, would not stimulate seedling growth (dry weight) (Ries, et al., 1977). As analytical techniques with GLC were refined, octacosanol was found to be contaminated with about 1% TRIA. The concentrations of octacosanol used in bioassays of its activity were sufficiently high that there was more than sufficient TRIA, as a contaminant, to stimulate growth. The obvious hypothesis to explain this observation was that the octacosanol was inhibiting TRIA activity. An extensive series of tests showed that equimolar concentrations of octacosanol, and all other long-chain compounds tested inhibited the activity of TRIA. Octacosanol also inhibited TRIA activity at less than equimolar levels. For example, 10^{-10} M octacosanol applied in the nutrient media with 10^{-8} M TRIA consistently inhibited TRIA activity. Later studies showed that octacosanol applied to the foliage of rice seedlings inhibited the activity of TRIA applied to the roots. The reciprocal experiment showed that octacosanol applied to the roots inhibited TRIA applied to shoots. Further research with rice showed that octacosanol inhibited TRIA activity if applied to the shoots prior to TRIA application to the roots, but not if applied to the shoots after TRIA application to the roots (Table 4). Thus, either TRIA or octacosanol moves very rapidly in rice seedlings, or it elicits a messenger that moves quickly to the site of TRIA activity. This rapid inhibition of TRIA activity by such low rates of very closely related compounds makes octacosanol and other long-chain alcohols excellent inhibitors for studies with TRIA.

TABLE 4

Response of 17-day-old rice seedlings, 24 h after treatment, to TRIA applied to roots before, after and at the same time as octacosanol is applied to the shoots

Chemical	Time of application (HR)		Dry weight	
	TRIA (100 µg/1 applied to roots)	Octacosanol (100 µg/1 applied to shoots)	(mg/shoot)	(% increase over control)
None	--	--	44.9	--
TRIA	1400	--	50.3	12.0
TRIA + octacosanol	1400	1400	48.1	7.1
TRIA + octacosanol	1400	1300	45.5	1.3
TRIA + octacosanol	1300	1400	50.3	12.0
LSD 5%			2.6	
LSD 1%			3.5	

Periodically, it has become difficult to reproduce the TRIA response in laboratory experiments. Further investigation has shown that residues of morpholine (tetrahydro-2H-1,4 oxazine), a strong oxidizing agent used to clean steam lines, was the cause of inhibition in our laboratory. Distilled water in our laboratory, as is often the case, was obtained from redistilling condensed steam. Distilled water now is prepared from tap water which has been demineralized prior to distillation.

The observation that the previously discussed chemicals inhibit TRIA activity led to the idea that lipophilic substances present in field sprayers may interfere with TRIA activity. Tests which were conducted to test this hypothesis established that some of the failures in the field were probably due to the presence of phthalates in sprayers and water. TRIA dispersions passed through two standard field sprayers and three of five different experimental small plot sprayers lost at least 30% of their activity as measured by the increase in maize seedling growth 4 to 7 days after TRIA application. Hexane extracts of water passed through polyvinyl chloride (PVC) tubing removed from these same sprayers contained more than 5.0 μg/l of 2-diethylhexyl phthalate. This phthalate ester was shown to inactivate the colloidal dispersion of TRIA at phthalate concentrations of 5.0 μg/l (Ries, et al., 1984). Sprayers equipped with natural rubber hoses did not inactivate the TRIA as measured by the same corn bioassay. The lack of consistent results by many researchers may be due to inhibitory substances (possibly phthalate esters) in their sprayers or in their water supply.

An attempt was made to establish a consistent environmental protocol for treating seedlings. Several tests showed that air temperature around maize and rice seedlings before treatment was important. Plants in growth chambers were subjected to 15, 25, or 35°C for 1 h before and after spraying with TRIA. Response was always positively linearly correlated with the ambient air temperature before spraying (Ries, et al., 1983). These and other tests in the greenhouse and field suggest that TRIA may be more effective if field applications are made on a warm day.

Other studies have shown that the time of day TRIA was applied may be critical. Tests were conducted with identical 16 h photoperiods in both the greenhouse and in growth chambers. Maize growth 7 days after treatment averaged 77% greater when TRIA sprays were applied at 1700 HR compared to applications at 0900 and 1300 HR in greenhouse experiments. In contrast, foliar applications to maize seedlings in growth chambers consistently showed that the maximum response from TRIA applications at 1300 HR averaged twice as much as from applications at 0900 and 1700 HR (Ries, et al., 1983). Field studies with wheat on time of TRIA application were conducted over a 3-year period. There was a small (4%) but statistically significant increase in yield from TRIA applications made at 1700 HR compared to 0900 and 1300 HR (Biernbaum, 1986).

Physiological effects

TRIA rapidly increases the total dry weight of rice and corn seedlings within a few minutes (Ries and Wert, 1982). The increase in dry weight is accompanied by similar rapid increases in leaf area, and concentrations of reducing sugars, free amino acids, soluble protein and reduced nitrogen (Ries and Wert, 1982). In one test with corn the increase for these parameters, 1 h after treatment, was from 20% to 45% more than untreated controls or the zero time treatments.

Plasmalemma-enriched Ca^{2+} and Mg^{2+} dependent ATPase activity increased after application of TRIA to the roots of barley seedlings *in vivo* and *in situ* (Lesniak et al. 1986) Ca^{2+}- and Mg^{2+}- dependent ATPase activity was 64% and 85% higher, respectively, in the roots of seedlings germinated in the presence of 1.0 μg/l of TRIA compared to the controls. Simultaneous additions of equimolar amounts of TRIA and octacosanol to cell-free extracts inhibited ATPase stimulation by TRIA. The addition of 10^{-7} M CAM to barley root membrane vesicles increased ATPase activity 45%. The further addition of TRIA to this mixture resulted in a further increase of 20% in ATPase activity

(Lesniak, et al., 1986).

TRIA applied to Chlamydomonas reinhardtii grown at 5% CO_2 increased cell density, total chlorophyll and photosynthetic CO_2 assimulation (Houtz, et al., 1985). TRIA also increased the specific activity of RUBP in Chlamydomonas cells. Octacosanol inhibited these effects.

TRIA messenger

Extensive tests have shown that octacosanol and all other long-chain compounds tested inhibited the activity of TRIA. Further research with rice showed that octacosanol inhibited TRIA activity if applied to the shoots prior to TRIA applications to the roots, but not if applied to the roots after TRIA applications to the shoots. Thus, either TRIA or octacosanol moves very rapidly in rice seedlings or it elicits a messenger that moves quickly to the site of TRIA activity.

Applications of octacosanol were made to the foliage of 15-18 day-old rice seedlings and the roots were harvested after 100 min. It was found that octacosanol elicited the formation of a water soluble compound (OCTAM) that inhibited the activity of TRIA, but did not stimulate plant growth. TRIA was found to elicit the formation of a water soluble compound (TRIM) that stimulates plant growth. TRIM is not inhibited by OCTAM or by octacosanol. The crude extract of these water soluble messengers is active at less than 10 mg/l.

The formation of TRIM in the roots and shoots of rice seedlings occurs within 1 min of application of TRIA to the shoots in the light. Applications of TRIA to seedlings growing in the dark slows down the appearance of TRIM, but does not stop it. This rapid formation of the messenger, and the previously reported rapid increases in plant metabolism after TRIA treatment, is difficult to explain. Efforts to find possible artifacts in our purification procedures and bioassays have been without success.

The formation of TRIM may be due to either the direct action of TRIA, or TRIA may elicit a signal that rapidly moves throughout the plant. Regardless of the mode of TRIA action, the rapidity of this action indicates that the formation of TRIM is due to a rather simple chemical reaction such as oxidation or hydrolysis of a chemical already present in the plant cells. Partial purification of TRIM using column and high performance liquid chromatography has shown that there are at least two hydrophillic, low molecular weight messengers (chemicals), which also increase the dry weight of whole plants.

DISCUSSION

TRIA and other long-chain compounds are present in the environment wherever there is organic matter. In fact, more TRIA is naturally in or on plants than the amount applied, although it is largely bound in a lipophillic matrix.

With our present knowledge, the most effective results in the field may be obtained by applying the colloidal dispersion formulation of TRIA at concentrations of 0.1 to 10.0 µg/l in 10 to 1000 l of water per hectare. The spray should be applied between 1200 and 1700 HR after a rain or irrigation, and on a day having a favorable temperature for the growth of the crop being treated. The best time of application, based on our present knowledge, is probably at any time when other conditions are optimum between the 3 to 4-leaf stage and anthesis, in the case of flowering crops. Any method of application that distributes the spray accurately is satisfactory. The water used for spraying should be free of other chemicals, particularly long-chain compounds and phthalate esters. Any PVC tubing on sprayers should be replaced, preferably with natural rubber. These same conditions will give the best results for both *in vivo* and *in vitro* studies in the laboratory or greenhouse.

The rates shown to be effective in increasing plant growth in the laboratory and crop yield in the field are extremely low. At the lower rates and volumes of the colloidal dispersion that have been shown to be effective, less than 1.0 mg/ha is needed. Under the same conditions an application of just a few grams per hectare would probably be ineffective. If 1.0 g/l proved to be the optimum rate for TRIA use on crops, it would be necessary to use only 2.0 mg of TRIA in a 200 l spray tank. It is conceivable that the lipophilic materials left as residues in sprayers would be nearly impossible to clean out of experimental or commercial sprayers and these could bind or inactivate TRIA. In this case, a radically different approach may be required for TRIA to be used successfully for increasing crop yields. This may require the development of a formulation that will be resistant to interference by other chemicals, or by applying the TRIA in small quantities via a very "clean" system.

Efforts have been made to further delineate the effect of TRIA on plant growth and metabolic responses. In many cases TRIA shows pronounced effects on plant growth, crop yield, and several physiological processes in plants but results are inconsistent. This has not been unusual during early investigations of other plant growth substances, particularly when searching for their definitive modes of action. The pervasiveness, low water-solubility, chemical contamination and the difficulty in formulating TRIA have undoubtedly contributed to this inconsistency. The voluminous research conducted on the known plant hormones has revealed a specific mode of action in only a few cases, such as the effects of gibberellins on barley aleurone layers. Even these cases are only valid for unique assays in a few species. The recognized modes of action explain only a few of the known effects of the plant hormones studied.

The rapidity of the TRIA responses suggests that TRIA may be quickly absorbed into the plant and probably is active in an unaltered form. TRIA may act on the membrane in such a way that an enzyme, enzymes or messengers are triggered causing a cascading effect resulting in increased metabolism and the accumulation of various critical intermediary metabolic compounds, which result in an increase in dry weight and growth. It is also clear from the inhibitor studies that the TRIA, or the messenger causing these effects, moves very rapidly in the plant both acropetally and basipetally.

An alternative hypothesis is that TRIA rapidly acts on the membrane which produces a signal that passes quickly throughout the plant. This signal causes the formation of TRIM, which in turn is responsible for the observed growth and metabolic changes measured.

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4. Grassland Management

Chairman: R. TAYLOR

BIOLOGICAL RESPONSES OF AMENITY GRASSES TO GROWTH REGULATORS

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ABSTRACT

Control of unwanted vegetation has long been a costly and time consuming job. With the advent of efficient mechanical mowers the ability to manage vegetation while maintaining an aesthetically pleasing landscape has been greatly increased. Herbicides have been used for total vegetation control and to adjust the landscape to more desirable species through broadleaf weed control and brush control. At low rates these products have been used to suppress growth of turf swards. However, most of the herbicides result in significant phytotoxicity. Growth regulators which do not exhibit phytotoxicity often result in an off color appearance of the sward. This off color has been documented to be a result of the natural life cycle processes within the grass plant. Thus the identification and characterization of amenity grass growth regulators have been complicated by the variation of biological response. The physiological state of the grass at the time of application may play a greater role in the erratic performance of growth regulators than the applied chemistry. This paper is involved with 1) the classification of vegetation, 2) characterizing the life cycle of perennial grasses, 3) classifying the growth regulators and 4) integrating all of the above to result in economically valuable and predictive biological responses of amenity grasses to growth regulators.

CLASSIFYING TURF AREAS

A most important step in understanding where to use growth regulators is to classify the areas according to levels of management. Figure 1 outlines a classification scheme as one method of describing relative management intensities.

Class A turf is that receiving high levels of input. Mowing is done on a frequent basis to maintain a groomed appearance at all times. Fertilizers are usually applied 2 to 4 times per year. Pests are generally controlled on a curative program and the areas are often but not necessarily irrigated. Examples of Class A turf include golf greens, tees and fairways, sportsfields, high quality home lawns, and improved sections of industrial grounds parks and cemetaries.

Class B turfs are those that for reasons of aesthetics need to be mowed on a frequent basis but generally do not have other management inputs. The key objective remains control of vegetation height as for Class A turfs. Perhaps once a year or every two years, these areas are fertilized and broadleaf weeds are controlled. Mowing frequency is equal to that for Class A turf when based on the growth rate of the turfgrasses but may be somewhat less based on calendar days. Examples of these areas include the major portion of industrial grounds, parks, cemetaries, golf course roughs, and home lawns.

Class C turf is mowed 2 to 3 times per year, usually never fertilized but control of certain broadleaf weeds may occasionally occur when infestations become severe. The key objective with this mowing frequency is to cut off seedheads which result in brown color and excessive vegetation which may harbor unwanted animals. An example of this type of turf area would be highway roadsides.

Class D areas can no longer be called turf and the mechanical or chemical brush and weed control cannot truly be called mowing. Vegetation control along these areas is usually done with a "brush-hog" or chemicals known as "total veg" control materials. Examples of this class include railroad and power line right-of-ways as well as the more obscure parts of highway right-of-ways.

CLASSIFYING CHEMISTRY

There are numerous ways which vegetation management chemistry can be classified and soil and plant residual action is one way. Certainly there are advantages of products that have a short residual action, long residual action and combinations of both. A key part of this discussion centers on amidochlor. Amidochlor has been researched under the MON-4620 and 4621 code numbers. As shown in Figure 2, it has the shortest life in the turfgrass biosphere. Amidochlor will slow vegetative growth for a period of six weeks and control seedheads of cool-season grasses if applied prior to seedstalk elongation. The biological effect seems to outlast the chemical effect as the plant reorganizes meristems toward vertical growth. Thus the rapid loss of amidochlor from the biosphere provided a unique tool to study growth and development of cool-season grasses.

As with many other growth regulators, amidochlor does not discolor rapidly growing, non-vernalized turf. In fact a darker green color results in suppressed tissue, although some natural tip dieback occurs as the leaves age beyond their natural life. Yet in the field, many examples of off-color were reported with the product.

In examining the question of off-color, it was proposed that certain physiological processes of turfgrass growth and development were obscured during the revolution of turfgrass science and management that occurred in the 1950's. Briefly, development of efficient mechanical mowers and fertilizers designed specifically for turfgrass resulted in a rapid improvement in the ease of maintaining aesthetic quality in large acreages of turf. Turfgrass research on the life-cycle of cool-season grasses, particularly on the seedhead or reproductive phase, more or less ended at that time as these processes could be rather easily obscured through mowing and other management practices.

For years researchers have said that mowing results in a series of developmental and physiological processes in the plant such as synthesis of the growth hormone ethylene at the cut end of leaf blades and subsequent stimulation of tiller development. It is now believed that these processes currently associated with mowing are again altered when the practice of mowing is reduced or eliminated. It is further believed these processes are important in describing a large part of the erratic turfgrass response to growth regulators both in turfgrass quality alterations and in growth suppression. The processes are involved in the annual life-cycle of perennial cool-season grasses which result in natural leaf aging, senesence and death.

It is proposed that descriptive stages of spring development be identified to provide for better markers of proper application timing and interpretation of growth regulator results. Research and observations at Monsanto have led to conclusions presented here that indicate the response of grasses to growth regulators can vary from excellent growth suppression with little or no turf quality loss to poor growth suppression with severe turf quality loss.

COOL-SEASON GRASS LIFE CYCLE

Figure 3 outlines the annual life-cycle of cool-season grasses and identifies proposed growth stages: I) Cold dormancy, II) Green-up, III) Rapid vertical growth, IV) Reproductive physiology, V) Revegetation, VI) Heat and drought dormancy and VII) Fall revegetation. Since spring is the preferred time for growth regulator application, only the first five stages are discussed.

Stage I. Dormancy or Pre-greenup

Pre-greenup is the appearance of the turf immediately following loss of snow cover. The appearance varies according to the kind of grass, the quality (color) of the turf the previous fall and the severity of winter effects. Within days after the snow melts and under full sun, existing leaves that were not excessively dammaged from winter effects will green up through chlorophyll synthesis. Leaves damaged beyond repair remain brown and fully visible until warmer temperatures hasten their degradation.

In certain areas of the Pacific north-west and mid-Atlantic east coast states, winter temperatures are mild and cool-season grasses only partially brown off. In this case the pre-greenup stage does not occur. Apparently this is also true of the British Isles and much of the Western European Continent.

Stage II. Greenup and initial growth

As temperatures begin to warm, new leaves grow from the crown apex (growing point) within existing leaves. Older leaves degrade and are replaced by new leaves. This process, known as greenup, may occur over a period of several weeks depending on how fast soil temperatures rise. If this stage is prolonged by continued cool temperatures, the turf may reach 100 percent greenup while achieving only minimal vertical growth.

Stage III. Rapid vertical growth

The beginning of Stage III is most easily characterized by the need to mow. The grass is beginning to grow so fast that weekly mowings often remove much more than the recommended 1/3 to 1/2 of the existing leaf height. If spring season temperatures warm rapidly and consistently, this stage can be entered before 100 percent greenup, and more than one mowing may be required before complete greenup has been achieved.

Near the end of this stage, the seedhead forms at the stem apex. The developing seedhead can often be felt as a bulge at the base of the plant. To identify this stage, a number of plants need to be examined since not all will develop a seedhead each year. For verification, the leaves can be stripped, exposing the young seedhead approximately 1/8 inch in diameter and 1/2 inch long. Stage III ends when the the first young, short seedheads appear in the turf area. While it is too late to control those seedheads, a high number of later forming seedheads can still be controlled. The duration of Stage III appears to vary according to climate and weather, but usually lasts 2-3 weeks.

Stage IV. Reproductive physiology

In this stage, the seedstalk below the seedhead has begun to elongate. In many cool-season grass species, about the time the seedhead becomes visible in a mowed turf, a natural plant hormone (signal) causes the leaves on the tiller that bears the seedstalk to stop growing and provide nutrients and energy to the developing seedstalk. Thus the plant is under the effect of a natural, internal plant growth inhibitor.

At this time a signal (perhaps the same one) also causes the lateral buds to start developing into tillers at a faster rate and to form a new crown apex. The aging leaves associated with the seedstalk discolor, senesce and die as the young tillers grow and expand.

Stage V. Revegetation

The turfgrass sward eventually replaces all the original plants through rapid growth of new tillers. The dead plants degrade and fall into the thatch. Thus, the green color of the lawn is maintained through development of new crown apices and new leaves.

LIFE CYCLE VARIATION AMOUNG SPECIES AND VARIETIES

Normally this transition (life-cycle) occurs in a lawn with minimal disruption of turfgrass quality. Grass varieties or species that have difficulty maintaining quality during transition are referred to as the "stemmy" types.

In the cool-season region, May and June are known as the stemmy months for the stemmy varieties. Turfgrass researchers and turf managers alike have known that the grasses are not attractive during the stemmy phase. However, while stemmyness seems to be well known, it has not been well researched.

For many years turfgrass researchers have suggested that the key improvement of the Kentucky bluegrass (Poa pratensis L.) varieties is improved resistance to Drecshlera (Helminthosporium) leafspot diseases. Leaf infections in the spring are thought to translate into the severe "melting-out" turf losses which become most evident in the common varieties.

However, both university and turfgrass seed company researchers have also known that one of the major differences between improved and common Kentucky bluegrass varieties is the ability to produce seed. Common varieties produce copious amounts of seed and many of the improved varieties are very poor seed producers. As an example, the variety Sodco was very attractive in the vegetative state in the lawn, but failed to produce enough seed for marketing. It is proposed that the severe turfgrass quality losses from the melting out phase in common Kentucky bluegrass are primarily a result of reproductive senescence of the of the leaves associated with the seedstalk and the leafspot organism invades leaf tissue that would have senesced anyway.

It is recognized that the more recently developed varieties, such as Baron, are both "improved" and have excellent seed production. Apparently the mowing regime (early seedhead removal) is quite effective in preventing these varieties from going through the destructive flowering physiology state.

As a cool-season species, tall fescue (Festuca arundinaceae Schreb.) is best adapted to the transition zone of the United States largely due to summer survival. Yet unmowed tall fescue develops a seedhead, matures and browns off while mowed tall fescue remains green. Thus it appears that a major contribution to summer "tolerance" of tall fescue is the fact that frequent mowing removes the seedhead before natural hormones kill the leaves and perhaps a portion of the roots.

Variation among species and varieties, in relative ease or difficulty living through reproductive transition appears to be associated with two factors: 1) the overall tendency of the species or variety to produce seedheads (percentage of the plant apices with potential to flower) and 2) the tendency of those plants to follow through with the flowering physiology state in spite of the mowing regime imposed on them (seedheads regularly mowed off).

CHARACTERIZING THE GROWTH REGULATORS

Growth is often defined as irreversible enlargement in size while development is transformation of apparently identical cells into diversified cells and plant organs. Based on these definitions, the current turf growth regulators can be divided into two types. Type I are those that suppress both growth and development. Development not only includes the transformations from a seed to a mature plant in an annual species, but also includes most of the stages outlined in Figure 3, of the annual life-cycle within a perennial plant.

The chemistry included in this group all suppress turfgrasses for about a six week period. Within the Type I group, amidochlor is a suppressor while the others such as mefluidide maleic hydrazide and chlorflurenol are usually labeled inhibitors. The inhibitors stop growth immediately after application while a suppressor allows for some growth. This may be partially due to the time it takes for amidochlor to be absorbed by the roots and partially due to its mechanism of action. Regardless, the end result is a gradual reduction of growth that eventually approaches inhibition. The concept of a suppressor is not to stop growth and mowing, but to permit slow replenishment of turfgrass leaf tissue and utilize trim mowings as needed.

Other chemicals known to inhibit growth and development of cool-season grasses are labelled the herbicide TYPE I regulators. These are defined as the herbicide type because all have a primary use as a herbicide. Examples of this type of chemical include glyphosate, sulfonyl ureas and imadazolinones. These compounds are characterized as having a very narrow margin of safety on cool-season grasses and accidental overdoses can quickly and easily kill turf.

However, the sulfonyl ureas and imidazinones will likely find use on roadsides as a grass growth inhibitor with the primary benefit of long term broadleaf weed control. Glyphosate is also effective as grass suppressant with short term weed control. Annual broadleafs are often eliminated. In addition tolerant grasses will survive and compete against more susceptible grasses as evidenced by bermudagrass (Cynodon dactylon L.) release from Johnsongrass (Sorghum halepense L.) infestations.

Type II turf growth regulators are those that suppress growth only. The developmental sequence of the plant continues, however new plant organs develop in miniature size. Examples of this type include paclobutrazol or PP-333 and flurprimidol or EL-500. These compounds are often referred to as anti-gibberellins because the primary action is the gibberellin biosynthesis inhibition. These regulators are effective internode elongation suppressors.

Fungicide Type II growth regulators are those that are primarily used as a fungicide but have a use as a growth regulator. Fenarimol was developed as a fungicide but is now being recommended for use on putting greens for selective suppression of annual bluegrass. On the other hand, applications of paclobutrazol have been documented as controlling Septoria species.

LIFE CYCLE RESPONSES TO THE REGULATORS

If a Type I regulator is applied at Stage I, the most noticeable effect is a delay of spring green-up. Since development is slowed as well as growth, the rate of appearance of new green leaves is slowed and the size of the leaves is diminished. Root active growth regulators are effective in reducing growth when applied at this stage while foliarly active compounds require green leaves to absorb the product.

Application at Stage II results in delay of further green-up and subsequent growth suppression. Application at this stage is desirable since the turf often has greened sufficiently and rapid spring growth has not yet begun.

Stage III is considered the optimum time for application of Type I regulators to provide good turfgrass quality and the normal 5-6 week duration of vegetative suppression or inhibition. Often there is a slight loss of turf quality from the 2nd to the 4th week from leaf aging, and enhanced dark green color from the 7th to the 10th week or longer. Seedhead control is usually greater than 90 percent for applications made during this stage.

Rapid vertical vegetative growth signals the beginning of Stage III. Seedhead elongation signals the end of the stage which is the latest application time for optimum results. As soon as the first seedhead is visible above the boot leaf, the application time is over, especially if a root absorbed product cannot be watered in immediately after application.

Applications of any Type I growth regulator at Stage IV can be detrimental to the appearance of the turfgrass area especially if the grass is a stemmy type. Growth regulators do not reverse the effects of the hormonal signal and in effect, work cooperatively with the signal to completely inhibit growth of existing leaves. Likewise they do not reverse the signal for tiller initiation but do greatly slow tiller development, at least for a time. Eventually one or more lateral buds, deep in the thatch and not having sufficient product, receive the signal. When that occurs these buds rapidly grow and develop into tillers.

Thus application of Type I regulators at Stage IV result in undesireable turfgrass responses: (1) excessive growth inhibition for a short period, (2) severe loss of turfgrass quality as leaves senesce and die, and (3) early termination of activity due to rapid growth of escaped tillers not affected by the product.

Turf quality enhancements resulting from proper Type I use

The significance of this signal reinforces the fact that Stage III is the preferred time for application. Since developmental inhibitors applied during Stage III prevent seedheads from developing, they also prevent the signal from being sent and prevent the negative turfgrass quality consequences of the signal. Therefore, these applications can actually result in improved turfgrass quality compared to a nontreated area undergoing the "stemmy" reproductive physiology phase. Further, the effect of preserving leaves seems to be accompanied by a preservation of existing roots. As a result, improved summer growth, color, rooting, and tolerance to summer stresses including heat, drought and diseases have been observed when using some Type I growth regulators.

Type II regulators

Because Type II plant growth regulators do not suppress plant development, applications at any of the stages from I through IV can result in (1) diminutive seedhead expression below the mowing height, (2) senescence and death of the main tiller and (3) suppression of the size of the new tillers that normally grow large enough to mask the dying leaves. Therefore no stage of application on stemmy varieties in the spring is acceptable for Type II plant growth regulators.

It is important to state that the Type II regulators do show acceptable results on non-stemmy, highly vegetative species and varieties. For instance, tall fescue seedheads apparently can quite easily be mowed off prior to the signal, even when stunted by a Type II regulator and good results have been achieved. Type II growth regulator use on Baron Kentucky bluegrass, however, has not been as successful. Apparently when the seedhead height is stunted, the mower does not remove the seedhead soon enough to prevent the natural signal and the leaves usually senesce and brown off rapidly during Stage IV. Finally, it should also be noted that Type II growth regulators have shown excellent performance in the fall season when perennial species do not exhibit the reproductive growth stage.

MATCHING REGULATORS WITH CLASSES OF TURF

Use of all turf growth regulators on Class A turf is extremely limited at this time, and more research is needed to reduce the visible off-color of aging turf and to insure protection from pests that often damage turf areas being managed at a high level. However, where slight off-color can be tolerated during the off-season in exchange for improved color and survival during the outdoor busy season, the regulators that result in minimal off-color have been used effectively.

In addition, there are several specialty uses in Class A turf for certain growth regulators. Mefluidide has been used successfully for seedhead control of annual bluegrass in intensively maintained turfs. While in some cases it has been reported that seedhead control has reduced annual bluegrass populations through reduced seed available for germination, others have reported greater annual bluegrass populations due to inadvertant conversion of an annual species into a perennial species by eliminating the reproductive and maturation phase of the plant. For those turf managers who desire to keep annual bluegrass rather than kill it, improved appearance from less seedheads and improved summer survival of this species from proper use of mefluidide in the spring has been valuable.

In addition, the Type II growth regulators have been used successfully to reduce annual bluegrass populations. This has been true for flurprimidol, fenarimol and paclobutrazol. These compounds have been reported to have both selective suppression of mature annual bluegrass in perennial grasses as well as elimination of competition from seedling annual bluegrass that develops in miniature and cannot compete in the existing turf.

The final consideration for use of growth regulators on Class A turf centers on hard to mow areas where mechanical mowing may result in more problems than the leaf aging off-color appearance associated with a growth regulator. For instance, spring rains often delay mowing schedules. When mowing resumes, wet conditions preclude the use of a mower on steep slopes, ditch banks, low spots and areas full of obstacles. If mowed mechanically, soil displacement and/or excessive wear and tear of the turf may occur. Thus areas of Class A turf that present these special problems can be preselected early in the season and treated with a growth regulator to prevent the mechanical dammage that usually far exceeds any off-color from a growth regulator.

Class B turf represents the greatest potential for cost effective use of growth regulators. Many areas of turf are mowed on a frequent basis for yet do not receive other management inputs. It is on these areas that growth regulators can blend into mechanical mowing programs and greatly reduce the costs, time consumption and headaches of maintaining these areas. That allows more time to attend to Class A turfs that are most important in projecting the desirable aesthetic image.

Most of the Type I and Type II growth regulators can be used successfully on Class B turf. However, because of overlap safety, uniform grass response and safety to ornamentals in the landscape, amidochlor is the easiest to apply correctly.

Type I, herbicide Type I and Type II growth regulators can be used successfully on Class C turf. However, the herbicide types can injure or kill cool-season grasses and should only be used with extreme caution.

As mentioned earlier, warm-season grasses have shown a greater tolerance for the herbicide type growth regulators. Thus, all growth regulators can be used effectively on warm-season Class C turf except for amidochlor. Because foliar uptake of amidochlor is ineffective, microbial degradation is rapid and warm-season grasses are deep rooted, the regulator cannot be effectively absorbed and translocated to the growing point.

The most widely accepted chemicals for Class D vegetation are the herbicide type, including those with residual action. For the most part, bare ground is considered acceptable, at least for a short perod of time. The biggest concern with residual types is associated with mobility of the products to surrounding desireable vegetation. Advantages exist in mixing residual with non-residual types because rates of residuals high enough to produce "burn down" have potential to injure and kill surrounding vegetation.

ENHANCED PERFORMANCE WITH GROWTH REGULATOR MIXTURES

Because of the different mode of biological action of Type I and Type II regulators on turfgrasses, it would appear beneficial to consider a combination of the two types. Type I regulators have been shown to 1) suppress growth for up to 6 weeks , 2) suppress seedheads, 3) slow the rate of ontogeny, 4) release from suppression effects rapidly and 5) result in etiolated growth of new tillers. On the other hand, Type II regulators have been shown to 1) suppress growth minimally for the first 4 weeks and suppress growth for up to 10 weeks, 2) suppress height of seedheads only, 3) have little to no effect on ontogeny, 4) release suppression effects slowly and 5) result in shorter thicker leaves on the new tillers. The following study was initiated to evaluate the combination of amidochlor and paclobutrazol.

Methods

The study was conducted on a mature stand of Merion Kentucky bluegrass at the Monsanto Research Farm at St Charles Missouri. Plot size was 1.8 X 3.0 m and the treatments were replicated 3 times. In addition, the study contained 12 control plots to insure detection of the natural variability that occurred across the plot area.

Treatments were applied on April 10, 1986. A 1.8 X 0.9 m portion of each plot received 107 kg/ha actual nitrogen on the day of treatment, at 2, and again at 4 weeks after treatment. Irrigation was supplied as needed to maintain active growth. The mowing height was 5 cm throughout the study. The fertilized portion of the plot was harvested every 2 weeks for 10 weeks, while the remaining unmowed portion was used for seedhead counts.

Clippings were harvested from a 1.27 X 0.56 m area, dried in a 60 C convection oven and weighed. The dry weight of clippings was computed to square meter. Seedheads were counted 8 weeks after treatment using a wire ring with a diameter of 25 cm. The seedhead count was based on 6 determinations per plot, computed to square meter and transformed to square root for analysis.

Results

The data indicate that a typical curve of spring growth was obtained in the control plots (Figure 4). Very little growth occurred during the first 2 weeks of the study. However, growth was rapid from week 2 through week 8. Growth from week 8 to week 10 was much reduced as warm temperatures slowed growth. The growth curves for amidochlor treated plots indicate very little difference between rates of 1.12 and 2.24 kg a.i./ha. This confirms the threshold type of growth suppression of amidochlor, where higher rates show no additional suppression effects. Amidochlor was effective at suppressing growth during the first 4 weeks, released the grass from suppression at 6 weeks and grew faster than the control plots at 8 weeks under these high nitrogen levels.

In Figure 5, increasing the rate of paclobutrazol from 0.22 to 2.24 kg a.i./ha resulted in incremental increases in amount and length of suppression of grass growth. Paclobutrazol was not very active for the first 4 weeks after application, but was very active from week 4 to week 6. The 0.67 kg a.i./ha rate was active at 8 weeks while the 2.24 kg a.i./ha rate was active for all 10 weeks.

When the two products are combined the effects are complementary. Since the rate of amidochlor has very little effect on growth, the following figures are selected based on the rate of paclobutrazol. At 0.22 kg a.i./ha, the combination exhibits equal or better growth suppression at the 2, 4 and 6 week harvests (Figure 6). Unfortunately this rate is not sufficient to overcome the growth enhancement by amidochlor at 8 weeks. The combination using 0.45 kg a.i./ha shows the same basic growth suppression except that the overgrowth with amidochlor alone is controlled with the combination (Figure 7). When 0.67 kg a.i./ha of paclobutrazol is used in the mixture, growth suppression was achieved at 8 weeks after treatment even under the high fertility conditions of the study (Figure 8).

The seedhead evaluation is shown in Figure 9. Paclobutrazol was found to have no effect on seedhead number at most rates of application, although two rates significantly stimulated seedhead numbers. As expected, seedhead numbers were significantly reduced at all rates of amidochlor application. However, when the two products were combined, a significant reduction of seedhead number occurred over that found for amidochlor alone.

Thus the two products are 1) complementary in type of growth suppression, 2) additive in amount and length of vegetative suppression and 3) synergistic in seedhead control.

Figure 1

Vegetation Management Classification

- **Class A**
  Frequent mowing, fertilization, and pest control; Often irrigated
- **Class B**
  Frequent mowing; Occasional weed control and fertilization
- **Class C**
  Infrequent mowing; Occasional weed control
- **Class D**
  No mowing, Occasional brush and weed control

Figure 2

Plant and/or Soil Residual Action

- <u>1 Month</u>
  amidochlor
- <u>1-2 Months</u>
  glyphosate
  mefluidide
  maleic hydrazide
  chlorflurenol
- <u>2+ Months</u>
  paclobutrazol
  flurprimidol
- <u>3+ Months</u>
  imadazolinones
  sulfonyl ureas

Figure 3

ANNUAL LIFE CYCLE OF PERENNIAL COOL-SEASON TURFGRASSES

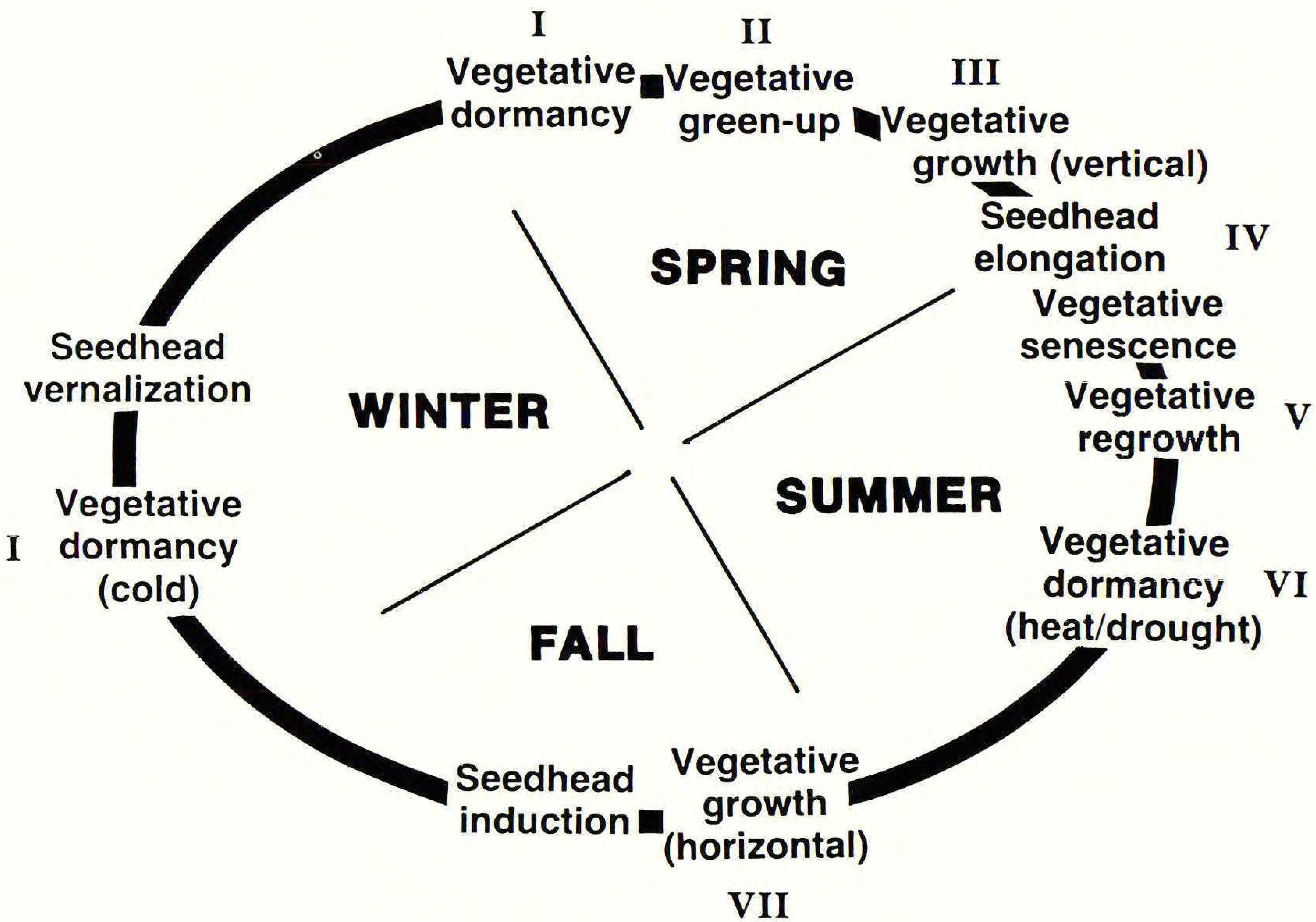


Figure 4

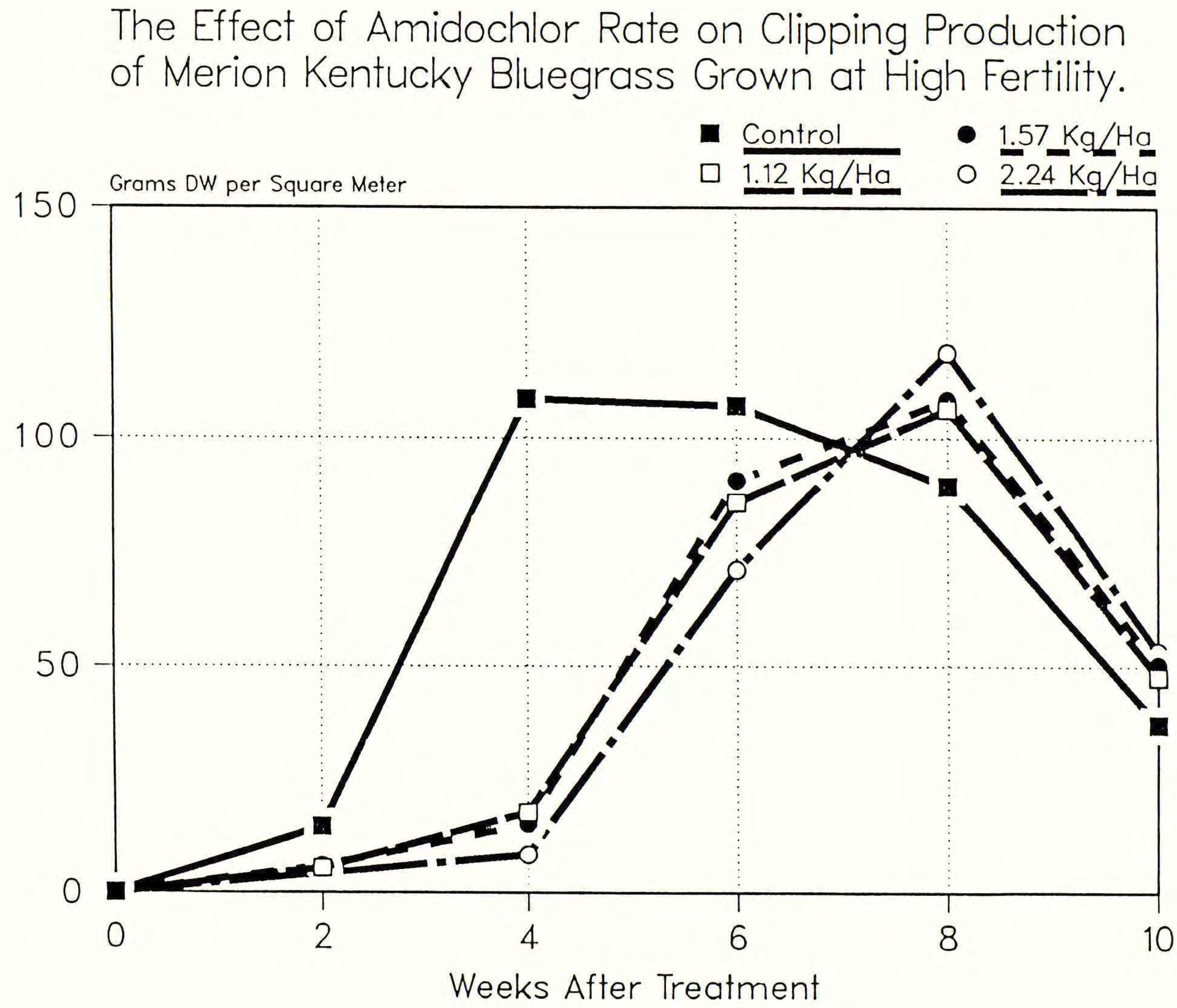

The Effect of Amidochlor Rate on Clipping Production
of Merion Kentucky Bluegrass Grown at High Fertility.
Control
1.12 Kg/Ha
1.57 Kg/Ha
2.24 Kg/Ha
Grams DW per Square Meter
150
100
50
0
0
2
4
6
8
10
Weeks After Treatment

Figure 5

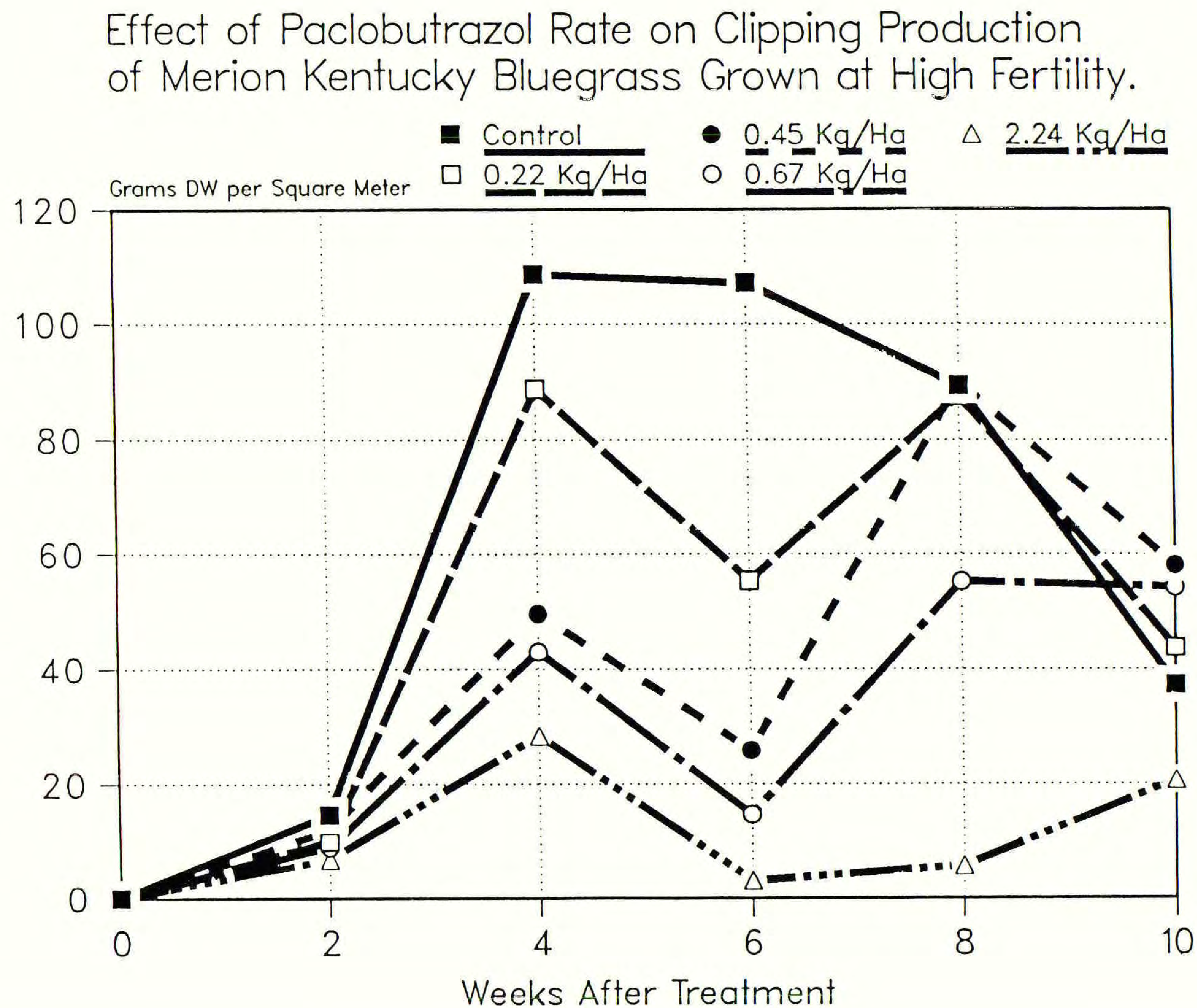

Effect of Paclobutrazol Rate on Clipping Production
of Merion Kentucky Bluegrass Grown at High Fertility.
Control
0.22 Kg/Ha
0.45 Kg/Ha
0.67 Kg/Ha
2.24 Kg/Ha
Grams DW per Square Meter
120
100
80
60
40
20
0
0
2
4
6
8
10
Weeks After Treatment

Figure 6

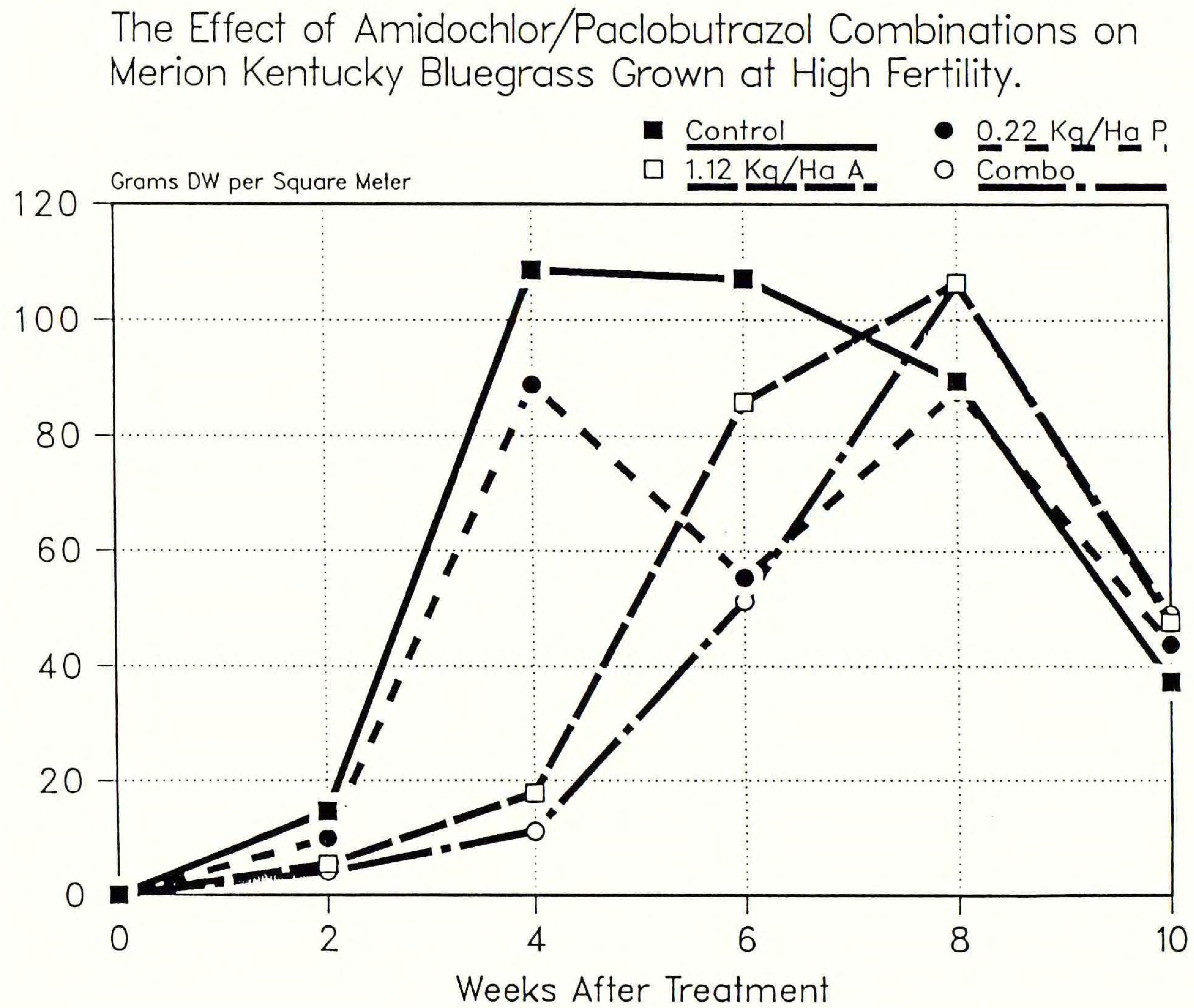

The Effect of Amidochlor/Paclobutrazol Combinations on Merion Kentucky Bluegrass Grown at High Fertility.
Control
0.22 Kg/Ha P
1.12 Kg/Ha A
Combo
Grams DW per Square Meter
120
100
80
60
40
20
0
0
2
4
6
8
10
Weeks After Treatment

Figure 7

Effect of Amidochlor/Paclobutrazol Combinations
on Merion Kentucky Bluegrass at Grown at High Fertility.

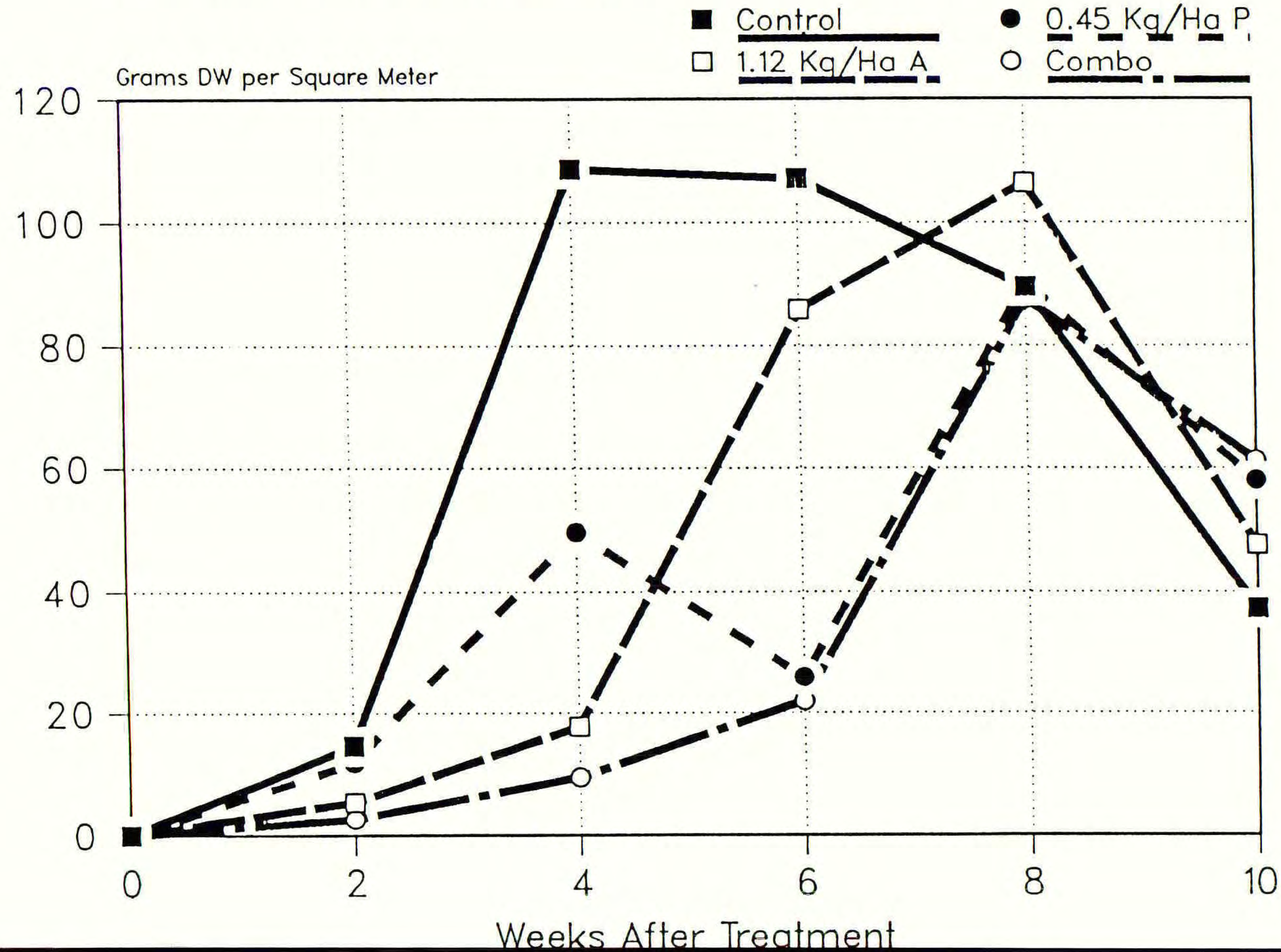
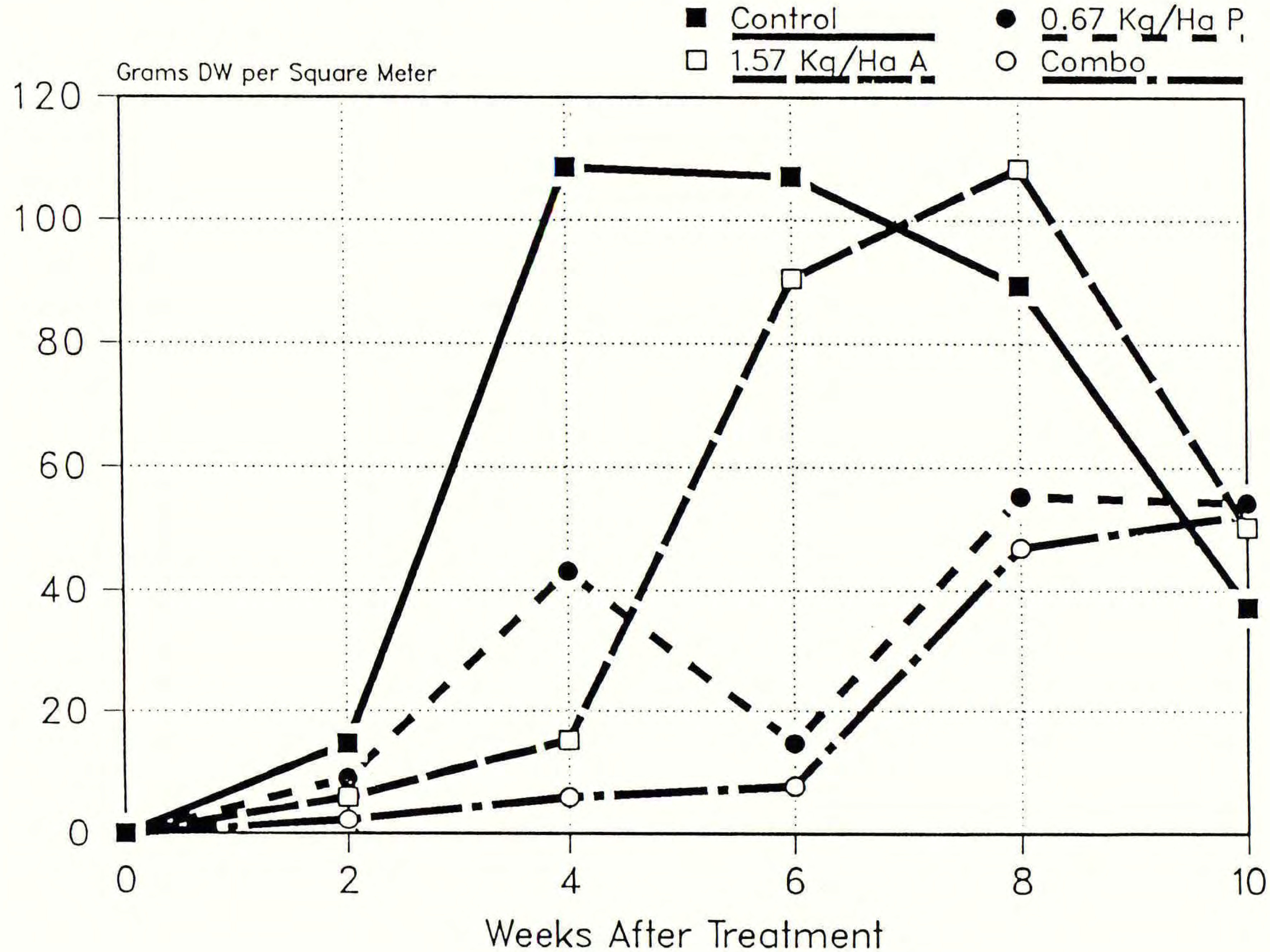


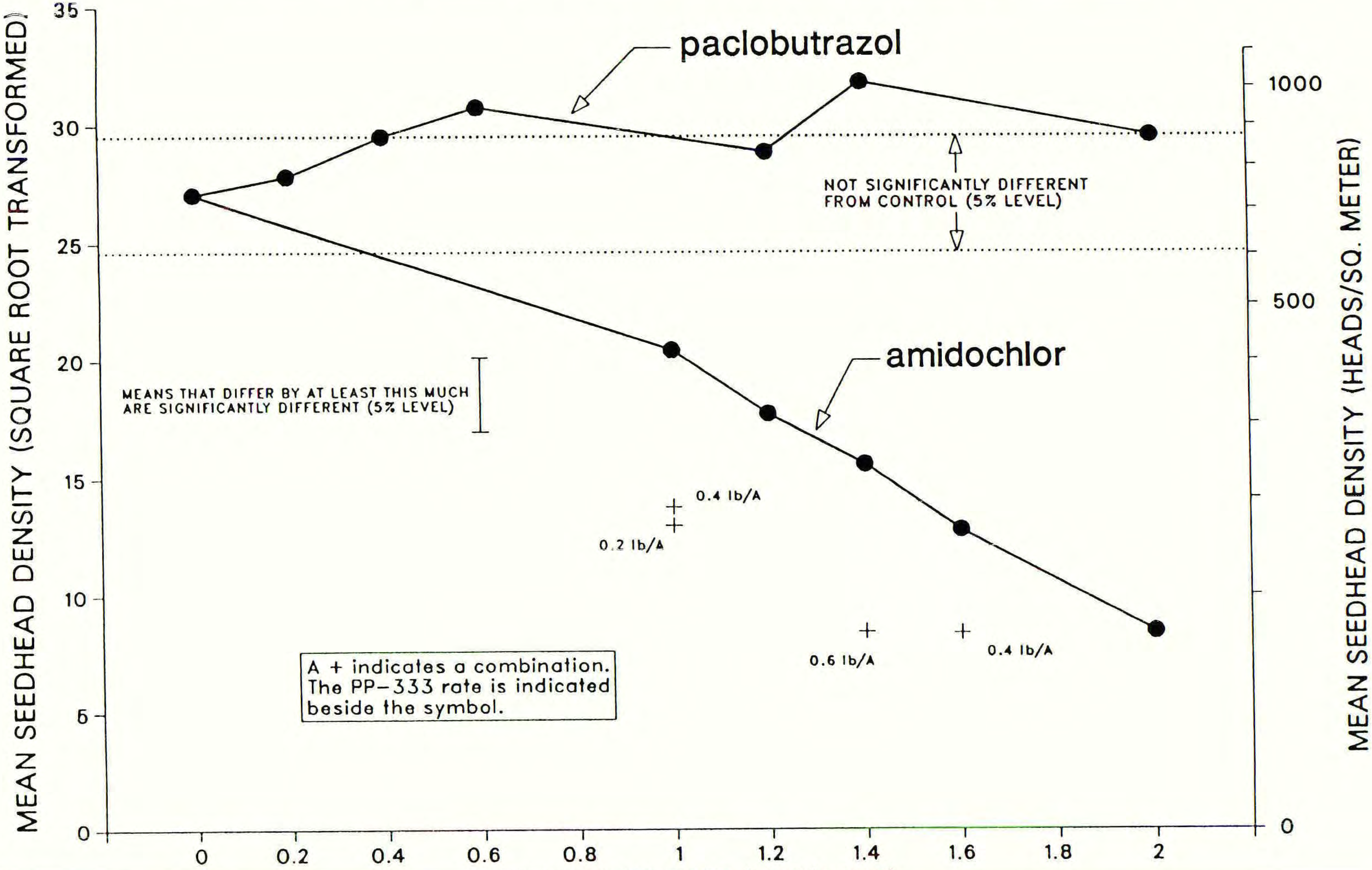
Figure 8

The Effect of Amidochlor/Paclobutrazol Combinations on Merion Kentucky Bluegrass Grown at High Fertility.



The Effect of Amidochlor and Paclobutrazol on Seedhead Density in Merion Kentucky Bluegrass

Figure 9



THE USE OF THE GROWTH RETARDANT MEFLUIDIDE FOR MAINTAINING ACCEPTABLE GRASS HEIGHT AND FOR IMPROVING SPECIES DIVERSITY IN A SWARD.

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ABSTRACT

In a set of tests carried out on rough swards in churchyards the growth retardant mefluidide was applied at the recommended rate one or more times each season. It was found possible to maintain acceptable grass height often with improved aesthetic appearance due to grass flowerhead suppression.

Using specific examples other aspects of chemical application are considered. Grass and broadleaved species sensitive to mefluidide can give rise to bare ground or a thinned sward, which with height suppression offers opportunity for the increase of species unaffected by the chemical. Additionally, new plants from the dormant seed population in the soil or from colonising species outside of the treated area can increase species diversity. Reduction of cutting by chemical application increases the chances of seeding, even from chemically-affected species.

Change in the sward composition following mefluidide application must be influenced heavily by location and environment. It is suggested as a general guide that application over a two year period would have maximum beneficial effect, followed by a cutting regime to assist development of the new balance.

INTRODUCTION

The application of a chemical growth retardant to such areas as amenity land, roadside verges and orchards is not a new idea. Maleic hydrazide, for instance, has been available for almost forty years. However, no chemical has completely satisfied the requirement of suppressing growth without undesirable side effects to the sward (Field, 1983) and their use in conservation work seems limited (Parr and Way, 1984).

Mefluidide (N-[2,4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]-phenyl]acetamide) was discovered and developed in the U.S.A. by the 3M Company during the 1970's (Price, 1984) and is sold in the U.K. as MOWCHEM by May & Baker Ltd. Work has shown mefluidide to give better growth retardation and less discolouration than maleic hydrazide (Atkin, 1984) with little effect on dicotyledonous plant species when applied in early spring (Marshall, 1983). Mefluidide has therefore subsequently been tested as an aid to conservation particularly for use in situations where grass height must be controlled. Such an area is often the typical parish churchyard which increasingly represents a last area of 'natural' grassland in many places.

This paper aims to highlight the major aspects brought about by chemical application by using selected plant species to illustrate this.

MATERIALS AND METHODS

Location and site details

The results presented have been drawn from a range of locations, mainly in churchyards, in south-east Essex. Sites were of a standard format and consisted of 1½m x 1½m plots; six replicate plots per treatment arranged randomly in blocks.

Treatment and application

At all sites treatment consisted of at least:

1. Mefluidide applied as 1.66 l/ha MOWCHEM in mid-late April of each year.
2. As (1) with further application in mid-June.
3. Control receiving similar management to that of surrounding area. This was usually in the form of infrequent cutting by rotary mower, gang mowing or little/no clearance at all. In all cases the management type had been carried out in a similar way for many years.

Additionally some sites contained further sets of plots to investigate the effect of three applications of mefluidide per year (April, June, August).

The effect of application in a previously uncut sward was also investigated. All plots were cleared during the winter months and were raked following cutting.

Chemical application was made using a 'Solo' hand-pump sprayer fitted with a 1½m boom and calibrated to 700 l/ha.

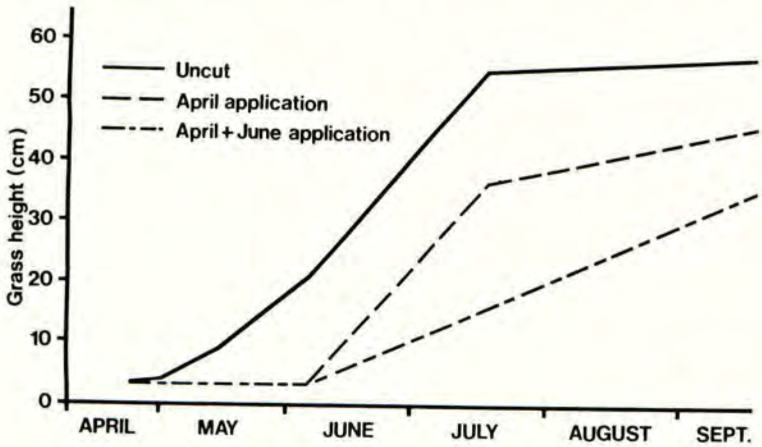
ASSESSMENT

All assessments were made within the central ½m^2 of each plot. Simple visual assessment of % ground cover of all species was carried out in April of each year before commencement of spring growth, using a grid quadrat. This covered the entire assessment area and was subdivided into 25 squares to simplify determination. Grass height was measured using a sward stick technique (Marshall, 1983) at frequent intervals throughout the season. Four readings were taken per plot. Other assessments were made as necessary. Results were analysed by two-way analysis of variance.

RESULTS

The typical effect of mefluidide on the grass height of a mixed species sward is illustrated in Figure 1.

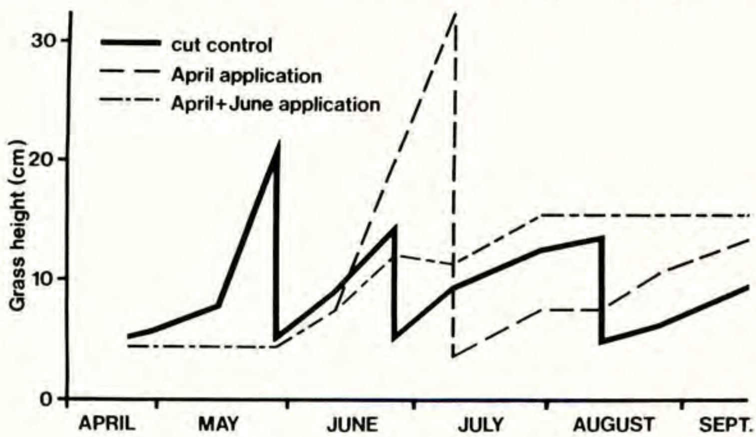
Fig. 1. Effect of mefluidide on grass height : sprayed v. uncut



Chemical inhibition was virtually total for several weeks following application in April, duration being dependent on species composition and climatic conditions. As the effect weakened, upward growth commenced although flowerhead suppression kept height significantly less than the unsprayed area. Further application in June continued to severely retard grass height for the remainder of the season.

Figure 2 gives an indication of the effectiveness of the chemical compared to typical infrequent management in a churchyard situation.

Fig. 2. Effect of mefluidide on grass height : sprayed v. cut



The sward consisting predominantly of Agrostis stolonifera and Poa sp. had been maintained using a rotary mower in May, June and August to keep an acceptable height of 15-20 cm. Single application of mefluidide in April eliminated the May cut and a single cut in July maintained the desired height; a second chemical application in June eliminated the need to cut at all.

Visual appearance is often as important as height control. Mefluidide application enhanced this by the often total inhibition of grass flowerhead formation (Table 1).

TABLE 1

Typical chemical effect on grass flowerhead formation

Species	Total flowerheads in six replicate plots ($3m^2$ per treatment)								
	May			July			September		
	A	A+J	C	A	A+J	C	A	A+J	C
Dactylis glomerata	0	0	11	0	0	1	0	0	0
Poa sp.	0	0	136	0	0	0	0	0	0
Holcus lanatus	0	0	0	25	1	21	0	6	0
Lolium perenne	0	0	0	13	21	12	-	-	-

Species such as Dactylis glomerata and Poa sp. flowered only in the control (C) plots. Total inhibition by April and June (A+J) applications was purely a chemical effect although the identical effect achieved by April (A) application only was possibly due to other influences such as daylength once chemical effect had diminished. Later flowering species such as Holcus lanatus were unaffected by the early application only. Lolium perenne represents one of the few species resistant to mefluidide (another being Elymus repens) and thus flowering was unaffected by either treatment.

As well as height mefluidide can also alter the ground area occupied by a species. Holcus lanatus in particular was found to be susceptible (Table 2). In this case both double and triple application drastically reduced % ground cover compared to the slight apparent natural decline recorded in the

TABLE 2

Change in % ground cover of Holcus lanatus over a two year period

Mefluidide application in 1984 + 1985	% ground cover		
	April 1984	April 1985	April 1986
April + June	40.0	24.1*	9.1*
April + June + August	48.9	17.5*	5.7*
Unsprayed control	51.0	45.2	39.2

*Significantly different (P = 0.05) to unsprayed control unsprayed areas. H.lanatus was the most sensitive species found in this study although it would appear that Poa, Agrostis and Hordeum are adversely affected. Broadleaved species may also show susceptibility. This, of course, can give rise to areas of bare ground or a thinning in the sward (Table 3).

TABLE 3

Typical % bare ground after one season following mefluidide application

	April	November
One application (April) + one cut (July)	2.2	10.0*
Two applications (April + June)	2.5	31.7*
Unsprayed (cut May, June, July, August)	2.3	2.2

*Significantly different (P = 0.05) to unsprayed control

Vegetatively, many species remained relatively unaffected by mefluidide. An example was Glechoma hederacea (Table 4). A natural spread appeared to be occurring during the course of the study so that over two years all plots contained the species. Note, however, that the normal management of the area - that of cutting approximately monthly throughout the growing season - ensured only small percentages of the plant in these plots. The changed conditions in the mefluidide-treated plots greatly encouraged the growth of Glechoma to make it a dominant species.

TABLE 4

The spread of Glechoma hederacea in two seasons - % ground cover

0 (17)	0 (27)	0 2	1 (25)	11 (19)	21 14
0 (1)	0 6	0 (21)	1 6	5 (13)	9 (32)
0 5	2 (8)	3 (16)	5 (16)	5 4	2 (58)

NB: Table 4 represents an entire site with a total of 18 plots. TOP FIGURES in each plot show the % ground cover in April 1984 prior to chemical application. LOWER FIGURES show the % ground cover in April 1986. Figures in brackets denote mefluidide-treated plots. Unbracketed figures are unsprayed control plot values.

It was found that the flowering and seeding of many broadleaved species was inhibited by chemical application, the degree of effect being dependent on timing. Despite this, mefluidide treatment often proved advantageous to the species especially when compared with the usual method of management for the area. For instance, Vicia sativa (Table 5), being a climbing annual, rarely realises its full potential in a managed area as it will be cut down before reaching full height or pod set (unsprayed control). Pods set seed only on the lower stems giving rise to a small population.

TABLE 5

Effect of mefluidide on Vicia sativa (common vetch) over a 2 year period

Mefluidide application in 1984 + 1985	% cover 4/84	Height (cm) 6/84	Height (cm) 7/84	Number of pods/½m² 7/84	% cover 4/85
April	15.4	4.3*	55.0*	126*	29.2*
April + June	12.8	5.2*	18.3	0.5*	3.5*
Unsprayed control	10.0	25.8	11.7	10	12.8

* Significantly different (P = 0.05) to unsprayed control

Single application of mefluidide (April) severely retarded growth (and flowering) through June although by July plants had recovered sufficiently so that seed was shed by the time that the grass was cut later in the month (Fig.2) resulting in a large 'flush' of seedlings the following year. Application in April + June (Table 5) retarded growth for the entire season so that seed set was minimal, this being reflected in the poor % cover the following year.

Colonisation of plants from outside the treated area was enhanced by formation of bare ground. The arrival of a successful coloniser, Senecio jacobaea, onto a damp site was recorded. The site was situated alongside an overgrown area where Senecio predominated (Table 6).

TABLE 6

The spread of Senecio jacobaea following mefluidide application

Mefluidide application in 1984 + 1985	Total number of plants in 6 replicate plots ($3m^2$ per treatment)		
	April 1984	April 1985	April 1986
April	0	3	2
April + June	0	9	28
Unsprayed control	0	0	1

Note that no plants were present prior to chemical application and that over two years only one plant was found in the unsprayed plots, due to the regular mowing management. Bare ground was minimal and no viable seed was recorded from soil samples taken prior to the start of the study. A single application of mefluidide (April) increased bare ground and the possibility of colonisation; however, competition from other species appeared to check any further Senecio plants. Application in April + June gave rise to increased bare ground offering more potential for colonisation; bare areas were still available in 1985 which gave rise to the large numbers in April 1986.

DISCUSSION

To maintain or improve species diversity and quality of a sward requires a plan of management specific to the flora present. In many situations, including that of the average parish churchyard, this may not be feasible; the alternative is often in the form of neglect or even headstone removal and mowing, either treatment destroying the original habitat.

The use of a chemical growth retardant offers another alternative and has the advantage of being easier to apply than mowing, especially in awkward situations. From this study mefluidide was proved to be extremely effective in the two main criteria of grass height suppression and appearance. Although the ground cover of some grass species was reduced by its application (e.g. Holcus lanatus) others such as Alopecurus pratensis were able to increase quickly at the expense of these.

The reduction in the frequency of grass cutting in most cases was obviously advantageous to many broadleaved plants, especially with seed production. Single application of mefluidide in April and cutting in July favoured early-flowering plants (e.g. Vicia sativa, V. hirsuta, Lathyrus nissolia) provided that flower inhibition was not too severe. Application in April + June (with no cutting) gave unacceptable extended inhibition of these but favoured later flowering plants (e.g. Achillea millefolium, Centaurea nigra). Most species suffered some flower inhibition or distortion (e.g. Ranunculus repens) especially when application was made just prior to the flowering period.

Vegetative spread of creeping species again depended on chemical sensitivity and whereas Glechoma hederacea was relatively unaffected other species (e.g. Veronica filiformis) could only increase ground cover as chemical effect diminished.

Colonisation by new plants derived either from the dormant seed bank in the soil or from seeding plants outside of the treated area was especially noticeable in the second year of treatment, following the formation of bare ground in the first. New arrivals, whether desirable meadow plants or rank weeds would be dependent on the environment. In natural or semi-natural grassland the latter will mainly colonise from other nearby areas and the indications from this study are that mefluidide application over only a two year period will minimise these, providing that a cutting regime follows to maintain the new balance and discourage undesirable non-grassland species. As with maleic hydrazide it is suspected that mefluidide would create problems if used as a complete alternative to grass cutting in a conservation situation. Species diversity may well increase but species quality might decline.

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LIMIT® TURF REGULATOR: COMMERCIAL INTRODUCTION

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ABSTRACT

Amidochlor was registered in the United States for use on non-residential turf in December, 1984. It was commercialized as Limit turf regulator in 1985, and positioned for use in the Greens Markets, i.e., on landscaped non-residential properties. Characterization of these markets establish their complexity and the hurdles to be overcome in introducing and establishing the success of Limit. Analysis of the experiences associated with Limit highlight 13 principles relevant to new product commercialization.

LIMIT® TURF REGULATOR

Amidochlor is a turf regulator called Limit®. This product was registered by the EPA for use on non-residential turf in December, 1984 and was commercialized in the United States in 1985. Non-residential turf is typified by golf courses, cemeteries, office sites, institutions and landscaped municipal property. In aggregate, these properties make up the Greens Markets. Note that residential and industrial properties such as highway rights-of-way are not included.

THE GREENS MARKETS

The Greens Markets are complex. An overview of the marketplace and the goals of a major chemical manufacturer are important to understanding the actions taken in commercializing amidochlor and the principles illustrated by the actions taken and experiences encountered in commercializing a specialty product in the Greens Market.

The commercialization of Limit began in 1982. At that time, the Greens Markets were characterized as follows:

1. Lacks national or regional statistical base needed to quantify the markets and justify commercialization of a new growth regulator in an undeveloped product category.

2. Tens of thousands of small end-users or consumers responsible for maintaining relatively small properties.

3. Segmentation by geographical, climatic and, ultimately, biological differences in turf species and cultures.

4. Segmentation by differences in land use, quality of landscapes and financial objectives of the owners and managers.

5. Significant variation among property managers within each segment in terms of technical knowledge, experience and motivations.

6. Management objectives of enhancing turf growth and simplified programs. Objectives that conflict with the use of a growth regulator.

7. Many managers prejudiced against trying a new turf regulator, based on perceived failures of trying previous growth regulator products.

These facts clearly illustrate the complexity of the Greens Markets and the difficulties confronted in commercialization of a new turf regulator. The market has changed little since then.

®Limit is a Registered Trademark of Monsanto Company

THE GREENS TRADE AND COMMERCIAL HURDLES

Added to these factors are the realities of the Greens trade, the cost of entry and the objectives of a modern corporation:

1. The distribution channel is composed of two major segments:

   A. Hundreds of small, localized, distributor-dealers often specializing in serving a specific segment and requiring a high profit margin to support the services required by the end-user.

   B. A large, efficient volume driven agricultural distribution system providing minimal service, and selling on a low margin.

2. Large areas of turf under the control of municipalities and other governing bodies that depend on specialists to introduce new technology, but, as product volume grows, solicit bids for the products and services they require.

   The small dealer is reluctant to invest resources in developing a new product only to lose a growing account to low margin dealers in the bidding process.

3. Extremely high product introduction costs. The regulatory requirements necessitate the expenditure of as much as $28 million dollars and years of testing to provide the data package required to establish the toxicological and environmental acceptability of a new chemical. Registration or governmental approval is required before the product can be sold.

4. In a large corporation, each new product must offer sufficient volume, profitability and competitive advantage in order to compete for limited investment capital required for commercialization.

One key to success today, and the future of the chemical business, is a product or line of products that are highly successful. Fortunately for Limit, Monsanto has both a successful line of agricultural products, including Roundup® herbicide, which provides a position of leadership in today's Greens Markets. This base enabled Monsanto to commercialize Limit and provides for continued research and development of the new products required by these markets.

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PRINCIPLES FOR NEW PRODUCT INTRODUCTION

With this overview of the Greens Markets, let's return to 1982. Monsanto's Research Department had established that amidochlor had unique features that justified commercialization:

1. Control of seedheads and 50% reduction of vegetation growth of cool season grasses.

2. Unique mode of action.

3. Outstanding turf safety.

The experience of developing Limit to commercial launch illustrates 6 principles associated with most new product introductions:

1. Know your product and the system it affects.

2. Employ experts in highly technical ventures.

3. Do the project right.

4. Learn from failures.

5. Maintain an open mind in the decision-making process.

6. Have product champions.

Research trials in 1982 had reached the point where future work had to involve a greatly expanded number of trials under diverse environmental and cultural conditions. The people that had developed the product through 1982 were better than average, but were not experts in the field of turf culture and physiology. Nevertheless, recommendations were written for application of the product, then coded MON 4621, and over fifty trials were established with turf managers across the United States.

The trials were observed at 30 and 60 day intervals, in a manner typical of herbicide trials. The results indicated that this product should be terminated. Success was not overwhelming. The majority of the failures involved unacceptable loss of quality and lack of performance. Management and the technical team were frustrated because previous trial results had shown good turf quality and consistent performance.

Fortunately, an expert in turf science had joined Monsanto a short while before the testing began. Dr. John Kaufmann, a speaker in this symposium, joined Monsanto's Research Department based, to a large extent, on his university research with amidochlor and his belief that this was the best, least phytotoxic and most consistent turf regulator ever tested. John painstakingly analyzed each failure during the summer of 1983 and by fall, could not only explain the majority of the failures but, in the process, developed the concept of growth stages of perennial grasses. This concept markedly improved our knowledge of turf regulator performance as affected by growth stages.

His work also highlighted the need for rain or irrigation to move the product to the root zone for plant uptake and the need to delay applications of regulators to quality turf that had already reached the stage of seedhead emergence.

The importance of seedhead emergence was fundamental to the future success of Limit. Improper timing of the application appeared to have caused the majority of the quality problems. In summary, unregulated turf produces seedheads, but does not lose quality because the senescencing, yellow to brown reproductive tissues are partially removed by mowing and the remainder are masked by new leaf tissues. We simply do not see the majority of the off-color tissue and, therefore, do not perceive a significant loss of green color.

Application of regulators to turf at the start of heading reduces the vertical growth, reduces the need for mowing, and reduces the production of new leaf tissue. As a result, the naturally senescencing, yellow to brown reproductive tissues are much more visible and, in highly reproductive turfs, the overall effect may be an off-brown or yellow color due to the density and appearance of the reproductive tissues. The regulator worked, but the timing was incorrect to produce the desired quality.

The interaction of MON 4621 with turf at the stage of seedhead emergence would have probably been evident had fewer trials been followed at weekly intervals. This would have provided data on both growth stages and quality. Growth regulators are not herbicides and must be studied differently.

The principles illustrated by these initial trials:

1. Understand the product, and the systems it affects. If the research area is poorly understood, expand the number of tests slowly and observe them carefully.

2. Employ experts. Above average people may not have the technical knowledge needed in a new area.

3. Do the project right. The large number of locations and the "best effort" were not wrong, but drawing a preliminary conclusion without in-depth analysis almost killed the product.

4. Learn from failures. Without them, we would not have advanced our understanding of turf and how to properly use this type of regulator.

5. Keep an open mind when making decisions. Be willing to hold final decisions until the facts are known.

6. Have product champions. People dedicated to the product that are willing to stick it out when the going is rough, and will not accept no for an answer when their experience indicates it is the wrong decision.

There are five more principles that emerge from the description of the market given at the start of the paper:

1. Be creative.

2. Focus

3. Know the customer.

4. Plan in-depth.

5. Be committal.

At the onset, statistical data on the Greens Markets was lacking. One segment, golf courses, was well documented. However, there were no statistics we deemed to be reliable for cemetaries, parks, institutions, etc. The solution was based on two facts:

1. Turf is a metropolitan or people related market. It is concentrated where people live and work, and

2. The United States is photographed from the air at regular intervals.

The solution to quantification of the market was to "read" the photographs and the results provided the necessary data after mathematical analysis. Simple in hindsight, but creative at the time.

It was obvious that a successful introduction would depend on focusing resources to reach those turf managers in the area where the product performed best, was needed most, and where the end-user or applicator had sufficient knowledge and experience to follow directions. Market knowledge and hard decision making caused us to focus on the northeastern quarter of the United States, having the greatest number of non-residential sites, cool season grasses, and high moisture levels in spring. The combination produces the greatest need to mow. The key end-users were targeted to be golf course superintendents, chemical applicators, and managers of cemeteries and landscaped municipal properties. Focusing conserves resources, creates the successful experiences needed to build confidence, and increases volume in the shortest time.

LIMIT POSITIONING AND LAUNCH

Limit will not be an overnight success. An understanding of the customers revealed that it would take 3-5 years to get past the introductory, testing stage and have a sufficiently large, experienced customer base to reach the adoption phase of a product life cycle where volume would grow rapidly. It was also clear that the burden for product introduction and demand creation would fall on Monsanto.

Because of Monsanto's method of going to the market, it was necessary for us to study both our customer, the distributor-dealer, and their customer, the end-user or consumer. We reached the above conclusions after learning that:

1. Other commercial turf regulators had failed to perform and the consumer had been disappointed. Frequently, the consumer's expectations exceeded the ability of other manufacturer's products to produce the expected benefits. The result was a high degree of scepticism.

2. The majority of turf managers were not on the leading edge of technology. Consequently, many managers considered Limit to be the same as previous regulators and considered it a failure before it was tested.

3. Awareness of regulators was very low in several markets.

4. Chemical usage was low in some key markets and managers were fearful that they would test or apply incorrectly.

5. When managers had been trained in turf management, they were instilled with the desire to maximize growth.

6. Many managers had no training in property management apart from on-the-job experience that was mostly of the how-to variety. New management concepts are sometimes hard to grasp and adopt to their situation.

7. The need for a turf regulator varies considerably among managers within a segment. Some have insufficient budgets, others lack adequate labor, need to simplify management, have hazardous mowing sites, etc. Because of this diversity, the initial product positioning and benefit statements had to be broad in scope rather than highly focused. In general, it was surprising to learn that the first benefit desired by most managers was not a reduction of mowing expenses, but the ability to utilize their manpower more effectively and thereby get the priority work done in a timely fashion.

8. Turf managers feared that Limit would injure turf. Market Research established that Managers would test the product on a small scale the first year and would continue to test on an increasingly larger area for several years until satisfied that the risk was acceptable and the benefits were obtained as promised.

9. The distributor-dealer will not make a market. There are exceptions, but for most, the time required to sell the first trial gallon was too time-consuming and cut into the time to sell larger volume products to a larger number of accounts.

To summarize, Monsanto would have to devote the resources necessary to create awareness, differentiate Limit from other unsuccessful regulators, set consumer's expectations at a level in keeping with the product's proven performance, stress show-and-tell selling, and follow-up to ensure that the consumer recognized that the performance and benefits were obtained as promised.

This was the type of selling that made Monsanto and its' products successful. We knew this product would take significant resources and patience, and we were prepared to go forward.

This brings us to the final two principles:

4. In-depth planning

5. Commitment

The need for these is obvious. In the case of Limit, we asked Management for approval to not only spend a substantial amount to commercialize, but also to commit to investing from commercialization until the adoption phase was reached and the product became profitable. To justify this, a detailed plan was required and our entire team had to be committed to making the product a success.

There are many other aspects of commercialization that are no less important but time does not permit a review of formulation, toxicology, registration, training a sales force, media placement and creating awareness. All are important aspects of developing and introducing a new product.

The principles illustrated by the commercialization of Limit should reinforce at least one concept. A technical success in early research trials does not ensure a commercial success. Commercialization is a complex, costly and high risk undertaking. Limit is progressing as planned only because Monsanto had the skills, market knowledge, people and financial commitment to support Limit pre- and post-commercialization.

RINGING THE CHANGES ON THREE GROWTH RETARDANT CHEMICALS FOR AMENITY GRASS MANAGEMENT

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ABSTRACT

Three grass growth retardants were applied singly and in all combinations to three categories of moderately fine turf. The growth retardants were maleic hydrazide, mefluidide and paclobutrazol. The turf categories were a lawn of mixed grass species, a predominantly fescue lawn and a predominantly perennial ryegrass lawn.

Mixtures in pairs gave equal or better results than single chemicals and made it possible to exploit the better qualities of each. Paclobutrazol in mixtures benefited from the seed head suppression and the influence towards finer species contributed by maleic hydrazide and mefluidide. Melfluidide was superior to maleic hydrazide in growth suppression and colour but mixing these two chemicals was often as beneficial as mixing either of these with paclobutrazol. Off-colour problems from any of these treatments were few and of short duration. Additional tests with promising results were made with paclobutrazol at a higher rate in mixtures in the context of areas such as airport fields which because of traffic are difficult to spray regularly.

Much can be done to reduce maintenance by designing grassed areas for wide machines and sowing dwarf cultivars. The additional use of tank mixes of growth retardants will add flexibility to maintenance programmes and give long term control.

INTRODUCTION

Though maleic hydrazide has been available for over 30 years as a grass retardant for roadside verges and areas which were difficult to mow (Waterhouse, 1980) it is only in the last decade that the use of growth retardants in a wider amenity grass context became a reality. Three developments were reponsible for this change; (a) the introduction of a formulation of maleic hydrazide which was less liable to cause off-colour and being rainfast was more reliable. (b) the energy crisis of the 1970s which caused the cost of maintaining amenity grass to previously accepted standards to escallate. (c) the development of two new grass growth retardants mefluidide and paclobutrazol.

Previously growth retardants were generally regarded as of limited application and confined to areas where the final appearance was not too important (Shearing and Batch, 1979; Marshall, 1982; Field, 1983). Now with improved formulations and new products available it seemed appropriate to examine the use of growth retardants on moderately fine areas representative of parks, lawns around school and factory buildings and the home lawn. It was with this in mind that the present investigation was initiated. Monostands of single species are uncommon on this side of the Atlantic. Hence a mixed species lawn at the

Research Centre was used. However adjacent, all but pure, stands of fescue and perennial ryegrass were used to verify the effect of the growth retardants on these individual species. A further area of mixed species but mainly perennial ryegrass was used for additional tests with a higher rate of paclobutrazol.

MATERIALS AND METHODS

Four lawn areas were used for these experiments:

Lawn A An "old lawn" sown originally with a bent fescue mixture but now containing an admixture of coarse grasses mainly perennial ryegrass (Lolium perenne).

Lawn B A "fescue lawn" sown in 1982 with a seeds mixture consisting of 80% Chewings fescue (Festuca rubra ssp. commutata) and 20% browntop bent (Agrostis castellana) in which fescue was now dominant.

Lawn C A "perennial ryegrass lawn" sown in 1982 with a seeds mixture consisting of 60% perennial ryegrass, 35% Chewings fescue and 5% browntop bent in which perennial ryegrass was dominant.

Lawn D An area sown with mixed grass species including perennial ryegrass and now dominated by the latter.

Three growth retardant chemicals were tested alone and in mixtures:
(a) maleic hydrazide 500 g/l of the monoethanolamine salt
(b) mefluidide 240 g/l
(c) paclobutrazol 250 g/l

The rates of use of these chemicals alone in mixtures are given in Tables 1-4 and these rates were maintained throughout the four years of the experiment except that in 1983 the chemicals were at lower rates in the mixtures than in 1984, 1985 and 1986. In 1983 and 1984 they were applied in 448 l/ha water and in 1985 and 1986 in 500 l/ha to lawns A, B, and C using a Drake and Fletcher Mistifier pressurised sprayer with five fan jet nozzles (Teejet 11005) on a 1.25m boom. In the case of Lawn D the chemicals were applied in 400 l/ha with a Dorman wheelbarrow sprayer with a 2m boom with seven fan jet nozzles. In all experiments the plots were shielded during spraying to prevent drift of chemical from one treatment to another.

Lawn A: This was divided into 21 plots each 15.0 m x 1.5 m and the experimental design was three randomised blocks of seven treatments. The experiment was commenced in August 1983 and concluded with the last application in June 1986. Over the four seasons the same treatments were applied a total of six times to the same plots, once in 1983 and 1986 and twice in each of the intervening years. One annual overall application of a 2,4-D/CMPP herbicide mixture was given to control broad-leaved weeds.

Assessments of colour were made visually. Species composition of the plots are agreed visual estimations made jointly by two persons. Height measurements were restricted to the finer grasses because of the differential effect of the growth retardants on the mixed species and the tendency for tufts of coarse grasses to develop in some plots. Retardation effects were quantified by taking fresh weight. In summer, yields were taken at two intervals from spraying - usually 4 to 6 and 8 to 11 weeks which involved splitting the plots. In autumn following the second application the entire plots were harvested 8 to 11 weeks from spraying except in 1983 when harvesting took place 4 weeks after spraying.

Lawns B and C: These areas were used to compare the effect on fescue and perennial ryegrass of the treatments applied to Lawn A. The same treatments were applied on the same dates and at the same rates to lawns A, B and C but they were not replicated on lawns B and C. These three lawns were usually mown at about 25 mm approximately one week before spraying. Retardants were applied when at least 24 hours without rain were expected.

Lawn D: This was a coarser area which had not been maintained to the same standard as the other lawns. It was used in these experiments to simulate grass at an airport which because of air traffic could not be mowed at regular intervals. The chemical mixtures tested here were different and at a higher rate than those used on lawns A, B and C and are given in Table 5. They were applied twice in 1985, on 18th June and 13th September. There were four mixtures of retardants and an unsprayed treatment and these were replicated three times in a randomised block design.

All data from the replicated experiments on lawns A and D were subjected to an analysis of variance and LSD's calculated.

RESULTS

Lawn A : Fresh weights of grass harvested after 4 and 8 to 11 weeks following the application of the retardants are given in Table 1. Except in 1986 when maleic hydrazide was the only effective treatment most other yields were significantly lower than the unsprayed. Paclobutrazol was generally less effective than the other treatments in the 4-week harvest and some results with mefluidide were poor in the extended harvests. Of the single chemicals maleic hydrazide was generally intermediate. Yields from plots treated with mixtures of retardants usually did not differ significantly from those treated with the single chemicals.

In leaf height control, maleic hydrazide alone was generally least effective (Table 2). Though mefluidide had a good retarding effect for 4 to 5 weeks, it lacked the longevity of paclobutrazol which remained effective through 9 weeks. The chemical mixtures had a good retarding effect particularly those containing paclobutrazol.

Large fluctuations in colour occurred as a result of some treatments (Table 3). These were usually of short duration and depending on the chemical they occurred at different times during the season. Plots sprayed with paclobutrazol had a good colour from the time of spraying until late autumn when the colour deteriorated. Mefluidide had the opposite effect causing a deterioration in colour initially which quickly changed to the best colour of any of the treatments. Colour in maleic hydrazide plots was usually similar to the unsprayed controls. The colour of plots sprayed with mixtures also varied and were generally no worse than those of the single chemicals.

TABLE 1

Effect of three growth retardants singly and mixed together in pairs on fresh weight (kg) of lawn A plots harvested 4 and 8 to 11 weeks after spraying

	1st spray to harvest interval (weeks)[1]						2nd spray to harvest (weeks)	
Treatments (kg a.i. ha)	4 1983[2]	4 '84	4 '85	8 '84	11 '85	11 '86	10 '84	10 '85
PB 1.5[3]	8.3	11.8	4.9	12.4	13.6	13.1	14.1	6.3
MF 0.48	4.7	3.7	3.6	8.6	17.0	11.5	17.2	5.0
MH 4.0	6.6	7.0	4.8	10.6	15.1	9.7	13.0	6.1
PB 1.0+MF 0.24	4.3	4.9	1.8	6.2	11.3	12.6	11.1	4.0
PB 1.0+MH 4.0	6.0	6.4	1.7	6.2	9.6	11.1	8.6	3.9
MF 0.24+MH 4.0	4.8	4.4	2.9	7.5	15.5	11.0	11.5	4.8
Unsprayed	13.3	13.9	9.7	13.9	19.1	14.1	28.0	12.5
LSD 5%	1.45	1.45	1.11	5.24	3.3	3.3	2.99	1.88

[1] Plots were split for harvesting after the first spray
[2] In 1983, the application rates for the mixtures were PB 0.75+MF 0.24, PB 0.75+MH 2.0 and MF 0.24+MH 2.0
[3] PB = paclobutrazol, MF = mefluidide, MH = maleic hydrazide

Species composition of the plots was assessed in November, 1985 by which time the growth retardants had been applied five times to each plot (Table 4). Maleic hydrazide had a marked effect in increasing the percentage of fescue in the plots whereas paclobutrazol increased the percentage of coarse grasses; mefluidide was intermediate. Of the mixtures only the mefluidide/maleic hydrazide combination had a significant effect on species composition.

Of the separate retardants seedheads were controlled best by mefluidide and poorest by paclobutrazol. All three mixtures were good for seedhead suppression. The height of the seedheads was reduced by all five chemical treatments by comparison with the unsprayed controls.

Lawn B : Yields from this fescue lawn were generally lower from plots sprayed with mixtures of retardants than from the single chemicals and the mefluidide/maleic hydrazide treatment was usually best. Maleic hydrazide alone was poorest at controlling leaf height but there was no outstanding difference between the remaining five chemical treatments. As in lawn A paclobutrazol resulted in good colour throughout the season till late autumn when colour deteriorated and mefluidide caused off-colour initially which rapidly returned to normal green. Maleic hydrazide and the unsprayed plots were generally alike in colour. Mixtures with paclobutrazol had a colour effect similar to that of paclobutrazol alone. The maleic hydrazide/mefluidide mixture usually caused some off-colour. Seedhead number was reduced by all chemical treatments except paclobutrazol but seedhead height was reduced by all the retardants and mixtures.

Lawn C : In this perennial ryegrass lawn the plots sprayed with the chemical mixtures were consistently lower in yield and were usually lower in leaf height than the single chemicals in all four seasons. The colourreactions of these ryegrass plots to the treatments were the same as those for fescue. No seedheads occurred in plots sprayed with mefluidide alone or with maleic hydrazide alone or in mixtures. In contrast many seedheads occurred in the paclobutrazol-treated plot and much fewer in the plot treated with the paclobutrazol/mefluidide mixture.

In yield, height, seedhead production and colour these fescue and ryegrass plots (lawns B and C) reacted to the retardants in much the same way as the mixed lawn plots (lawn A).

Lawn D : All chemical treatments to this predominantly ryegrass lawn reduced yield and height significantly ($p < 0.05$) compared with the unsprayed (Table 5). Though all plots had reasonably good colour during the summer, the mefluidide/paclobutrazol treated plots retained colour best in late autumn while those treated with maleic hydrazide deteriorated. In May 1986 eight months after an autumn application there was a highly significant ($p < 0.001$) difference in height between the plots which were chemically sprayed and the unsprayed controls and colour was better than in the controls (Table 5).

DISCUSSION

With the development and release of mefluidide and paclobutrazol a new era for growth retardants dawned. The properties of these two chemicals have already been described (Price, 1984; Shearing and Batch, 1979). Most work on growth retardants has been done in relation to rough and difficult areas such as roadside verges, steep banks and cemeteries andseveral workers have expressed the view that it is in such areas where mowing frequency is low, appearance is not of prime importance and maintenance costs are high that the present range of growth retardants will be most readily accepted (Shearing and Batch, 1982; Marshall, 1982; Field, 1983; Atkin, 1984). However, this implies that they have some potential for use on finer areas and the present investigation shows that there need no longer be a question over whether growth retardants can be used successfully on moderately fine turf such as in parks, school and factory lawns and urban grassed areas (Kavanagh, 1986).

In general, mixtures of retardants in pairs gave good results and made it possible to exploit the better qualities of each particularly in relation to seedhead control and longevity. The relative ineffectiveness of paclobutrazol for seedhead control is well known (Shearing and Batch, 1982) but tank mixing it with maleic hydrazide or mefluidide overcame this weakness though there was some evidence that the 0.24 kg rate of mefluidide was too low. These mixtures also helped to counteract the tendency which paclobutrazol has for encouraging coarse grasses (Marshall and Craine, 1984) but the latter chemical gave longevity to the mixtures (Atkinson and Crisp, 1985).

No mowing was done during these experiments except when the plots were harvested for yield. Consequently, an uneven appearance developed because the species grew at different rates (Atkin, 1984) and were differentially affected by the retardants (Shearing and Batch, 1982). A topping over of the plots with a rotary mower every few weeks from spraying would have improved their appearance and removed any seedheads which developed. In the future the uneven effect caused by differential growth of coarse grasses which invade fine lawns may be prevented by sowing herbicide tolerant cultivars and maintaining them free from weed grasses by spraying with a broad spectrum herbicide (Johnston and Fisher, 1985).

Colour is an important quality in lawn turf and in general this was only temporarily impaired by any of the chemical treatments. Temporary off colour occurred with mefluidide shortly after application and with paclobutrazol in late autumn. These effects were very clearly shown by the separate fescue (lawn B) and ryegrass (lawn C) plots (Kavanagh, 1986).

The success of the higher rate of paclobutrazol in mixtures on lawn D indicates that long term control of grass growth could be achieved at airports where because of air traffic, spraying more often than once a year may be difficult. Even higher rates of both paclobutrazol and mefluidide would be desirable for long term control judging by the results of Atkinson and Crisp (1985) in orchards.

Two major problems for managers of extensive areas of amenity grass are growth peaks and escallating costs. Growth retardants can be of assistance in dealing with both. These chemicals can flatten out growth peaks and give flexibility in the management of the grass maintenance programme (Haggar, 1976; Atkinson and Crisp, 1985). They can also reduce costs substantially when because of obstacles or slopes pedestrian mowers must be used (Atkin, 1984). Designing a grassed area for large machines whether mowers or sprayers is an economical approach to grass maintenance (Parker, 1982). If, in addition, slow growing species and dwarf cultivars are sown costs will be further reduced (Johnston and Faulkner, 1985). It can be anticipated that in the future plant biotechnology will be applied towards the production of dwarf cultivars of several grass species use of which will reduce maintenance costs still further.

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TABLE 2

Leaf height (mm) of grasses[1] in lawn A treated with three growth retardants alone in mixtures on 30 August, 1983; 24 April and 24 August 1984; **17 May** and 6 September 1985 and 5 June, 1986

Treatments ($kg\ a.i.\ ha^{-1}$)	1983[2]	1984			1985			1986
	28 Sept	1 June	18 June	19 Oct	14 June	22 July	12 Nov	26 June
PB 1.5[3]	110	90	100	55	60	40	35	57
MF 0.48	77	53	123	103	70	120	77	67
MH 4.0	90	113	177	97	100	160	97	93
PB 1.0+MF 0.24	60	50	73	50	63	37	43	53
PB 1.0+MH 4.0	83	80	87	37	67	47	47	57
MF 0.24+MH 4.0	73	63	120	87	83	127	77	73
Unsprayed	187	160	167	160	130	180	107	100
LSD 5%	22.0	20.9	26.8	23.9	26.8	78.6	24.7	22.0

[1] Ignoring tufts of coarse grasses

[2] In 1983 the application rates for the mixtures were PB 0.75+MF 0.24, PB 0.75+MH 2.0 and MF 0.24+MH 2.0

[3] PB = paclobutrazol, MF = mefluidide, MH = maleic hydrazide

TABLE 3

Effect of three growth retardants singly and mixed together in pairs on colour[1] of lawn plots sprayed on 30 August, 1983; on 24 April and 24 August, 1984; 17 May and 6 September, 1985 and 5 June, 1986 (lawn A)

Treatment	1983[2]	1984			1985			1986
kg a.i. ha^{-1}	23 Sept	9 May	18 June	30 Oct	17 June	11 Oct	12 Nov	26 June
PB 1.5[3]	6.0	5.0	5.0	4.0	7.0	6.0	3.5	6.0
MF 0.48	3.7	3.0	6.3	6.0	5.3	5.3	5.3	4.7
MH 4.0	4.0	5.0	6.0	6.0	5.0	4.7	5.7	5.0
PB 1.0+MF 0.24	3.3	4.0	4.0	4.7	6.3	5.7	3.7	6.0
PB 1.0+MH 4.0	5.0	4.7	4.0	4.7	6.3	5.7	3.3	6.0
MF 0.24+MH 4.0	3.7	3.7	6.3	6.0	5.0	5.0	6.0	5.0
Unsprayed	8.0	5.0	5.0	6.0	5.0	6.3	5.7	5.0
LSD 5%	0.77	0.96	0.59	1.14	0.77	0.74	0.89	0.39

[1] Visual colour scale 1-9, 9 = very green
[2] In 1983, the application rates for the mixtures were PB 0.75+MF 0.24, PB 0.75+MH 2.0 and MF 0.24+MH 2.0
[3] PB = paclobutrazol, MF = mefluidide, MH = maleic hydrazide

TABLE 4

The effect of three growth retardants singly and mixed together in pairs on number and height of seedheads and on species composition (%) of lawn A

Treatments (kg a.i. ha^{-1})	Seedheads June 1984		Assessed November 1985[1]		
	No. of heads[2]	Height (mm)	Fescue	Bent	Coarse grasses[3]
PB 1.5[4]	4.0	205	50.0	32.5	17.5
MF 0.48	7.0	137	77.7	10.0	12.3
MH 4.0	5.0	300	93.7	6.0	0.3
PB 1.0+MF 0.24	5.7	183	70.0	22.7	7.3
PB 1.0+MH 4.0	6.7	193	72.7	22.3	5.0
MF 0.24+MH 4.0	7.0	163	86.7	11.7	1.7
Unsprayed	4.0	367	65.7	26.7	7.7
LSD 5%	0.92	36.1	11.9	11.4	6.2

[1] Following five applications/plot of each treatment between August 1983 and September 1985
[2] Based on a 1-9 scale, 9 = no seedheads
[3] Coarse grasses present were *L. perenne* but also *Holcus lanatus*, *Poa trivialis*, *Poa annua* and *Cynosurus cristatus*
[4] PB = paclobutrazol, MF = mefluidide, MH = maleic hydrazide

TABLE 5

The effect of applying paclobutrazol in mixtures with maleic hydrazide, mefluidide or both on 18 June and 13 September, 1985 to a predominantly perennial ryegrass lawn (lawn D)

Treatments (kg a.i. ha^{-1})	Assessed August 1985			Assessed November 1985			Assessed May 1986	
	Yield kg/plot	Height (mm)	Colour[1]	Yield kg/plot	Height (mm)	Colour[1]	Height (mm)	Colour[1]
PB 1.75+MF 0.24[2]	14.1	113	6.0	5.9	93	4.0	170	6.0
PB 1.75+MH 4.0	10.0	110	5.3	3.7	83	2.7	157	6.0
PB 1.75+MH 2.0	13.8	127	5.3	4.3	77	2.7	163	6.0
PB 0.88+MH 4.0+MF 0.24	21.5	147	5.0	4.3	83	2.7	287	5.0
Unsprayed	41.5	347	4.7	15.5	190	5.7	443	4.0
LSD 5%	10.38	32.5	1.17	2.69	19.9	1.06	46.6	0.0

[1] Visual colour scale 1-9, 9 = very green
[2] PB = paclobutrazol, MF = mefluidide, MH = maleic hydrazide