STUDIES ON THE INFLUENCE OF SOME AUXIN HERBICIDES ON GRASS-SEED CROPS

K. M. SEN

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STUDIES ON THE INFLUENCE OF SOME AUXIN HERBICIDES ON GRASS-SEED CROPS

ONDERZOEKINGEN OVER DE INVLOED VAN ENKELE HERBICIDE GROEISTOFFEN OP VOOR DE ZAADWINNING GETEELDE GRASSEN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS, IR. W. F. EIJSVOOGEL,
HOOGLERAAR IN DE HYDRAULICA, DE BEVLOEIING,
DE WEG- EN WATERBOUWKUNDE EN DE BOSBOUWARCHITECTUUR
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
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THEOREMS

I

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VI

Removal of all „Laxton's superb" pear trees in the Netherlands, proposed by VLASVELD in order to avoid the fireblight disease, will have no effect in this country.

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VII

The problem of cattle population in India is more than anything else a sociological one and for the solution of the problem, more attention should be paid to this aspect in the rural communities of India.

VIII

It is in the first place the impact of technological changes and not legislation or religious reforms, which will bring about the dissolution of the Caste system in India.

IX

The pre-treatment of maize seedlings with shortday gives no solution of the difficulties to grow „kernel maize" in the Netherlands.

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Ultimate lodging or the degree of lodging in cereals depends on the interactions of many factors concerned and not on a particular factor. Knowledge about this interaction at any growth stage of the plant is still inadequate.

Proefschrift K. M. SEN
Wageningen 1960

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To my parents and my wife

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Dit proefschrift verschijnt tevens als No. 66.11 in de reeks Verslagen Landbouwkundige Onderzoekingen

1. INTRODUCTION

1.1. Description of the weed problem in growing grass seeds in the Netherlands

In the Netherlands most grass species grown for seed production are perennial plants. For crop rotation at farms most of these species are grown as wintering „biennial" crops. After harvesting the seeds, a varying percentage of the fields depending on the species is maintained for one or more growing seasons. The annual species *Lolium multiflorum* Lamk. *westerwoldicum*, grown for seed-production purposes, is included in crop rotation as a spring-sown crop and is harvested in the summer of the same year.

The method in which grasses for seed production are grown partly determines to what extent weeds may influence the development of these plants. Many grass-seed crops, e.g. *Festuca rubra* L., *Dactylis glomerata* L. and *Poa pratensis* L., are sown under a cover crop in spring, e.g. peas, fiber flax, poppy seed or spinach grown for seed. The relatively open and not too competitive character of these crops allows a slow establishment of the grass seedlings. On loam and clay soils, spring wheat, barley or oats are seldom used as cover crops for grass-seed crops, but on sandy soils winter rye is a common cover crop for *Festuca ovina* L.

After harvesting the cover crop, the development of young grass plants may be considerably influenced by weeds.

After harvesting, fertilizer applications to promote the development of grass may stimulate weed growth. Therefore, weed control in the stubble at the end of summer and in early autumn may be of vital importance to the success of the crop.

In the Netherlands grass-seed crops are commonly grown on medium and heavy soils, although sandy soils are also utilized for some species, e.g. *Festuca ovina*. Heavier soils generally have a rather broad weed spectrum, in which annual as well as perennial weeds play an important part. Among the dicotyledonous annual weeds *Stellaria media* (L.) Vill. ssp. *media*, *Veronica* (L.) spp., *Capsella bursa-pastoris* (L.) Med., *Matricaria* L. spp., *Sonchus asper* (L.) Hill are most frequent. As perennials *Cirsium arvense* (L.) Scop., *Sonchus arvensis* L., *Tussilago farfara* L. and (on sandy soils) *Rumex acetosella* L. can be troublesome. The most prominent annual grass weeds are *Poa annua* L. and *Alopecurus myosuroides* Huds.

In grass-seed growing the commercial value of the product is of course greatly influenced by the absence of weed seeds and undesirable grass seeds. In the Netherlands the growing of grass-seed crops is allowed under the regulations of the N.A.K. (Nederlandse Algemene Keuringsdienst voor Landbouwzaden en Aardappelpootgoed, General Netherlands Inspection Service for Seeds of Field Crops and for Seed Potatoes). The contracts between growers and seed firms are usually based upon the „Algemene Teeltvoorwaarden" („General Growing Agreement"). In both sets of regulations the purity of grass seed and its germination capacity are important items in determining the value of grown seeds and the financial result to the farmer.

In order that seed yields of high purity may be reached and that growers may remove undesirable grasses and weeds, the fields are investigated on the presence of weeds twice during the growing season by officers of the N.A.K. To allow seed firms to employ the best suited cleaning procedures in handling the seeds, the final report or certificate on the harvested seeds mentions all impurities found. The possibility of removing impurities mechanically in some cases makes the presence of a small percentage of weed seeds acceptable.

Some grass-weed seeds specially mentioned as being objectionable are *Alopecurus myosuroides* HUDS., *Avena fatua* L., *Agropyron repens* P.B., *Poa annua* L. (NAK-Circulaire No. 121, 1960). The following dicotyledonous weeds are listed: *Lapsana communis* L., *Centaurea Cyanus* L., *Sonchus* spp., *Matricaria* spp.

In terms of cleaning techniques each species of grass seed has its own troublesome weeds but from the preceding paragraph the general conclusion may be drawn that the absence of weeds in grass-seed crops will also be reflected in the purity of the seeds harvested and the value of the product.

1.2. APPROACHES TO THE SOLUTION OF THE WEED PROBLEM

1.2.1. Mechanical weed control

Growing grass-seed crops on soils with a limited weed flora and using weed-free seed to establish the crop usually do not result in fields in which weed-control practices are no more required. These provisions are, however, of importance as preventive measures in the weed control programme.

Hand hoeing and other mechanical methods of weed control are connected with several disadvantages. Although grass-seed crops are generally sown in rows, inter-row mechanical weed control immediately after harvesting the cover crop causes too much damage to the grasses, owing to the small size of the plants (EVERS and WOLFERT, 1959a, b; TOLNER, 1960). After the young grass plants have been hardened off, mechanical weed control is possible, but if tillering is well under way great care has to be taken in order to prevent damage to the crop. Soil cultivation in autumn is not effective because the soil is frequently too wet then. Weeds like *Poa annua* and *Stellaria media* are retarded in growth only. These weeds may develop rapidly in late autumn and winter, when mechanical weed control is no longer possible.

The fact that mechanical weed control procedures are of little importance is certainly well demonstrated by grass-seed crops which are sown without cover crop in late summer or early autumn. *Lolium perenne* and *Poa trivialis* are grown in this way. Perennial weeds generally do not cause any trouble in the year of sowing, but in severe cases annual weeds may smother the young grass seedlings completely.

In early spring soil cultivation may cause a serious yield depression (EVERS and SONNEVELD, 1956; TOLNER, 1960).

Nowadays mechanical weed control methods are replaced more and more by the use of herbicides. Only for the control of undesirable grasses hand hoeing is still practised, as the herbicides available at present are not sufficiently selective to enable a chemical control of these grass weeds.

1.2.2. Chemical weed control

It may be stated that with several other crops as well as with grass-seed crops the introduction of herbicides has allowed the development of improved methods of weed control and has contributed to an increase in area covered by these crops (TOLNER, 1960). In the history of chemical weed control in grass-seed crops in the Netherlands initially sodium arsenite and calcium cyanamide have been the chemicals used most commonly.

The use of sodium arsenite (8–12 L of a formulation carrying 50% As_2O_3 per L) in autumn applications has never been supported by sufficient research data, but practical experience has established the value of these treatments. The toxicity of the product and its tendency to accumulate in the soil has led to the introduction of DNOC (5–6 kg a.e. per ha) and of mixtures of DNOC and small amounts of arsenite. The general effect of these treatments is a more or less satisfactory control of annual weeds, but only a temporary control of *Stellaria media* due to a restricted residual effect.

For good results this product has to be applied in high volumes of spraying liquid and under the right weather conditions (cloudy sky, relative humidity of the air at least 70% and no rain) (EVERS and WOLFERT, 1959). The product is not very effective with some weeds. *Polygonum aviculare* L., *Veronica* spp., *Solanum nigrum* L. and perennial weeds are only temporarily checked in their growth (ZONDERWIJK, 1959).

Calcium cyanamide can be of particular importance in the control of *Stellaria media* in the dormant season of the grass-seed crops. With this herbicide too the success of the application depends on the right weather conditions. It is recommended to apply the powdered product early in the morning after a night-frost before a sunny day. Even under optimal conditions, however, results are not always completely satisfactory. Moreover, the cost of the treatment is considerable, the residual effect is short and the fertilizing effect of the nitrogen is frequently questionable.

The importance of the auxin herbicides 2,4-D and MCPA for weed-control purposes in cereals and pastures (ROBBINS, CRAFTS and RAYNOR, 1952; LEOPOLD, 1955; AUDUS, 1959) and the similarity of these crops and grass-seed crops is well-known. It is therefore quite understandable that these products may also be used for the control of dicotyledonous weeds in grass-seed crops. For a profitable use it is important, however, that it has been demonstrated in cereals that the reaction of a plant on such a treatment will be determined partly by the stage of growth of the plant at the moment of spraying and by the weather conditions shortly before, at and after spraying (ROBBINS, CRAFTS and RAYNOR, 1952). Moreover, species and even varieties of species may react differently upon a specific application.

Realizing that grass-seed crops are perennial species (and not annual species like cereals) with a genetically more heterogeneous character than cereals usually have, some difficulties may have to be envisaged in comparing the data on susceptibility obtained from cereals with those obtained from grass-seed crops.

In 1957 at the beginning of our investigations experience with auxin herbicides in grass-seed crops was limited. In several spring-sown species 2,4-D (amine salt) was used as a rule for the control of *Tussilago farfara* and MCPA for the control of *Cirsium* *arvense*. Just at this moment 2,4,5-TP was introduced as a herbicide of possible value for the control of *Tussilago farfara*. It moreover had the additional advantage that it was of great effectiveness with *Stellaria media*.

With some species *(Lolium perenne, Festuca rubra)* applications of MCPA were carried out shortly before shooting. These treatments never were officially recommended due to the insufficient knowledge on the influence of the treatments on yield and seed quality.

1.3. PURPOSE OF THE PRESENT STUDY

Considering the practical importance of auxin herbicides in modern weed control and the great risks involved in their correct application in several crops, a study of the effect on grass-seed crops of some commonly used herbicides, belonging to this group, appeared to be desirable. The more so as studies carried out in other countries give neither sufficient nor satisfactory information on auxin herbicides to make a reliable recommendation possible with regard to the use of these herbicides in grass-seed crops.

In many publications, observations, made in experiments and in practice, on the effects of auxin herbicides on the morphological development and the seed production of grasses have been interpreted on the basis of the moment of application and the height of the plants with rather broad indications concerning the stage of development of the crop (c.f. for example ÅBERG and HAGSAND, 1951; HALLIDAY and TEMPLEMAN, 1951; MÖLLER NIELSON, 1951; EVERS and SONNEVELD, 1953; SLAATS and STRYCKERS, 1953, 1955). From these studies, which are of a rather practical nature, it may be concluded that the botanical nature of the grass species and the variety concerned, its stage at the moment of treatment, the auxin herbicide and the dosage used as well as possible weather conditions at the moment of spraying all influence the final effect of the treatment upon the morphological development of the crop and its seed production.

In England JEATER (1956, 1958) succeeded in giving a better description of the stage of growth of the grass plants at the moment of spraying, by studying the morphological development of the growing point of the shoots. Thus closer comparison became possible with knowledge of similar studies on the effect of auxin herbicides on cereal plants (c.f. FRIESEN, 1949; SEXSMITH, 1949; DAVIDSON, 1949; ALLEN, 1952). Moreover, this approach allows a better comparison between the reaction of different grasses. Unfortunately the development of the growing point of our cultivated grasses in relation to environmental factors such as temperature and light has been studied on a limited scale and only recently more attention has been paid to it (COOPER, 1950, 1952; GARDNER and LOOMIS, 1953; HANSON and SPRAGUE, 1953; BOMMER, 1959; PETERSON and LOOMIS, 1959). Consequently it appeared desirable to extend this type of study to grasses grown under the climatological conditions of the Netherlands. Moreover, a closer correlation between leaf and inflorescence abnormalities caused by auxin herbicides and data on seed yield and quality seemed to be lacking.

The present investigation has taken all these factors into consideration and also included the more recently developed auxin herbicide 2,4,5-TP. The investigation has been restricted to grass-seed crops grown on clay soil.

2. PROCEDURE OF THE EXPERIMENTS

To achieve the purpose of the investigation, field experiments and experiments under controlled temperature and light conditions of either artificial or natural light were carried out. In the following paragraphs the methods of research are described in detail.

2.1. Growing the plants

2.1.1. Field trials

The size of the individual plots in these experiments did not permit any yield determination to be carried out. The main purpose of these experiments was to obtain sufficient material to study the influence of treatments with auxin herbicides upon the morphological development of the grasses and upon the seed quality.

Field experiments were carried out in the North-East-Polder in 1957–58 and in the Haarlemmermeerpolder in 1958–59. The grasses studied were grown according to the normal agricultural routine from seeds. They were usually sown under a cover crop. In the experiments approved strains of Dutch grass breeders were used except for *Poa trivialis* and *Poa pratensis* of which only commercial seeds were available. In 1957–58 the following grasses were investigated: *Lolium perenne*, *Festuca pratensis*, *Festuca rubra*, *Poa pratensis*, *Poa trivialis* and *Dactylis glomerata*. Except for the last species all grasses were studied again in experiments during the season 1958–59. *Festuca pratensis* was studied in two different trials with different dates of sowing. In Table 1 all field trials have been mentioned.

In 1957–58 the unit size of each experimental plot was 2 m by 1 m, generally giving 4 rows of grass per plot. In the experiments of 1958–59 the plot size was 2 m by 2 m.

Table 1. Field experiments on the influence of auxin herbicides on the morphology and seed characteristics of grasses

Growing season and grass species	Month of sowing	Cover crop	Month of harvesting the cover crop
1957–58			
Dactylis glomerata	April, 1957	Peas	July, 1957
Festuca pratensis	Ditto	Ditto	Ditto
Festuca rubra	Ditto	Ditto	Ditto
Lolium perenne	early September, 1957	–	–
Poa pratensis	April, 1957	Peas	July, 1957
Poa trivialis	September, 1957	–	–
1958–59			
Festuca pratensis	April, 1958	Oats	August, 1958
Festuca rubra	Ditto	Peas	early August, 1958
Poa pratensis	Ditto	Ditto	Ditto
Festuca pratensis	end July, 1958	–	–
Lolium perenne	late August, 1958	–	–
Poa trivialis	late September, 1958	–	–

There were two replicates of each treatment in both years. A lattice design was used in all experiments.

Simultaneously with the experiments described other experiments were planned in which the influence on seed yield and quality was studied for various treatments. These studies were made in co-operation with the Department of Weed Control, Institute for Biological and Chemical Research on Field Crops and Herbage (I.B.S.) and the Department of Grassland and Forage, Research and Advisory Institute for Field Crops and Grassland Husbandry (P.A.W.) at Wageningen. In these experiments the plot size was 4 m by 10 m or 4 m by 8 m and each treatment had three replicates.

2.1.2. Pot experiments in the field

These experiments were carried out in earthenware pots in the garden of the Institute for Biological and Chemical Research on Field Crops and Herbage during the growing season 1957–58. In August 1957 the clones of two grass species – *Lolium perenne* and *Festuca rubra* – were separated into individual tillers. Four tillers at the same stage of development were planted in the pots. The pots were treated with auxin herbicides according to a statistically projected plan; each treatment had three replicates. The soil in the pots received an initial fertilization during filling and in early spring of 1958 ammonium nitrate was given to stimulate development.

2.1.3. Experiments in environmental chambers and greenhouses

The size of the environmental chambers and the vernalization requirement of many common grasses did not permit the experiments to be carried out with perennial grasses. Therefore experiments were made with the annual grasses *Poa annua* L. and *Lolium multiflorum* LAMK. *westerwoldicum* (Strain Barenza) under artificial light (environmental chamber), and with the weed *Poa annua* under natural daylight conditions (greenhouse experiment). The main purpose of the experiments was to obtain information on the influence of temperature after spraying on the effect of auxin herbicides on grass plants.

Five experiments were carried out in the environmental chambers and one in the greenhouses. For the experiments with *Poa annua* wooden crates were covered on the inside with a black plastic sheet and filled with moistened peat. Small earthen pots of approximately 10 cm in diameter were filled with local sandy soil mixed with compost and then placed inside the peat-filled crate in such a way that the peat surrounded the pots completely. The bottom holes of the pots were filled up with cement to prevent the roots growing into the peat.

Poa annua seeds were sown and from all seedlings emerging in each pot three plants were allowed to develop. Nutrients (Hoagland solution) were applied twice during the experiments one and three weeks after emergence. Water was added regularly to the peat and this kept the pots sufficiently moist.

For the experiments with *Lolium multiflorum westerwoldicum* Mitscherlich pots filled with a mixture of local sandy soil and compost were used with five plants in each pot. Nutrients were added in the same way as in the experiments with *Poa annua*. Water was added regularly in order to keep the soil sufficiently moist.

The plants were grown at various temperatures until the moment of spraying. After spraying the plants were kept at the same temperature or transferred to another temperature according to the scheme described under 2.2.3.

The intensity of light in the chambers was 6×10^4 ergs. cm^{-2} sec^{-1} between 400 and 700 mμ. The type of lamp used was a Philips High Pressure Luminescent Lamp (400 W). The total number of light hours in the rooms was 17 hours, alternating with a dark period of 7 hours. The relative humidity fluctuated between 65 and 100 percent. In the greenhouses with controlled temperatures the plants received natural daylength during the period 9th of June until 9th of August, 1959.

2.2. Treatment of the plants

The plants were treated with the auxin herbicides 2,4-dichlorophenoxyacetic acid (amine salt), 4-chloro-2-methylphenoxyacetic acid (potassium salt) and α-(2,4,5-trichlorophenoxy)-propionic acid (low-volatile ester). In this publication these chemicals will be abbreviated to 2,4-D, MCPA and 2,4,5-TP respectively.

Commercial products have been used. 2,4-D was applied as „Duphar-2,4-D", a product of Philips-Duphar N.V. containing 500 gm acid equivalent (a.e.) per liter. MCPA-K salt was applied as „Agroxone-Extra", a product of Imperial Chemical Industries N.V., Holland containing 400 gm acid equivalent per liter. 2,4,5-TP was applied as „AAtussil", a product of Landbouwbureau Wiersum N.V., carrying 428 gm acid equivalent per liter. Dependent on the experiment different amounts of acid equivalent of the auxin herbicides were applied, the usual rates being 1 and 2 kg a.e. per hectare.

2.2.1. *Field trials*

The auxin herbicides studied in these experiments were 2,4-D, MCPA and 2,4,5-TP. In the field experiments of 1957–58 only one dosage rate for each herbicide was used – 1 kg a.e. of MCPA per ha, and 1.5 kg a.e. of 2,4-D and 2,4,5-TP per ha. In 1958–59 two dosages rates – 1 kg and 2 kg a.e. of each herbicide per ha were used. The volume of spraying liquid was 1250 liters per ha. Each plot was sprayed separately with a hand sprayer. During spraying the plots were enclosed by a wooden shield to prevent drift. Yield experiments were sprayed with a knapsack sprayer.

The herbicides were applied at various stages of development of the grasses. The methods used to determine these stages are described under 2.4.1. In 1957–58 the grasses were sprayed at various times from August 1957 to June 1958 (harvest year) to cover as many stages of growth as possible. In 1958–59 the sprayings were reduced to a limited number of applications in autumn and spring. This appeared to be justified on the basis of 1957–58 experiments. In all experiments the moments of spraying as well as the weather conditions were chosen in such a way that optimum weed control effects could be expected.

2.2.2. *Pot experiments in the field*

Pots were sprayed with a hand sprayer with 2,4-D, MCPA and 2,4,5-TP within a wooden frame of 2 m by 1 m in size. Two dosage rates were used, viz. 1 kg and 2 kg a.e. per ha.

The spray volume amounted to 1250 liters per ha. Pots to be sprayed were kept under shelter 2–3 days prior to and 2–3 days after the moment of spraying. As in the field experiments, the herbicides were applied at various moments throughout the season so as to cover as many stages of growth as possible.

2.2.3. Experiments in environmental chambers and greenhouses

These experiments were only carried out with 2,4-D. The concentration of the herbicide used was 2 kg a.e. per ha. The method of spraying employed was exactly the same as with the pot experiments except for the plants being taken from the chambers or greenhouse at different stages of development and being returned immediately after spraying to chambers maintained at the same, a lower or a higher temperature than the original one. In one experiment, the plants after spraying were transferred to another temperature more than once.

2.3. Harvest of the plants

With the field experiments the two middle rows of the sprayed area from each plot were harvested. With the pot experiments the pots were harvested individually. The harvests of the individual plots or pots were put into paper bags, and brought to the laboratory to be dried, threshed and cleaned. Before threshing a hundred tillers from the field experiments and fifty from the pot experiments were selected at random from the material harvested to be kept separately to study inflorescence abnormalities. The remainder was threshed and cleaned mechanically.

The greenhouse and environmental-chamber experiments were only undertaken to study the morphological abnormalities of plants caused by auxin herbicides. The duration of these experiments was roughly two months. They were harvested to study the dry weight production, the number of tillers, the number of ear-bearing tillers, the number of abnormal tillers etc. All the shoots of every plant were examined.

2.4. Study of the effects

2.4.1. Criteria of the morphological development of grass plants

2.4.1.1. Introduction

In studying the responses of plants to environmental factors such as, for instance, temperature, day length, application of fertilizers or herbicides, or in following the development of plants in studies on agricultural techniques, for instance the sowing time of crops, seed rates etc., it is necessary to have criteria to describe the stage of development of the plants in relation to their growth cycle. In discussing the criterion adopted in our investigations to describe the morphological development of grasses it is of interest to mention briefly some of the approaches followed in working out similar standards for cereals.

The height of the plant with spring and winter cereals is not a reliable indication of the important morphological changes occurring in the shoot apices at the moment of inflorescence initiation (WITTENROOD, 1959). The number of visible leaves may give a satisfactory indication. As has been shown for spring cereals by ANDERSEN (1955), the average leaf number of a population of cereal plants shows a steady increase with time, with definite short intervals, however, during which this number remains constant. During these intervals the development of the vegetation point is not interrupted. For investigations with a high degree of accuracy a study on the stage of development of the growing point is required.

The scale developed by FEEKES (1941) also is of little use in the early stages of development of the plant. The system based on examining the morphological development of leaves and tillers, and nodes during tillering and stem extension, and the development of the spike from the time of heading onwards is of value after the formation of the inflorescence has been finished.

The following general conclusion may be drawn from the work on cereals. Direct observation with a microscope of the stage of development of the growing point is the most justified procedure with all investigations in which an influence is to be expected on the morphological development of the shoot apices and inflorescence structures. Research on the ontogeny of the inflorescence in cereals is sufficiently advanced to allow an identification of stages in this morphological development (ESAU, 1953).

For our studies perennial and intensively tillering grasses were used on which neither one nor more main tillers could be distinghuished in early spring. Consequently, the counting of leaves on main tillers was impossible. The height of the plant (ÅBERG and HAGSAND, 1951) is an unsatisfactory criterion too, as it depends on many factors such as the age of the stand, the cover crop under which the grass was sown, the level of fertility, and the management of the crop.

Therefore, in these grasses as well as in cereals the morphological development of the growing point of the shoot apex seems to be the best criterion for practical use in investigations.

The importance of some stages in the development of the growing point as a criterion for a description of the physiological status of the grass plant was clearly demonstrated by DE VRIES (1957). His studies were based on the discovery of VAN DE SANDE BAKHUYZEN (1947) that stages can be recognized in the life cycle of wheat and several other plants in which the ratio between the fresh weight and the dry weight of shoots remains constant. For two types of *Lolium perenne* and for *Festuca rubra* DE VRIES showed that sudden changes in this ratio are correlated with the initiation of the reproductive stage. For a closer study of morphological changes in the growing point the mentioned ratio does not seem to give further indications. The ratio as such does not serve the purpose of a criterion in distinguishing in detail between the different stages of the growing point during the vegetative or reproductive stage of the plant.

In several grass species attention has been paid to the stages of the growing point during the development of the inflorescences. EVANS and GROVER (1940) studied many grasses. SHARMAN (1947) studied *Phleum* spp., *Lolium perenne*, *Agrostis* spp.; JEATER

(1956) studied *Lolium perenne*, *Festuca rubra* and *Dactylis glomerata;* KLEINENDORST and TEN HOVE (1957) studied *Lolium perenne* and *Dactylis glomerata*. Recently BOMMER (1959) studied many grasses in detail.

2.4.1.2. Sampling of the material

All these studies mentioned above aim at the description of the development of a single shoot apex during and after flower initiation. If in practical studies a system of the stages of development, recognizable with a microscope is used, there will arise the problem of a rather wide variation in shoot apices from different plants and even from different tillers from one single clone. Within the scope of this study the system followed by JEATER as well as by KLEINENDORST and TEN HOVE has been adopted in sampling the material. Due to the many grasses involved it has not been possible to pay special attention to the variations mentioned.

2.4.1.3. Dissection of the growing points

A binocular microscope and sharpened spear-shaped and straight needles were used to dissect the shoot apices of the grasses (KLEINENDORST and TEN HOVE, 1957).

When shoots were taken from a tillering clump for dissection each shoot was removed as deep as possible in order to obtain the apex, which is always well protected. The few outer leaves of the selected shoot were removed without the use of lens or needle. In view of the experience acquired previously about the location of the apex at different stages of development, the part of the leaves above the expected location was cut off with a spear-shaped needle. The remaining part was then placed on black paper, mounted on a slide on moistened filter paper in order to prevent the apex from drying up. The slide was subsequently put under the microscope and by keeping the material in position with one hand dissection could be carried out with a needle held in the other hand. For the final touch in making the shoot clearly visible, the straight needle was used. The shoot apex was removed when it had to be photographed or when it was not too clearly observable.

Microphotography took place in a dark room with a relatively low temperature. A relatively weak lamp and a projection lamp were placed in front of a microscope. All accessories required for microphotography with an Exa camera were attached to this microscope. An opaque, black glass plate was marked with two very small parallel lines in the center. This was done in such a way that when it was placed under the microscope in standard position the two lines running parallel came under the camera objective in the correct position without any loss of time. The apices were placed between the parallel lines under a binocular microscope. The focussing for photography was done with a weak light, in order to prevent the material from drying up. Focussing and taking the photographs only took few seconds, but had to be done quickly to prevent the loss of turgor of the material due to the heat of the strong microscope light. The film used was Agfa Isopan FF 13°/10 Din.

2.4.1.4. Stages recognized in the morphological development of the shoot apex

As mentioned under 2.4.1.1., the number of stages in the development of the shoot apex distinguished in the present investigation has been selected in such a way as to have a limited number of stages with quite distinct morphological characteristics. Every time a spraying was carried out the shoot apices of the control plants were examined. The final classification, mentioned below, is based upon preliminary studies on all the grasses, touching on almost all major morphological changes. Although wide variations in the type of inflorescence exist in grasses, ranging from a spike with *Lolium perenne* to the much-branched panicle with *Dactylis glomerata*, the morphological development of all grasses follows much the same pattern. Grasses like *Dactylis glomerata* and *Festuca pratensis* have multiple-branched types of inflorescence, the secondary reproductive primordia of which, different from grasses such as *Lolium perenne*, do not normally produce spikelets but elongate to form branches on which primordia of higher order are formed.

The stages of development used are those found in the majority of the growing points of the tillers sampled at random at one particular time. Individuals of the group may also show different stages in different parts of the developing inflorescence. In this case, the specimen is noted down according to the most advanced stage observed. The final assessments of the morphological stage of development of the plants, therefore, are relative rather than absolute. Instances in which two stages are found in equal or almost equal proportion, both stages are mentioned with a hyphen in between. For example, Vegetative-Secondary means that vegetative as well as secondary reproductive primordial stages are present in equal or almost equal proportion.

The scale of the stages of development, followed in this study, is given in TABLE 2. The distinction of five stages is convenient in the present investigation for reasons already mentioned before. The sub-stages under C also occur in the sequence indicated, but the type of inflorescence determines after which sub-stage the next stage (i.e. D) begins.

TABLE 2. Scale of developmental stages in the apices of grasses represented in the experiments (FIGS. 1–5)

A. VEGETATIVE PRIMORDIAL STAGE (Abbreviated: V.P.)

The stage is characterized by the presence in the apex of transverse ridges, occurring in the same plane of the leaves (FIG. 1). During the development of the growing point the number of visible ridges increases. Therefore the number was counted and the vegetative stage is expressed by the number of ridges at a particular moment. For instance 1–, 2–, or 5– Vegetative Primordial Stage, which may be abbreviated as 1 V.P., 2 V.P., or 5 V.P. respectively.

B. DOUBLE RIDGE STAGE (Abbreviated: D.R.)

This is the transitional stage during which the apex changes from the vegetative primordial stage into the reproductive primordial stage (FIG. 2). In the axils of some of the leaf primordia, visible in the vegetative primordial stage, buds start to develop. The alternate rows of „double bumps" on the shoot apex characterize this stage.

TABLE 2 (continued)

C. REPRODUCTIVE PRIMORDIAL STAGE (Abbreviated in the sub-stages)

After the formation of double ridges, the axillary buds grow out to form the branches of the inflorescence of higher order. The degree to which this order continues depends on the type of inflorescence of the various grasses. On the last order of branches the rachillae are borne.

Under the following sub-headings different orders are described, comprising all grasses under investigation. Each grass will follow the sequence upto the point characteristic to the species and then will change over to the next stage of „Floral Parts Formation".

C-I. Secondary reproductive primordial stage (Abbreviated: Sec.)

The rachis being the primary axis of the inflorescence, the branch formed by the development of the axillary bud is taken as „Secondary" to the primary axis (FIG. 3). Thus the stage is characterized by the presence of the second order of branching as the most advanced stage of development.

C–II. Tertiary reproductive primordial stage (Abbreviated: Tert.).

In their turn secondary axes may give rise to branches. These are of tertiary order and the stage is characterized by the development of the tertiary order of branching (FIG. 4).

C–III. Quaternary reproductive primordial stage (Abbreviated: Quat.).

D. FLORAL PARTS FORMATION (Abbreviated: Floral).

After the successive orders of branching specific to the type of inflorescence are completed, floral parts are formed, and differentiated (FIG. 5).

Finally, glumes and lemmas grow out and envelop the developing florets. In the present investigation, all stages of development of inflorescence between the initiation of floral parts and their completion have been included under the stage of „Floral Parts Formation".

E. EMERGENCE OF THE INFLORESCENCE (Abbreviated: Em. Infl.)

In the present investigation the emergence of the inflorescence is characterized by the partial or complete emergence of the inflorescence from the flag leaf or the final stage of enclosure within the flag leaf.

2.4.2. Review of morphological abnormalities observed in grass plants after treatment with auxin herbicides

Though innumerable papers are available on the gross morphological responses of cereals to auxin herbicides (reviewed by KAUFMAN, 1953), literature pertaining to grass-seed crops is rather limited (ÅBERG and HAGSAND, 1951; MÖLLER NIELSON, 1951; KERSTING, 1955; JEATER, 1958). Moreover, it does not cover all grass-seed crops included in our investigations. In view of this fact, an attempt has been made to group the different types of symptoms observed in this experimental material.

For the quantitative assessment of abnormalities observed in our experiments, i.e. a determination of the percentage of abnormalities, only the symptoms described below were taken into account. Descriptions and illustrations are derived from studies in environmental chambers, greenhouses and the field, and from all grasses included in the experiments. In some grasses teratological aberrations also appeared in the non-treated control plants and were recorded duly. It must be admitted that a detailed knowledge of the developmental morphology of leaves and inflorescence of the different grasses is required to obtain a more fundamental approach in the grouping of malformations. Such a study has not been possible within the scope of our investigation.

For the quantitative assessment of malformations, 100 tillers from field experiments, 50 from pot experiments and all tillers from the experiments in environmental cham-

FIG. 1. Vegetative primordial stage *(Lolium perenne)*

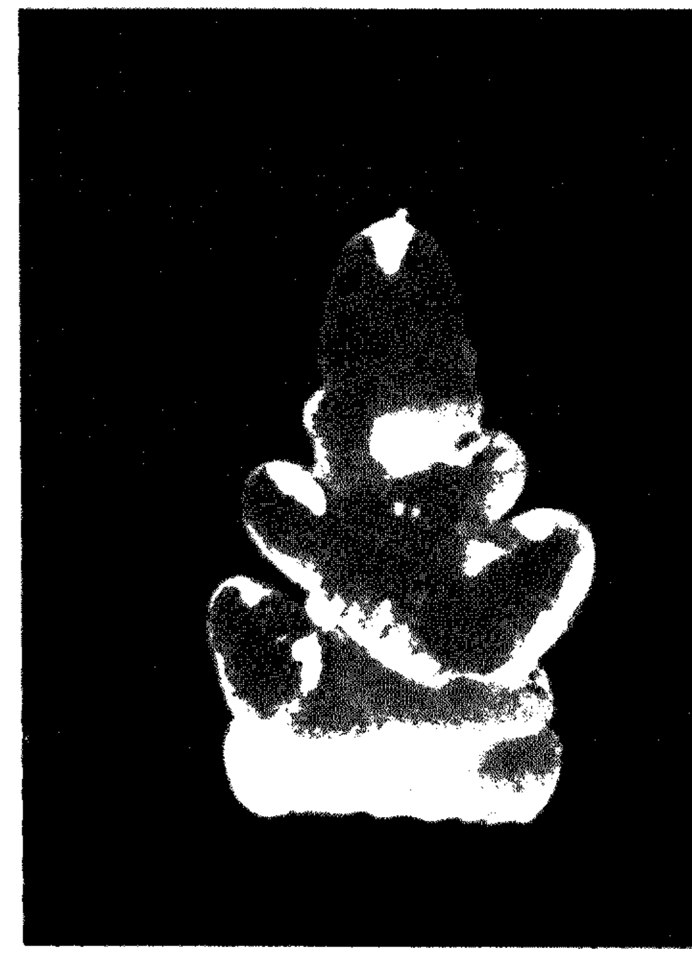


FIG. 3. Secondary reproductive primordial stage *(Festuca rubra)*

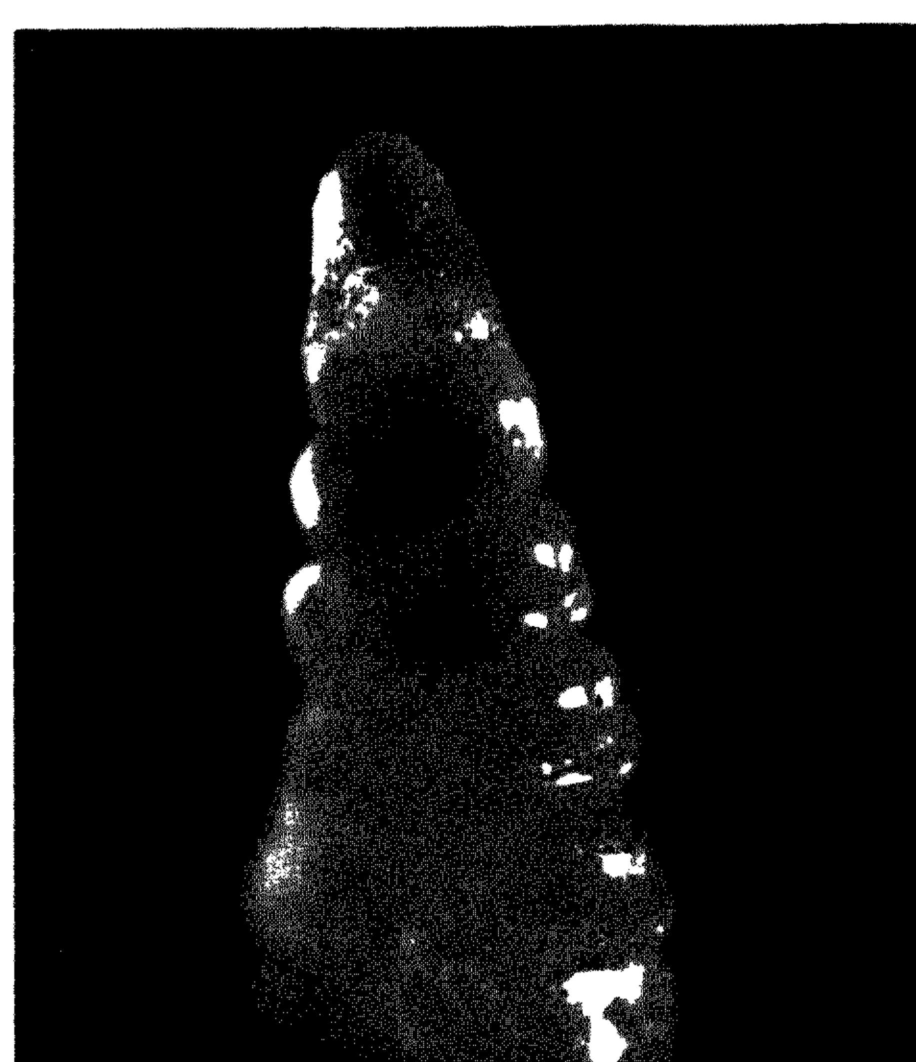


FIG. 2. Double ridge stage *(Dactylis glomerata)*

Fig. 4. Tertiary reproductive primordial stage *(Festuca rubra)*

Fig. 5. Floral parts formation *(Festuca rubra)*

bers were examined. This involved, on an average, the examination of 10,000 ear heads for each field experiment, and 15,000 ear heads for each pot experiment. Of the field and pot experiments, tillers were selected at random from the harvested plot and kept in labelled paper bags until they were examined. The paper bags of each plot were stored in a dark room at low temperature (2–4 °C).

In the season 1958–59, three to four weeks prior to the harvesting date, samples from the two outer rows of sprayed plots were brought to the laboratory to assess leaf and stem abnormalities. In this case the samples were divided at random into three groups, each group containing twenty tillers. The groups were assessed separately. Based on field observations several treatments were chosen to be examined for leaf and stem abnormalities.

In TABLE 3 the morphological aberrations observed are listed under three headings. In the description of symptoms no attempt has been made to coin a special terminology, but the most logical description was aimed at. In many descriptions the very useful review of KAUFMAN (1953) has been followed. Several symptoms are closely related to each other or may give rise to other abnormalities, but it has not been tried to establish relationships between abnormalities.

TABLE 3. List of morphological abnormalities observed in grass plants after treatment with auxin herbicides (FIGS. 6–9)

I. LEAF ABNORMALITIES

I–A. Development of onion leaves or rolled leaves or folded leaves

The leaves in cross section are solid or partially hollow and form cylindrical structures for a part or the complete length of the longitudinal axis. They resemble onion leaves. Otherwise, the leaf may remain rolled and does not unfold or the leaf margins only are rolled and form tubular structures. In some cases the two longitudinal halves remain folded along the midrib.

Examples have been found in the field experiments with *Festuca pratensis, Festuca rubra, Lolium perenne* and *Poa trivialis* and in the environmental chamber experiments with *Lolium multiflorum westerwoldicum* and *Poa annua.* This symptom is a dominating feature amongst all abnormalities caused.

I–B. Development of giant and dwarfed leaves

Giant leaves may either be longer and narrower, longer and wider or just longer than those of normal plants. In some instances of giant leaf only the leaf sheath is broader than it usually is with normal plants. Dwarfed leaves are shorter and narrower than those of normal leaves. Sometimes the lower portion of the lamina is a narrow strip while the tip expands normally.

The frequency of occurrence of this symptom has been very low. It has been observed in *Festuca rubra, Poa annua, Poa trivialis* and *Lolium multiflorum westerwoldicum.*

I–C. Development of abnormally stretched leaves

Abnormal emergence of leaves may occur owing to various obstructions caused by onion, rolled or folded leaves. These phenomena and also the unequal growth of the different parts of leaves may result in various ways and degrees of twisting, curling, zigzagging etc. of a part or of the whole leaf. Slit, distorted and wrinkled leaves may appear. Longitudinal folding of the blade and excessive elongation of the leaf-sheath may take place. The lower parts of the leaf may remain attached to two nodes.

This abnormality has been specially observed in *Lolium multiflorum westerwoldicum* and *Poa annua,* to a lesser extent in *Festuca pratensis* and *Poa trivialis.*

I–D. Failure of the normal emergence of leaves

This symptom results from the development of onion, rolled or folded leaves. The leaf may be enclosed partly or completely by an older leaf of the same tiller. In its turn the trapped leaf may

TABLE 3 (continued)

inhibit the normal emergence of younger leaves. Young tillers may emerge abnormally because of abnormalities in the leaf structure of older leaves. In some instances these tillers may pierce the other leaves and become entangled in the split leaf bases or intertwine with other tillers. Rosette-like bunched leaves may also influence the normal emergence of young emerging leaves. Particularly the *Poa* species and *Lolium multiflorum westerwoldicum* showed this abnormality.

I–E. Bunching of leaves

Spirally coiled leaves or onion leaves may emerge in the same place of the axis or rather normally shaped leaves may come out in rosette form. The symptom has mainly been observed in *Festuca pratensis*, *Lolium multiflorum westerwoldicum* and *Poa annua*. It occurred in *Lolium multiflorum westerwoldicum* more frequently than in other grasses.

I–F. Cohesion or fusion of leaves

"Sheaths of two leaves appear as one sheath and the blades are irregularly fused longitudinally. The blades are usually oriented at right angles to each other so that the leaf appears to have two blades" (KAUFMAN, 1953). Sometimes the fusion of two leaves, oriented at right angles to each other, may take place without the development of a common leaf sheath. Fused leaves may be rectangular or tubular in form. The emergence of normal successive leaves may be influenced by fused leaves; this has been frequently observed in *Poa annua*.

Leaves, sometimes tubular, may be longitudinally fused to other leaves along the margins, thus making the margins rounded or tubular. In other instances, sheaths of many leaves may be fused causing a rosette appearance. Tillers may be held together due to the cohesion of their respective leaf-sheaths. *Lolium multiflorum westerwoldicum* and *Poa annua* showed this symptom quite often but it also occurred in *Festuca pratensis*, *Poa pratensis* and *Lolium perenne*.

I–G. Cohesion or fusion of leaf and inflorescence

Leaves may be fused to the main axis of inflorescences along the margins, or the axis of the branch may be fused to the lamina for variable lengths. It has been observed particularly in *Festuca rubra* and to a smaller extent in *Poa annua*.

II. STEM ABNORMALITIES

II–A. Abnormal texture of stem

Stems may be softer, more fleshy, thicker, stiffer or more pliable than normal. The symptom occurred very rarely in *Festuca pratensis*, *Poa annua* and *Poa trivialis*.

II–B. Cohesion of stems

This phenomenon is characterized by the partial or complete fusion of one or more tiller stems to the main axis of the stem. Sometimes the fusion may be so complete that the inflorescence of the tiller stem appears to emerge from one of the main-stem nodes. This symptom too is not of frequent occurrence, but has been observed in the *Lolium* and *Poa* species (FIG. 7).

II–C. Retarded or inhibited development of stems

The stems do not elongate the normal way and appear longitudinally folded, twisted, curled, coiled like a spring, zigzagged, bent at right angles, or otherwise malformed. Some of the symptoms may be seen in earlier stages of development when the stems appear malformed owing to their being enveloped by tubular leaves.

This abnormality is very predominant in *Lolium perenne* and *Poa trivialis* but has also been observed in *Festuca rubra*, *Lolium multiflorum westerwoldicum* and *Poa annua*.

II–D. Abnormal growth and the proliferation of nodes and internodes of the stem

The node may be regularly or irregularly thickened or it may be elongated, bent, twisted around, proliferated and bifurcated. The internode may be swollen at its base, flattened or may form a pit. *Festuca rubra* showed a higher degree of this abnormality than *Festuca pratensis*, *Lolium multiflorum westerwoldicum*, *Poa annua* or *Poa trivialis*.

II–E. Cohesion of nodes

This is characterized by the fusion of two nodes with a very small twisted internode in between. It has been observed in *Poa annua* and *Poa trivialis* even though it is of rare occurrence (FIG. 6).

TABLE 3 (continued)

II–F. Bunching of tillers

In this instance tillers may be found in clusters at the base of the main axis. This symptom could only be assessed in *Poa annua*.

III. INFLORESCENCE ABNORMALITIES

III–A. Retarded or inhibited development of the inflorescence or of the branches of the inflorescence due to a failure of emergence

Retarded or inhibited development of the inflorescence results from the formation of tubular flag leaves or a distorted sheath of the flag leaf. The inflorescence may be enclosed partly or completely by the onion leaves. In the first case, the axis of the inflorescence or its branches may pierce the tubular flag leaf in various ways. In some instances more than one leaf may be involved and these leaves may be cohesed or of the onion type.

When the emergence is completely inhibited various forms of bending of the main axis and branch axes may result.

Branches of inflorescences may also be enveloped by or entangled in the hollow straw-like internodes of the main axis.

The symptom is very common in almost all grasses but particularly noticed in *Festuca pratensis, Festuca rubra, Lolium perenne, Poa annua, Poa pratensis* and *Poa trivialis*.

III–B. Cohesion or fusion of leaf and inflorescence

This symptom has been described under „leaf abnormalities".

III–C. Abnormal shape and structure of the main axis of the inflorescence and of its branches

A part or the whole main axis of the inflorescence may be thicker than normal, tubular, stiff, straw-like or in any other way divergent from the usual structure (FIG. 8). This may also be the case with branch axes and rachillae. *Poa annua* and *Festuca rubra* showed the symptom very clearly.

Sometimes there is an excessive elongation (e.g. with *Festuca rubra)* or shortening (e.g. with *Poa trivialis)* of the internodes of the inflorescence axis. In other instances excessively elongated branches may take over the function and form of the main axis or in the same way a secondary branch may replace a primary one, as has been observed in *Lolium perenne* and *Festuca rubra* (FIG. 9).

Inflorescence axes and their branches may show the phenomenon of ramification. Instead of the axis concerned two or more axes occur in its place (e.g. with *Festuca rubra*). In *Lolium perenne* the rachillae may be abnormally elongated and may carry more florets than usual.

Sometimes an abnormal ramification of the main axis diverges from the main part of the inflorescence (e.g. with *Festuca rubra* and *Poa pratensis)*. This may be due to excessive elongation of the main inflorescence axis internode and its subsequent divergence from the main axis, as explained by KAUFMAN (1953).

III–D. Development of dwarfed inflorescences and branches

Inflorescences in which spikelets were initiated but failed to develop resulted in a small inflorescence, which may sometimes be only three mm in length. Examples have been found with *Festuca rubra, Lolium perenne* and all *Poa* species.

III–E. Absence of the alternation of spikelets, florets and branches

This abnormality is characterized by opposite spikelets or opposite florets instead of normal alternate arrangements on their respective axes. These may also emerge on the same side of the node, one above the other or in the same place. These abnormal spikelets may consist of one pedicelled and one sessile one, thus departing from uniformity. The phenomenon has been of frequent occurrence in *Lolium perenne* but has also been observed in *Festuca rubra* and *Poa pratensis*.

III–F. Bunching of spikelets and whorling of branches

This abnormality is characterized by similar structures occurring more often than usual in the same place or around the same place of the node. These symptoms may give rise to a special shape and form of the inflorescence, like a sun-ray type or scaly whip-like branches, reducing the length of the inflorescence sometimes to a few cm.

In *Festuca rubra, Lolium perenne* and *Poa trivialis* the symptom was very frequent, but it also showed up in *Poa annua* and *Poa pratensis*.

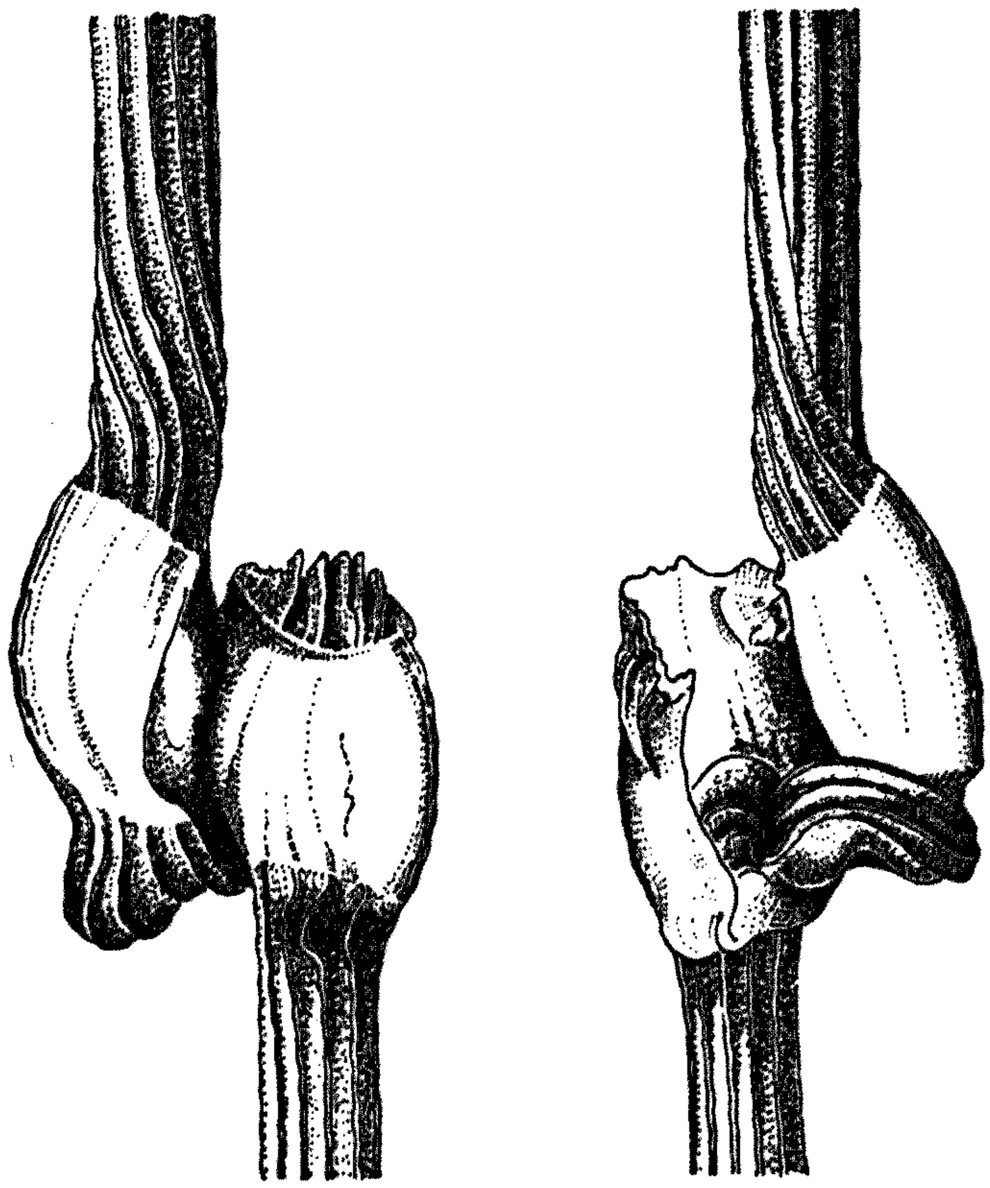


FIG. 6. Cohesion of nodes *(Poa trivialis)*

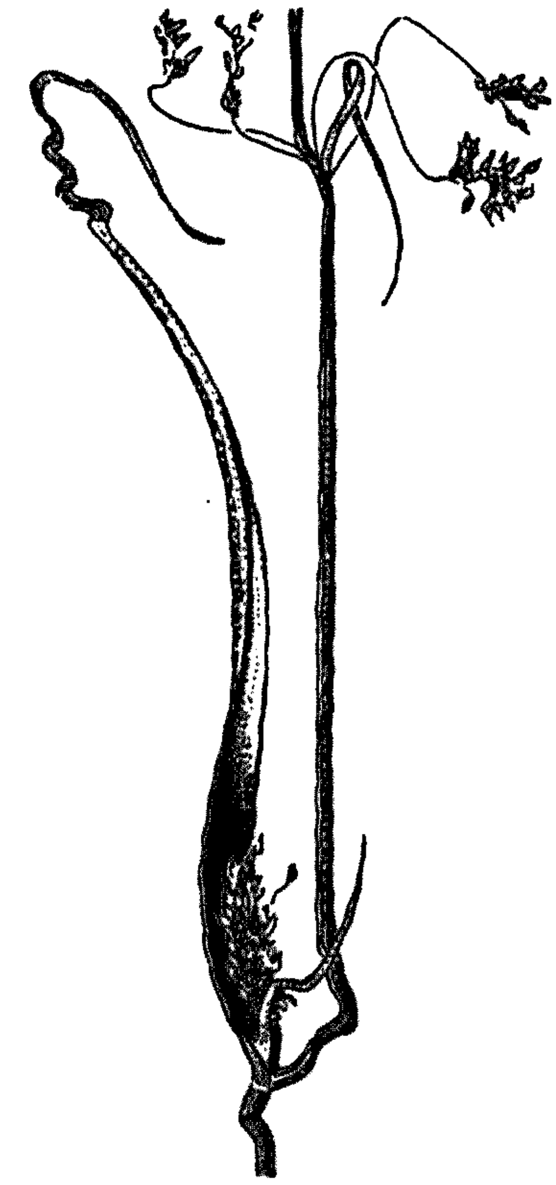


FIG. 7. Cohesion of tillers *(Poa trivialis)*

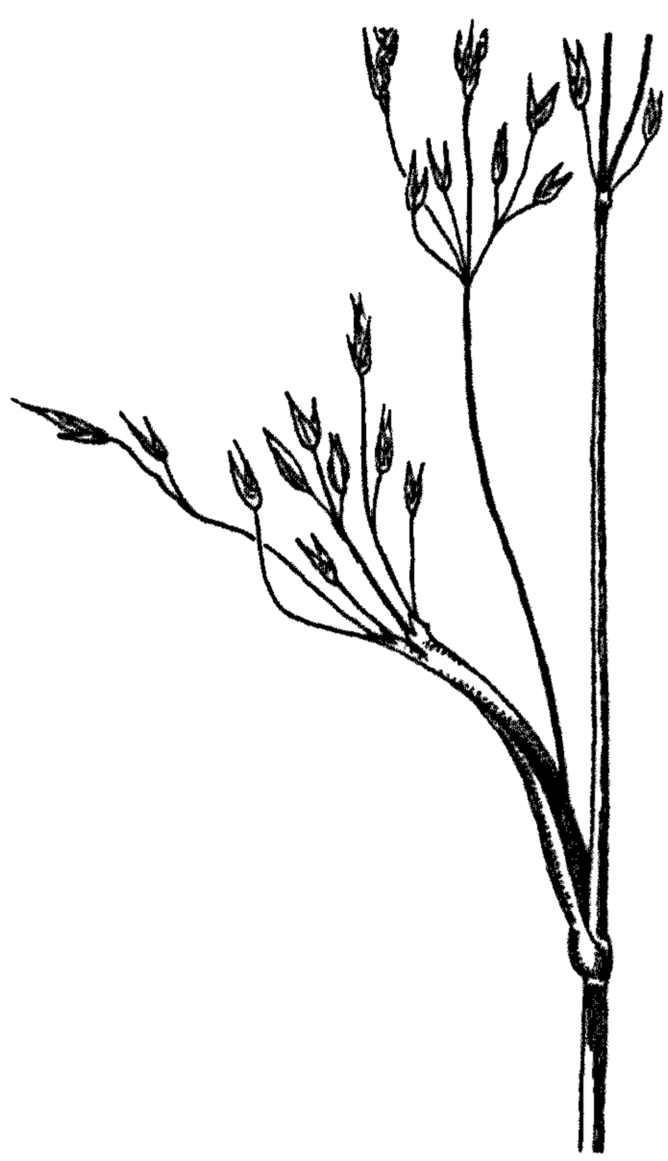


FIG. 8. Straw-like, boat-shaped structure of secondary axis *(Poa trivialis)*

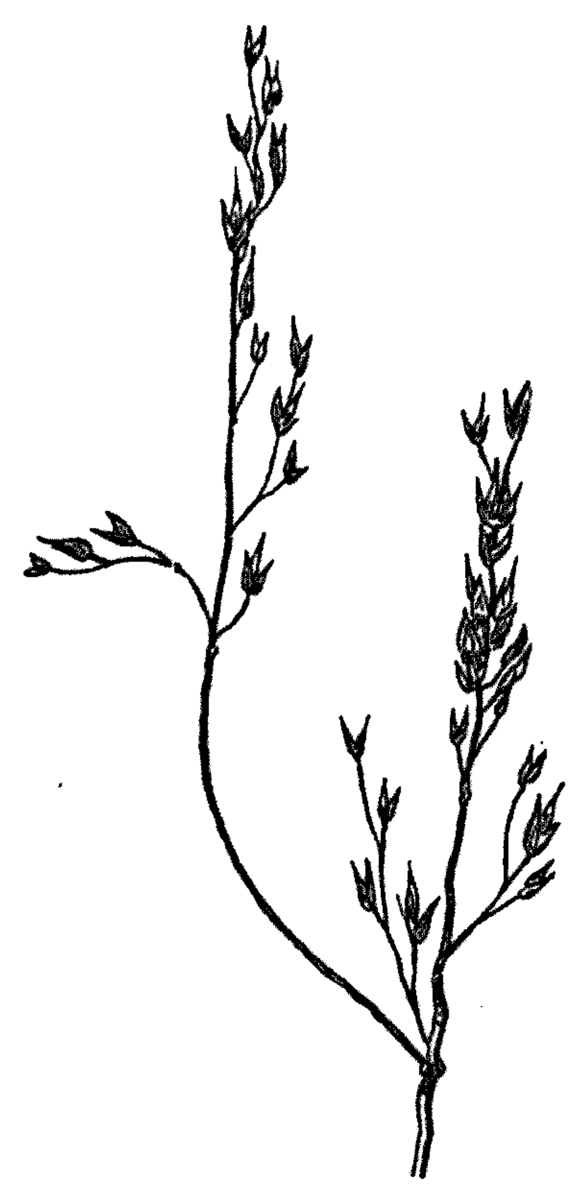


FIG. 9. Excessively elongated internode of main inflorescence axis *(Festuca rubra)*

In his studies JEATER (1958) assessed leaf abnormalities by expressing the number of single plants showing leaf abnormalities in a percentage of the total number of treated plants. Using this method he could come to a hundred per cent abnormalities. In the experiments discussed presently this system could not be followed. Our experiments were carried out in the fields of growers, not with selected individual plants. Due to this greater heterogeneity of the plant material and moreover to the system of studying single tillers and not individual plants, our percentage of abnormalities does not necessarily have to agree with the data mentioned by JEATER.

If it is assumed that all growing points which can be induced to form malformed („onion") leaves have indeed been influenced and that in all these tiller apices initiation of flower formation takes place later on, 100 per cent abnormality may be expected in the shooting tillers. This assumption is permitted, because tillers in which abnormal leaves are developing remain capable of being reproductive. The herbicide acts primarily on newly initiated leaves and not to the same extent, or not at all on the apical meristem proper (KAUFMAN, 1955).

The fact that we do not observe this high percentage of abnormalities, but a considerable smaller number in many cases may have several reasons. In the first place the assessment of the stage of development of the growing point has been made on basis of well developed tillers, disregarding many young tillers that are less advanced in their development. It has been indicated (c.f. KLEINENDORST and TEN HOVE, 1957) that the differences in the development of the apices of tillers may be particularly great in the vegetative and early reproductive stage of growth. These differences gradually disappear until at the time of flowering individual inflorescences are almost equally advanced. We have to presume that in addition to the normal tolerance to herbicides demonstrated by plant species (best expressed in the dosage-response curve, indicating the kill or the growth reduction at increasing dosages) (BLACKMAN, 1950), the individual differences between various plants and tillers of the same plant cause a lower abnormality percentage than 100. This variation in susceptibility may not be the same at all spraying times and will certainly become smaller according to the more uniform development of the individual tiller apices. This could cause a higher percentage of abnormal tillers, the closer the inflorescence initiation is approached.

2.4.3. Yield determination

Yield determinations were made only in the experiments carried out by research officers of I.B.S. and P.A.W. The experiments were statistically analysed. The plots were harvested by hand and after field drying the material was threshed and field-cleaned by a threshing machine especially constructed for field plot investigations.

2.4.4. 1000-Kernel weight

From all but the environmental-chamber experiments, the harvested seeds were cleaned and from each treatment two times two hundred and fifty seeds were counted. Each sample was weighed separately and 1000-kernel weight calculated. The replicates of each treatment were treated separately. Results were statistically analysed.

2.4.5. *Germination energy and germination percentage*

The seeds used for 1000-kernel weight determination were utilized for germination experiments. Fifty seeds taken at random from the 250-seeds' samples of each treatment were used.

The germination experiments were carried out in environmental chambers according to a plan statistically projected, using the Copenhagen tank system. The scheme of germinating seeds by alternately changing from low temperature (15 °C) and 7 hours light to high temperature (20 °C) and 17 hours darkness was followed. The rules concerning the number of days indicated by the Rijksproefstation voor Zaadcontrole van Zaaizaden (1951), after which the counting of seeds should take place for the determination of energy or germination percentage were followed as closely as possible, but with regard to experience not strictly adhered to. In TABLE 4 it has been mentioned what system was followed in determining the germination energy and power of different grasses.

TABLE 4. Number of days after which counts were made for germination energy and germination percentage

Grass species	Germination energy	Germination percentage
Dactylis glomerata (from seeds)	8	14
Festuca pratensis (from seeds)	6	14
Festuca rubra (from seeds)	6	14
Lolium perenne (from seeds)	4	7
Lolium perenne (seeds from clones)	5	7
Poa pratensis (from seeds)	9	21
Poa trivialis (from seeds)	7	14

From the 1957–58 field experiments seeds from all treatments were tested on germination. On basis of field observations of the same year and experience gathered with the previous year's experiments only some treatments were taken into account from the 1958–59 experiments. Owing to the failure of seed setting in *Festuca rubra* in the pot experiment, germination test for this experiment were not undertaken.

2.4.6. *Other data*

Apart from the observations made during the growing period in both field and environmental chamber experiments, the following post-harvest data on the latter experiments were recorded:

1. Total number of tillers
2. Number of tillers with inflorescence
3. Dry weight of shoots

2.4.7. Statistical analysis

As mentioned in the previous paragraphs, in several instances experimental data were statistically analysed. For Analysis of Variance and F-test the standard statistical methods were followed. In other cases the Student-Newman-Keuls-multiple range-test was used, as described by FEDERER (1955).

If the system of collecting data or of carrying out the experiments did not allow statistical analysis it has been indicated in the presentation of the results.

3. RESULTS

3.1. Field and pot experiments

The results obtained from the experiments are given in the following paragraphs. The dosage rate of herbicides (2,4-D, MCPA or 2,4,5-TP) applied in „kg acid equivalent per ha" is indicated as „kg".

As stated in chapter 2, the experiments on the influence of auxin herbicides on seed yield in different grasses were carried out by research officers of the I.B.S. and P.A.W. at Wageningen. In regard of the practical importance of these experiments, the results were published as soon as possible after harvesting the plots (Evers and Sonneveld, 1953, 1954, 1956a, 1956b; Evers, 1958, 1959, 1960).

It may stated here that only the results of those germination energy experiments which showed statistically reliable difference between the treatments, have been presented in this dissertation.

3.1.1. Poa trivialis

3.1.1.1. Leaf and stem development

This was particularly influenced by applications of 2,4-D and MCPA shortly after winter at the stage of development 3 V.P. (Table 5). 2,4,5-TP applied at any stage did not cause any malformation. Both rates of application of 2,4-D and MCPA had influence, but the effect of 2,4-D was twice as severe as the effect caused by MCPA. The influence of 2,4-D slightly showed in treatments at the stage of development Sec.

Table 5. Percentage of leaf and stem abnormalities caused in *Poa trivialis* by auxin herbicides

Date of spraying 1958–59	Stage of development of growing point	MCPA		2,4-D	
		1 kg	2 kg	1 kg	2 kg
30.10	–	0	0	0	0
20.11	2 V.P.	0	0	0	0.8
4. 3	3 V.P.	22.5	30.0	50.8	61.8
3. 4	Sec.	0	0	1.7	1.7
22. 4	Em. Infl.	0	0	0	0
12. 5	Em. Infl.	0	0	0	0
control	–	0	0	0	0

3.1.1.2. Structure of the inflorescence

MCPA at the rate of 1 kg and 2,4,5-TP up to 2 kg caused no or only little effect at any moment of application (Table 6).

2 kg of MCPA caused slight damage in spraying at the stage of development 3 V.P. (first week of March). At the same stage (2–3 V.P. between the second week of February and the second week of March) 2,4-D at the rate of 1.5 and 2 kg caused considerable damage.

With both MCPA and 2,4-D the highest percentage of leaf and stem and inflorescence abnormalities was observed at the same developmental stage of the growing point (2–3 V.P.) and with both herbicides a higher dosage caused an increased percentage of abnormalities.

TABLE 6. Percentage of inflorescence abnormalities in *Poa trivialis* by auxin herbicides

Date of spraying 1957–58	Stage of development of growing point	MCPA 1 kg	2,4-D 1.5 kg	2,4,5-TP 1.5 kg
8.11	2 V.P.	0	0	1.5
29.11	2 V.P.	0.5	0	0
12. 2	2 V.P.	6.5	30.5	0
19. 3	2 V.P.	0	24.5	0
26. 3	5 V.P. (Sec. visible)	2.0	6.0	0.5
2. 4	V.P.–Sec.	2.0	7.0	0
15. 4	40% V.P.–60% Sec.	0	1.0	1.0
22. 4	Sec.	0.5	0.5	1.5
6. 5	Floral	0.5	0	5.5
13. 5	Floral	1.5	0	9.0
19. 5	Em. Infl.	3.0	0	0
28. 5	Em. Infl.	0.5	0.5	2.5
control	–	0	0	0

Date of spraying 1958–59	Stage of development of growing point	MCPA		2,4-D		2,4,5-TP	
		1 kg	2 kg	1 kg	2 kg	1 kg	2 kg
30.10	–	0	0	0	0	0	0
20.11	2 V.P.	0	0	0	1.0	0	0
4. 3	3 V.P.	6.5	23.0	19.5	38.5	0	0
3. 4	Sec.	1.0	1.5	3.0	3.0	0	0
22. 4	Em. Infl.	0	0	0	0	0	5.5
12. 5	Em. Infl.	3.0	1.0	1.5	0	0	0
control	–	0	0	0	0	0	0

3.1.1.3. Yield

Data obtained from I.B.S.-P.A.W. experiments with MCPA, 2,4-D amine and 2,4,5-TP are summarised in TABLE 7.

From this table it can be concluded that in the early growth stages due to direct killing of the young grass plants and growth retardation a considerable decrease in yield can be caused. Applications in November did not cause a statistically reliable difference in yield with any of the three chemicals. In the early regrowth stages after winter a tendency of yield depression can be noticed. Shortly before and during the emergence of the inflorescence great decreases in seed yield are produced by MCPA and 2,4-D. 2,4,5-TP has not been investigated in this respect.

3.1.1.4. 1000-Kernel weight

In the experiments of I.B.S. and P.A.W. (TABLE 8) no influence was found in any of the treatments. At early inflorescence emergence, MCPA and 2,4-D tend to increase the 1000-kernel weight. There was no experiment with 2,4,5-TP at this stage of growth.

In our experiments in 1958, the average 1000-kernel weight of seeds harvested from plots sprayed with different auxin herbicides on May 19th at the stage Em. Infl. showed a significant increase in comparison to the control at 5% level. The increase was mainly due to an increase at plots treated with 1.5 kg of 2,4,5-TP. In 1959, similar results were obtained.

TABLE 7. Influence of MCPA, 2,4-D and 2,4,5-TP on seed production in *Poa trivialis* (as percentages of non-treated plots), according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA					2,4-D			2,4,5-TP
Growing season	1954–55			1955–56	1956–57	1954–55			1956–57
Experiment No.	CI 1821	CI 1822		CI 2265	CI 2473	CI 1821	CI 1822		CI 2473
Rate of application (a.e./ha)	1 kg	1 kg	2 kg	1.5 kg	1 kg	1 kg	1 kg	2 kg	1.5 kg
Time of application in order of stage of development									
Early stage									
1. Pre-emergence	47.2**					34.4**			
2. 2-leaf stage	77.1*					72.9*			
3. 4-leaf stage	93.2				94.8	100.3			102.6
Regrowth after winter									
4. ± 6 cm high	93.2			110.5		99.8			
5. ± 15 cm high	100.0				74.1	94.6			91.5[1]
6. just before inflorescence emergence				81.6					
Inflorescence emergence									
7. early infl. emergence		56.5**	52.4**	79.0			89.3	75.2*	
8. (intermediate stages)				65.9					
9. (intermediate stages)				44.4**					
10. (intermediate stages)				57.9*					
11. full infl. emergence				90.8					
12. full bloom		86.3	94.2	70·0			84.6	80.6	
Yield of non-treated control in kg/ha	909	844	844	860	773	909	844	844	773

* Significant difference at 5% level.
** Significant difference at 1% level.
[1] Sprayed: once at stage No. 3 and once at stage No. 5.

TABLE 8. Influence of MCPA, 2,4-D and 2,4,5-TP on 1000-kernel weight (as percentages of non-treated plots) of *Poa trivialis*, according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA				2,4-D			2,4,5-TP
Growing season	1954–55	1954–55		1956–57	1954–55	1954–55		1956–57
Experiment No	CI 1821	CI 1822		CI 2473	CI 1821	CI 1822		CI 2473
Rate of application a.e./ha	1 kg	1 kg	2 kg	1 kg	1 kg	1 kg	2 kg	1.5 kg
Time of application in order of developmental stage								
Early stage								
1. Pre-emergence	102.3				101.0			
2. 2-leaf stage	99.4				100.3			
3. 4-leaf stage	98.6			97.9	98.3			97.9
Regrowth after winter								
4. ± 6 cm high	98.9				103.9			
5. ± 15 cm high	101.5			99.5	102.0			97.9[1]
Inflorescence emergence								
7. early infl. em.		107.2	104.8			104.8	106.7	
12. full bloom		98.6	100.5			99.0	98.1	
1000-Kernel weight of non-treated control (gm)	0.2042	0.2090	0.2090	0.1950	0.2042	0.2090	0.2090	0.1950

[1] Sprayed: once at stage No. 3 and once at stage No. 5.

3.1.1.5. Germination percentage

TABLE 9 giving the results of the yield experiments of I.B.S. and P.A.W. shows that MCPA in particular causes a highly significant decrease when applied during the emergence of the inflorescence. Also our own plots sprayed at this stage showed this effect. In 1959 this decrease was statistically significant in the 1 % level. It was primarily caused by MCPA applied at the rate of 2 kg. In 1958, there was only a tendency of a lower germination percentage when applied at the rate of 1.5 kg at the same stage of development. In this instance, it did not cause a statistically reliable difference. MCPA, however, had the greatest effect.

3.1.2. Poa pratensis

3.1.2.1. Leaf and stem development

Abnormalities were only observed after applications of 2,4-D at the stage of development 2 V.P. in October and November. No influence of dosage rate was noticeable (TABLE 10).

3.1.2.2. Structure of the inflorescence

Relatively few abnormalities were caused by applications of any of the three chemicals under investigation (TABLE 11). In both seasons of investigations maximal

TABLE 9. Influence of MCPA and 2,4-D on germination (as percentages of non-treated plots) of *Poa trivialis*, according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA				2,4-D		
Growing season	1954–55	1954–55		1955–56	1954–55	1954–55	
Experiment No.	CI 1821	CI 1822		CI 2265	CI 1821	CI 1822	
Rate of application (a.e./ha)	1 kg	1 kg	2 kg	1 kg	1 kg	1 kg	2 kg
Time of application in order of stage of development							
Early stage							
1. Pre-emergence	97.9				100.0		
2. 2-leaf stage	100.0				96.8		
3. 4-leaf stage	97.9				98.9		
Regrowth after winter							
4. ± 6 cm high	98.9			97.7	98.9		
5. ± 15 cm high	98.9				100.0		
6. just before infl. emergence				89.8			
Inflorescence emergence							
7. early infl. emergence		89.4	87.2**	86.4		98.8	93.6
8. (intermed. stages)				76.1			
9. (intermed. stages)				85.2			
10. (intermed. stages)				93.2			
11. full. infl. emergence				98.9			
12. full bloom		101.1	100.0	101.1		98.9	98.9
Germination percentage of the non-treated control	95	94	94	88	95	94	94

** Significant difference at 1% level.

TABLE 10. Percentage of leaf and stem abnormalities caused in *Poa pratensis* by 2,4-D

Date of spraying 1958–59	Stage of development of growing point	2,4-D 1 kg	2,4-D 2 kg
13.8	2 V.P.	0	0
18.9	2 V.P.	0	0
24.10	2 V.P.	5.8	4.2
24.11	2 V.P.	5.8	5.0
4.3	64% V.P.–16% Tert.	0.8	0
24.4	Em. infl.	0	0
12.5	Em. infl.	0	0
control	–	0	0

susceptibility occurred at the development stage 2–3 V.P. in the second half of November or in December. 2,4-D caused the highest number of abnormalities; abnormalities recorded for the control plots amounted to a higher than normal number of secondary axes.

In comparison to other grasses studied *Poa pratensis* appeared to be the most resistant to the effect of auxin herbicides.

TABLE 11. Percentage of inflorescence abnormalities caused in *Poa pratensis* by auxin herbicides

Date of spraying 1957–58	Stage of development of growing point	MCPA 1 kg	2,4-D 1.5 kg	2,4,5-TP 1.5 kg
22. 8	–	0	0	0
26. 9	–	0	0	0
8.11	1–2 V.P.	0	1.5	0
12.12	2–3 V.P.	0	5.5	0
4. 2	3 V.P.	0	0	0
19. 3	V.P.–Sec.	0	0	0
26. 3	Sec.	0	0	0
2. 4	Sec.	0	0	0
19. 3	Sec.–Tert.	0	0	0
15. 4	Tert.–Quat.	0	0	0
22. 4	Floral	0	0	0
6. 5	Floral	0	0	0
13. 5	Em. Infl.	0	0	0
19. 5	Em. Infl.	0	0	0
28. 5	Em. Infl.	0	0	0
control	–	0	0	0

Date of spraying 1958–59	Stage of development of growing point	MCPA		2,4-D		2,4,5-TP	
		1 kg	2 kg	1 kg	2 kg	1 kg	2 kg
13. 8	2 V.P.	1.0	0	0	0.5	0.5	3.0
18. 9	2 V.P.	0.5	0.5	0	0	0	0.5
24.10	2 V.P.	0.5	1.5	3.5	0.5	0	2.0
24.11	2 V.P.	1.5	2.0	3.5	12.0	0	0.5
4. 3	64% V.P.–16% Tert.	0	0	2.5	0	0	1.5
24. 4	Em. Infl.	0	2.5	0	0	0.5	0
12. 5	Em. Infl.	0	0	1.5	0	0	0
control	–	0.25	0.25	0	0.25	0.75	0.75

3.1.2.3. Yield

In the experiments of I.B.S. and P.A.W. autumn applications of MCPA, 2,4-D and 2,4,5-TP on the average did not cause any reduction in yield (TABLE 12). In spring before shooting all three herbicides generally caused depressions, particularly pronounced in 2,4,5-TP. At the early flowering stage, MCPA and 2,4-D caused a depression, statistically reliable at the 5% level.

3.1.2.4. 1000-Kernel weight

In the yield experiments of I.B.S. and P.A.W., an application of 1 kg of 2,4-D after winter when the plants were still dormant and an application of about 1 kg of 2,4,5-TP on plants just prior to shooting caused an increase in 1000-kernel weight (TABLE 13). In the author's experiments, 1000-kernel weight was not influenced by any treatment.

3.1.2.5. Germination percentage

The data obtained from the experiments of I.B.S. and P.A.W. do not point to definite specific influences, although MCPA applied prior to shooting and 2,4-D during Infl. Em. caused some reduction (TABLE 14).

In the author's experiments, no statistically reliable influence could be ascertained with any of the treatments.

TABLE 12. Influence of MCPA, 2,4-D and 2,4,5-TP on seed production (as percentages of non-treated plots) of *Poa pratensis*, according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA					2,4-D						2,4,5-TP					
Growing season	1955–56		1957–58	1958–59		1955–56		1956–57		1957–58		1956–57		1957–58		1958–59	
Experiment No.	CI 2115	CI 2392	IBS 118	IBS 265	IBS 267	CI 2115	CI 2392	CI 2466[1]		IBS 118		CI 2466		IBS 118		IBS 265	IBS 267
Rate of application a.e./ha	1 kg	1 kg	1.25 kg	1 kg	1 kg	1 kg	1 kg	1 kg	2 kg	1.25 kg	2.5 kg	1 kg	2 kg	0.08 kg	1.15 kg	1 kg	1 kg
Time of application in order of stage of development																	
Early stage																	
1. 8 cm high . . .								107.8	120.2*	99.0	104.6	109.3	117.1	108.9	91.9		
2. 10 cm high . . .								96.1	112.4			113.2	107.8				
3. 5–7 leaves, 2 tillers of 1st order	87.2					91.9				104.6	107.6			104.4	106.8		
Regrowth after winter																	
4. still dormant . . .	82.8*					81.3*											
5. 5–7 cm high . .	93.2					92.7											
6. 7–10 cm high . .	93.3			98.0		91.5						77.5**	70.5**			78.2	
7. 10–20 cm high, irregular								106.2	79.8**								
8. 20 cm high, just prior to shooting	97.3	101.0	84.8		93.1	94.0	95.4			88.5				77.7*	59.5**		53.3**
Inflorescence emerging																	
9. 90% infl. emergence		98.5					95.3										
10. fully emerged		100.1	97.0				97.3										
11. early flowering stage		77.9*					80.3*										
Yield of non-treated control in kg/ha . .	1431	1617	1362	1678	1736	1431	1617	1290	1290	1362	1362	1290	1290	1362	1362	1678	1736

* Significant difference at 5% level.
** Significant difference at 1% level.
[1] Pure acid formulation applied.

TABLE 13. Influence of MCPA, 2,4-D and 2,4,5-TP on 1000-kernel weight (as percentages of non-treated plots) of *Poa pratensis*, according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA			2,4-D			2,4,5-TP			
Growing season	1955–56	1958–59		1955–56	1956–57		1956–57		1958–59	
Experiment No.	CI 2115	IBS 265	IBS 267	CI 2115	CI 2466[1]		CI 2466		IBS 265	IBS 267
Rate of application (a.e./ha)	1 kg	1 kg	1 kg	1 kg	1 kg	2 kg	1 kg	2 kg	1 kg	1 kg
Time of application in order of stage of development										
Early stage										
1. 8 cm high					95.9	97.6	98.3	100.7		
2. 10 cm high					95.2	100.0	98.6	100.0		
3. 5–7 leaves, 2 tillers of 1st order	102.0			99.3						
Regrowth after winter										
4. still dormant	102.4			108.2**						
5. 5–7 cm high	102.0			102.0						
6. 7–10 cm high	102.0	97.2		101.4					106.9	
7. 10–20 cm high, irregular					102.7	97.3	105,5	103.4		
8. 20 cm high just prior to shooting	100		104.6	99.3						128,3**
1000-Kernel weight of non-treated control (gm)	0.291	0.317	0.304	0.291	0.292	0.292	0.292	0.317	0.317	0.304

[1] Pure acid formulation applied. ** Significant difference at 1% level.

TABLE 14. Influence of MCPA, 2,4-D and 2,4,5-TP on germination (as percentages of non-treated plots) of *Poa pratensis*, according to experiments of I.B.S. and P.A.W.[1]

Auxin herbicide	MCPA			2,4-D	2,4,5-TP	
Growing season	1955–56	1958–59		1955–56	1958–59	
Experiment No.	CI 2392	IBS 265	IBS 267	CI 2392	IBS 265	IBS 267
Rate of application (a.e./ha)	1 kg	1 kg	1 kg	1 kg	1 kg	1 kg
Time of application in order of stage of development						
Regrowth after winter						
6. 7–10 cm high		98.9			101.1	
8. 20 cm high, just prior to shooting	98.9		94.6	102.2		101.1
Inflorescence emerging						
9. 90% infl. emergence	98.9			97.8		
10. fully emerged	101.1			98.9		
11. early flowering stage	100.0			90.0		
Germination percentage of the non-treated control	90	91	92	90	91	92

[1] Statistical analysis not available.

3.1.3. *Festuca pratensis*

First of all it has to be mentioned that in 1959 one experiment was made in an early sown crop (under cover crop) and the other later in the season without any cover crop. In autumn the stages of development of the two experiments were not the same. This difference may not be determined only by sowing date, but growth site conditions and varietal breed may have influenced the apical development in the two environments.

In 1958 data were taken only on 1000-kernel weight and germination percentage.

3.1.3.1. Leaf and stem development

In both experiments 2,4,5-TP hardly showed any influence on the grass, while 2,4-D at both dosages caused a considerable percentage of malformations in the growth stage 2–3 V.P. in October and November (TABLE 15). Also MCPA caused a high abnormality percentage at the stage 2–3 V.P., but in this case the susceptibility of the grass to the herbicide was considerably lower with a slightly more advanced development of the growing point at the end of November.

There was no difference between the reactions at the two sowing times.

3.1.3.2. Structure of the inflorescence

As to these abnormalities 2,4,5-TP neither showed much activity (TABLE 16). At applications in growth stage 2–3 V.P. (3rd week of November) 2,4-D caused a higher percentage of abnormalities than MCPA and increase in dosage caused an increase in malformation percentage. A slight effect of sprayings of 2,4-D early after winter was more pronounced in one experiment than in the other.

TABLE 15. Percentage of leaf and stem abnormalities caused in *Festuca pratensis* by auxin herbicides

Date of spraying	Stage of development of growing point	MCPA 1 kg	MCPA 2 kg	2,4-D 1 kg	2,4-D 2 kg	2,4,5-TP 1 kg	2,4,5-TP 2 kg
1958–59 (Sown in April)							
22. 8	–	0	0	0	0	0	0
19. 9	1 V.P.	0	0	0	2.5	0	0
24.10	2 V.P.	5.8	16.7	14.1	19.2	0	0
20.11	2–3 V.P.	7.5	5.8	15.8	18.3	0	0.8
4. 3	3 V.P.	0	0.8	2.5	1.7	0	0
24. 4	Tert.	0	0	0	0	0	0
12. 5	Em. Infl.	0	0	0.8	0.8	0	0
control	–	0	0	0	0	0	0
1958–59 (Sown late July)							
20. 8	–	0.8	0	0	0	0	0
19. 9	1–2 V.P.	0	0	0	0	0	0
24.10	2–3 V.P.	11.7	15.8	20.8	25.8	0	0
20.11	Ditto	2.5	5.0	14.2	18.3	0.8	0
4. 3	3 V.P.	0	0	0	2.5	0	0.8
24. 4	60% Tert.–40% Floral	0	0.8	0	0	0	0
12. 5	Em. Infl.	0	0	0	0	0	0
control	–	0	0	0	0	0	0

TABLE 16. Percentage of inflorescence abnormalities caused in *Festuca pratensis* by auxin herbicides

Date of spraying	Stage of development of growing point	MCPA 1 kg	MCPA 2 kg	2,4-D 1 kg	2,4-D 2 kg	2,4,5-TP 1 kg	2,4,5-TP 2 kg
1958–59 (Sown in April)							
22. 8	–	1.0	0	0	0.5	0	0
19. 9	1 V.P.	0	0	1.5	1.0	0	0.5
24.10	2 V.P.	0	5.5	4.0	6.0	0	0
20.11	2–3 V.P.	5.0	4.0	11.0	18.5	1.0	1.0
4. 3	3 V.P.	0	0	0.5	2.0	0	0
24. 4	Tert.	0	0	0	0.5	0.5	0
12. 5	Em. Infl.	0	0	0	0	0	0.5
control	–	0	0	0	0	1.0	0.5
1958–59 (Sown late July)							
20. 8	–	0	0.5	0	0	0.5	2.5
19. 9	1–2 V.P.	0	1.5	4.5	2.0	1.0	0
24.10	2–3 V.P.	2.0	0	1.5	26.5	0	1.0
20.11	2–3 V.P.	0	0	14.5	26.5	0	2.5
4. 3	3 V.P.	2.0	2.5	4.0	5.5	0	1.0
24. 4	60% Tert.–40% Floral	2.0	2.0	1.5	0.5	0	0
12. 5	Em. Infl.	0	1.5	0	2.0	0	1.5
control	–	0	0	0	0	0	0

3.1.3.3. Yield

Yield determinations are rather scarce (TABLE 17). Late autumn applications with MCPA, 2,4-D and 2,4,5-TP did not result in statistically reliable yield reductions, although there is a definite tendency to lower yields with MCPA and 2,4-D. The same was true for MCPA and 2,4,5-TP, applied prior to inflorescence emergence.

TABLE 17. Influence of MCPA, 2,4-D and 2,4,5-TP on seed production (as percentages of non-treated plots) of *Festuca pratensis*, according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA		2,4-D	2,4,5-TP	
Growing season	1958–59		1958–59	1958–59	
Experiment No.	IBS 236	IBS 266	IBS 236	IBS 236	IBS 266
Rate of application (a.e./ha)	1 kg	1 kg	1 kg	1 kg	1 kg
Time of application in order of stage of development					
Early stage Late autumn ± 18 cm high	95.3		96.4	100.5	
Regrowth after winter Just prior to inflorescence emergence, 45–60 cm high		92.9			98.1
Yield of non-treated control in kg/ha	1142	1276	1142	1142	1276

3.1.3.4. 1000-Kernel weight

In the yield experiments of I.B.S. and P.A.W. (TABLE 18), 1000-kernel weight was not influenced. In the author's experiments, two year's results did not show either any statistically reliable influence on 1000-kernel weight.

TABLE 18. Influence of MCPA, 2,4-D and 2,4,5-TP on 1000-kernel weight (K.W.) and on germination (G.P.) (as percentages on non-treated plots) of *Festuca pratensis*, according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA				2,4-D	2,4,5-TP			
Growing season	1958–59				1958–59	1958–59			
Experiment No.	IBS 236		IBS 266		IBS 236	IBS 236		IBS 266	
Rate of application (a.e./ha)	1 kg		1 kg		1 kg	1 kg		1 kg	
	K.W.	G.P.	K.W.	G.P.	K.W.	K.W.	G.P.	K.W.	G.P.
Time of application in order of stage of development									
Early stage Late autumn ± 18 cm high	104.5	99.0			102.6	99.3	102.1		
Regrowth after winter Just prior to inflorescence emergence, 45–60 cm high			103.0	100.0				102.8	100.0
K.W. (in gm) and G.P. of non-treated control	2.001	97	1.614	92	2.001	2.001	97	1.614	92

3.1.3.5. Germination percentage

In the experiments of I.B.S. and P.A.W. (TABLE 18) treatments of 1 kg of herbicides did not show any influence. In our experiments dosage rates of 1 kg and 1.5 kg had the same result. 2 kg of auxin herbicides had no influence in the experiment sown in July. However, in the plants sown in April, this dosage lowered the germination percentage at 5% level when sprayed in the first week of March (growth stage 3 V.P.).

3.1.4. Festuca rubra

The experiments were carried out with two subspecies: *genuina* and *commutata*. As there did not appear to be any difference in behaviour between the two, the experimental results have been presented together.

3.1.4.1. Leaf and stem development

Leaf and stem abnormalities were assessed in one experiment only (TABLE 19). MCPA and 2,4,5-TP at dosage rates of 1 and 2 kg did not cause abnormalities, but with 2,4-D the applications at the stages of development 1–2 and 2–3 V.P. resulted in many malformations.

This experiment was carried out with the non-creeping selection of *Festuca rubra*.

3.1.4.2. Structure of the inflorescence

Also in this case 2,4-D caused the highest abnormality percentage (TABLE 20). MCPA and 2,4,5-TP had considerably less effect at the same stages of development (1–2 and 2–3 V.P.). A relatively great effect of 2,4,5-TP occurred in both experiments with applications at the stage just prior to or during emerging of inflorescence.

TABLE 19. Percentage of leaf and stem abnormalities caused in *Festuca rubra* by auxin herbicides

Date of spraying 1958–59	Stage of development of growing point	2,4-D 1 kg	2,4-D 2 kg
20. 8	1 V.P.	0	0
19. 9	1 V.P.	0	0
24.10	1–2 V.P.	12.5	17.5
20.11	2–3 V.P.	18.3	11.7
4. 3	75% V.P.–25% Sec.	0	0
24. 4	Em. Infl.	0	1.7
12. 5	Em. Infl.	1.7	0
control	–	0	0

TABLE 20. Percentage of inflorescence abnormalities caused in *Festuca rubra* by auxin herbicides

Date of spraying 1957–58	Stage of development of growing point	MCPA 1 kg	2,4-D 1.5 kg	2,4,5-TP 1.5 kg
26. 8	–	0	3.0	0
26. 9	–	1.5	1.0	0.5
8.11	1–2 V.P.	1.5	9.5	1.0
12.12	1–2 V.P.	5.0	12.0	0.5
4. 2	V.P.–Sec.	0.5	2.5	5.5
4. 3	Ditto	1.0	5.0	1.0
10. 3	Ditto	1.0	2.5	0.5
19. 3	Ditto	2.0	4.5	0
26. 3	Ditto	3.0	2.0	1.0
2. 4	Ditto	2.0	1.0	1.5
9. 4	Sec.	1.0	3.0	0.5
15. 4	Tert.	0	2.5	2.0
22. 4	Tert.	0	0	0.5
6. 5	Floral-Em. Infl.	0	1.0	5.0
13. 5	Em. Infl.	0.5	0	1.0
19. 5	Ditto	0.5	0.5	2.5
control	–	0	0	0

Date of spraying 1958–59	Stage of development of growing point	MCPA 1 kg	MCPA 2 kg	2,4-D 1 kg	2,4-D 2 kg	2,4,5-TP 1 kg	2,4,5-TP 2 kg
20. 8	1 V.P.	0	0	1.0	4.0	0.5	1.0
19. 9	Ditto	0	0	2.0	0	0.5	0
24.10	1–2 V.P.	0	0	2.5	6.0	1.0	0
20.11	2–3 V.P.	1.5	1.5	6.5	10.0	0	0
4. 3	75% V.P.–25% Sec.	0.5	4.0	6.5	2.0	0	0
24. 4	Em. Infl.	0	0.5	0	0	0	4.0
12. 5	Ditto	0	0	1.0	0	1.5	2.5
control	–	0	0	0	0	0	0

In a pot experiment with clonal plants (TABLE 21) the same trend as in field trials was observed, but the degree of sensitivity was much higher. Again with 2,4-D the highest percentages were observed in the vegetative growing stages and the early reproductive stages with a considerable increase when the dosage applied was increased. The MCPA effect was only pronounced at the 2 kg application in an early reproductive stage. The effect of 2,4,5-TP, already noticed in the field experiment, was particularly noticeable at the Floral stage.

3.1.4.3. Yield

In the experiments carried out by the I.B.S. and P.A.W. (TABLE 22), MCPA applied at various moments in spring did not cause statistically reliable yield reductions. 2,4-D, although not having a statistically reliable influence on the yield, shows a tendency to decrease yield production when applied at the early emergence of inflorescence. An autumn application of 2,4,5-TP did not cause any yield depression either, but an application at the first emergence of inflorescence was extremely harmful to the seed production, the decrease being significant at 1 % level.

3.1.4.4. 1000-Kernel weight

In I.B.S. and P.A.W. experiments (TABLE 23), an application of 2,4,5-TP at the dosage rate of 1.5 kg at the time of first emergence of inflorescence caused an increase reliable at 1 % level. In both our experiments the increase in 1000-kernel weight occurred at the stage Em. Infl. This was mainly caused by treatments with 2,4,5-TP at both dosage rates. However, in one year it was significant at 5 % level and in the other at 1 % level.

TABLE 21. Percentage of inflorescence abnormalities caused in *Festuca rubra* by auxin herbicides (pot experiment with clones)

Date of spraying 1957–58	Stage of development of growing point	MCPA		2,4-D		2,4,5-TP	
		1 kg	2 kg	1 kg	2 kg	1 kg	2 kg
15.11	1–2 V.P.	0	0	12.7	24.0	1.3	0
15. 1	3 V.P.	0.7	0	12.0	34.7	0	0
21. 2	75% 3–4 V.P.–25% Repr. (1/3 Sec.)	0.7	13.3	16.0	20.0	0	0
12. 3	70% V.P.–20% Sec.	0	0	0	9.3	0	0
22. 3	V.P.–Sec.	0	0.6	0	9.0	0	0
28. 3	Ditto	0.7	0	8.0	22.0	0	0
2. 4	Sec.	0	0.6	0	2.0	0.7	0
11. 4	Tert.	0	1.3	0	4.0	0.7	0.7
19. 4	Ditto	0	0.6	0	0.7	0	4.0
26. 4	Floral	0	1.3	0	0.7	15.3	16.0
5. 5	Floral	0	0	0	0	6.7	14.7
13. 5	30% Floral– 50% Em. Infl.	0	0	0	0	0	1.3
20. 5	Em. Infl.	0	0	0	0	0	0
30. 5	Ditto	0	0.6	0	0	0	0
3. 6	Ditto	0	0	0	0	0	0.7
control	–	0.5	0.5	0.5	0.5	0.5	0.5

TABLE 22. Influence of MCPA, 2,4-D and 2,4,5-TP on seed production (as percentages of non-treated plots) of *Festuca rubra*, according to experiments of I.B.S. and P.A.W.

Auxin herbicide	MCPA						2,4-D				2,4,5-TP
Growing season	1953–54			1958–59	1957–58	1958–59	1953–54			1958–59	1958–59
Experiment No.	CI 1607			IBS 221	VI D[1]	II C[1]	CI 1607			IBS 221	IBS 221
Type of grass	ssp. *genuina*			ssp. *commutata*			ssp. *genuina*			ssp. *commutata*	
Rate of application (a.e./ha)	0.5 kg	1 kg	2 kg	1 kg	1.25 kg	1 kg	0.5 kg	1 kg	2 kg	1.5 kg	1.5 kg
Time of application in order of stage of development											
Early stage											
1. 3–5 leaves, 1–2 tillers				98.9						100.1	97.7
Regrowth after winter											
2. still dormant	91.0	100.0	98.2				98.2	102.7	99.1		
3. regrowth	93.7	101.8	98.2				109.9	99.1	99.1		
4. shooting	100.0	92.8	96.4		101.8	94.5	100.0	72.1	72.1		
Inflorescence emergence											
5. 1st emergence of inflorescence				88.9						88.0	36.7**
Yield of non-treated control in kg/ha	1110	1110	1110	1843	958	1390	1110	1110	1110	1843	1843

** Significant difference at 1% level
[1] Statistical analysis not available.

TABLE 23. Influence of MCPA, 2,4-D and 2,4,5-TP on 1000-kernel weight (K.W.) and germination (G.P.)[1] (as percentages of non-treated plots) of *Festuca rubra* spp. *commutata*

Auxin herbicide	MCPA		2,4-D		2,4,5-TP	
Growing season	1958–59		1958–59		1958–59	
Experiment No.	IBS 221		IBS 221		IBS 221	
Rate of application (a.e./ha)	1 kg		1.5 kg		1.5 kg	
	K.W.	G.P.	K.W.	G.P.	K.W.	G.P.
Time of application in order of stage of development						
Early stage						
1. 3–5 leaves, 1–2 tillers	99.2	96.8	101.7	102.1	100.8	102.1
Inflorescence emergence						
5. 1st emergence of inflorescence	105.0	102.1	102.5	94.7	126.7**	93.6
control	1,200 gm	94	1,200 gm	94	1,200 gm	94

[1] Statistical analysis of germination percentage not available. ** Significant difference at 1% level.

TABLE 24. Percentage of inflorescence abnormalities caused in *Lolium perenne* by auxin herbicides

Date of spraying 1957–58	Stage of development of growing point	MCPA 1 kg	2,4-D 1.5 kg	2,4,5-TP 1.5 kg
4.10	–	15.0	2.0	2.0
17.10	–	7.5	8.0	7.5
8.11	1 V.P.	6.0	15.0	7.5
12.12	2 V.P.	0	8.0	10.0
12. 2	4–5 V.P.	12.0	13.5	0
4. 3	5–6 V.P.	2.5	40.0	7.5
10. 3	Ditto	9.5	32.5	11.5
19. 3	Ditto	15.0	37.0	1.5
26. 3	Ditto	2.5	9.5	5.5
2. 4	5–6 V.P. (D.R. visible)	9.0	16.0	3.0
9. 4	D.R. (40–50%)	6.0	12.0	8.5
15. 4	Ditto	10.5	12.0	5.5
22. 4	Sec.	4.0	3.0	2.0
28. 4	Floral	0	0	0
6. 5	Ditto	0	0	0
13. 5	Em. Infl.	0	0	4.5
28. 5	Ditto	4.5	1.0	0
5. 6	Ditto	3.0	4.0	2.0
control	–	0	0	0

Date of spraying 1958–59	Stage of development of growing point	MCPA 1 kg	MCPA 2 kg	2,4-D 1 kg	2,4-D 2 kg	2,4,5-TP 1 kg	2,4,5-TP 2 kg
26.11	–	1.0	6.0	3.0	2.0	3.0	3.0
4. 3	1–2 V.P.	7.0	1.5	3.0	2.0	4.5	3.5
3. 4	4–6 V.P.	3.0	6.5	5.0	10.5	2.5	2.0
13. 4	80% V.P., 20% Sec.	1.5	4.5	2.5	1.5	2.5	1.5
24. 4	Sec.	1.5	3.0	6.5	4.0	0	1.5
12. 5	Floral	4.0	1.0	2.0	1.5	4.0	3.5
2. 6	Em. Infl.	4.5	8.5	2.5	2.0	0	5.5
control	–	0	0	0	0	0	0

3.1.4.5. Germination percentage

In one experiment of I.B.S. and P.A.W. (TABLE 23), and in our own experiments, no statistically reliable influences were found.

3.1.5. Lolium perenne

3.1.5.1. Structure of the inflorescence

Lolium perenne showed a relatively high susceptibility to auxin herbicides. Like *Poa trivialis* it showed a maximum percentage of inflorescence abnormalities after winter.

In spite of the difference in the degree of susceptibility in the two field experiments, spraying at the stages 4–6 or 5–6 V.P., occurring in March-early April, caused the highest percentage of abnormalities (TABLE 24). This is particularly true for 2,4-D; this herbicide also influenced the inflorescence structure in the developmental stage D.R. In considering the differences between the growing seasons it will be of importance to note that two different strains were used in the two experiments.

In the pot experiments with clones of *Lolium perenne* a similar trend was found (TABLE 25).

3.1.5.2. Yield

The single experiment of I.B.S. and P.A.W. reported, concerning the influence of auxin herbicides on the yield of *Lolium perenne*, did not show any influence of MCPA or 2,4,5-TP. This was due to the occurrence of many weeds in the non-treated plots, resulting in low yields (TABLE 26).

TABLE 25. Percentage of inflorescence abnormalities caused in *Lolium perenne* by auxin herbicides (pot experiment with clones)

Date of spraying 1957–58	Stage of development of growing point	MCPA 1 kg	MCPA 2 kg	2,4-D 1 kg	2,4-D 2 kg	2,4,5,-TP 1 kg	2,4,5,-TP 2 kg
15.11	2–3 V.P.	6.0	2.0	3.3	0.7	1.3	0
15. 1	Ditto	7.3	12.7	10.0	7.3	0	2.0
21. 2	4–5 V.P.	7.3	5.3	12.0	18.7	2.7	2.0
12. 3	4–6 V.P.	7.3	2.0	10.7	30.0	0	0
22. 3	Ditto	2.3	1.3	7.3	25.0	0.7	0.7
28. 3	Ditto	2.0	2.7	18.0	26.0	0.7	1.3
2. 4	Ditto	1.3	5.3	2.0	15.3	2.0	1.3
11. 4	4–6V.P.(D.R.visible)	2.0	0	20.7	22.0	0	2.0
19. 4	4–7V.P.(D.R.visible)	2.3	2.7	8.0	13.3	0.7	0
26. 4	80% 7 V.P. – 20% D.R.	1.3	3.3	7.3	9.3	0.7	0
5. 5	V.P.–Sec.	1.3	1.3	0.7	0	0	0
13. 5	Sec.–Floral	0	0	0	0.7	0	4.0
3. 6	Em. Infl.	4.7	0	0	0	0	0
14. 6	Em. Infl.	2.7	1.3	1.3	0	0.7	0
30. 6	Em. Infl.	1.3	0.7	2.7	0.7	0.7	1.3
control	–	0	1.3	0.7	1.3	0	6.0

3.1.5.3. 1000-Kernel weight

In the experiment of I.B.S. and P.A.W. (TABLE 26), no reliable differences could be found. In the authors' field and pot experiments, even no difference at 5% level could be established at any time of application.

TABLE 26. Influence of MCPA and 2,4,5-TP on the seed production (yield), 1000-kernel weight (K.W.) and germination (G.P.) (as percentages of non-treated plots) of *Lolium perenne*, according to an experiment of I.B.S. and P.A.W.

Auxin herbicide	MCPA			2,4,5-TP		
Growing season	1958–59			1958–59		
Experiment No.	IBS 235			IBS 235		
Rate of application (a.e./ha)	1 kg			1 kg		
	yield	K.W.	G.P.	yield	K.W.	G.P.
Time of application						
4 leaves (2 tillers, 5–7 cm high)	128.2	102.0	99.0	124.0	100.4	102.1
Values of non-treated control plots: yield in kg/ha and K.W. in gm	1558	0.1563	97	1558	0.1563	97

3.1.5.4. Germination energy and Germination percentage

In the I.B.S. and P.A.W. experiment (TABLE 26), though the statistical analysis was not available, the values did not warrant to assume any possible effect. No statistically reliable differences at 5% level could be established in both our field experiments.

In the pot experiment with clones the germination energy of seeds from pots sprayed at the stages 4–6 V.P., 80% 7 V.P. - 20% D.R. and Em. Infl. was significantly lower (at 5% level) than that of the control. As to the germination percentage, seeds from plants sprayed at Sec. - Floral and full Em. Infl. stages were statistically inferior to the control (at 5% level), and those at the early inflorescence stage even at 1% level.

3.1.6. Dactylis glomerata

Only one field experiment with *Dactylis glomerata* was conducted. The small plot size (1 m $\times$ 2 m) and the presence of only a relatively little number of plants on some of the plots did not allow the harvesting of great numbers of shoots with inflorescences for the determination of abnormality percentage. From the harvested seeds only 1000-kernel weight, germination energy and germination percentage were recorded. Although no statistically reliable influence of MCPA (1 kg), 2,4-D (1.5 kg) and 2,4,5-TP (1.5 kg) could be established, there was a tendency for all three chemicals to decrease 1000-kernel weight in applications at the stage of development of V.P. - Sec. (15th April).

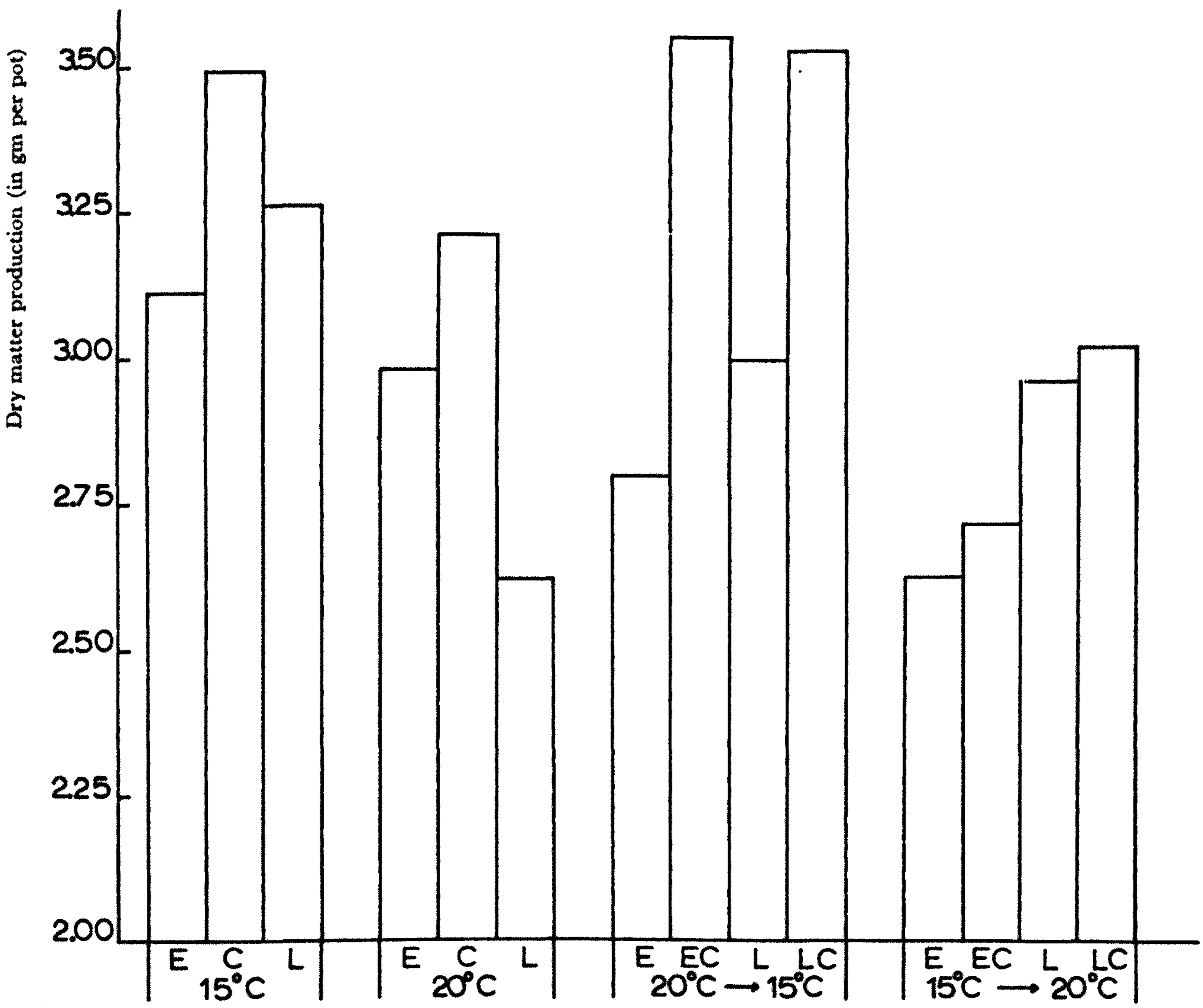
3.2. Greenhouse and environmental-chamber experiments

3.2.1. Lolium multiflorum westerwoldicum

3.2.1.1. Dry matter production (Fig. 10)

a. *Non-treated plants.* Under constant temperature conditions at 15 °C, non-treated plants produced more dry matter than plants at 20 °C. A drop in temperature from 20 °C to 15 °C at the stages of development 1–2 V.P. and 90% V.P.–10% D.R. resulted in the same dry matter production observed in plants grown continuously at 15 °C. A rise in temperature from 15 °C to 20 °C at 1–2 V.P. and Floral–Em. Infl. stages gave a dry matter production which was lower than that of plants growing continuously at 20 °C.

Fig. 10. Influence of temperature on the dry matter production of *Lolium multiflorum westerwoldicum* due to 2,4-D treatment



Explanation of the symbols

15 °C: constant temperature conditions
E: plants treated at 1–2 V.P. stage
L: plants treated at Floral.–Em. Infl. stage
C: control

20 °C: constant temperature conditions
E: plants treated at 1–2 V.P. stage
L: plants treated at 90% V.P.–10% D.R. stage
C: control

20 °C→15 °C: a drop in temperature after treatment
E: plants treated at 1–2 V.P. stage
EC: contrl foor E
L: plants treated at 90% V.P.–10% D.R. stage
LC: control for L

15 °C→20 °C: a rise in temperature after treatment
E: plants treated at 1–2 V.P. stage
EC: control for E
L: plants treated at Floral-Em. Infl. stage
LC: control for L

b. *Treated plants.* All treatments with 2,4-D gave a reduction in dry matter production in comparison to the respective controls. At constant temperature conditions of 20 °C, the influence of the treatment was particularly noticeable at the stage 90% V.P.–10% D.R.

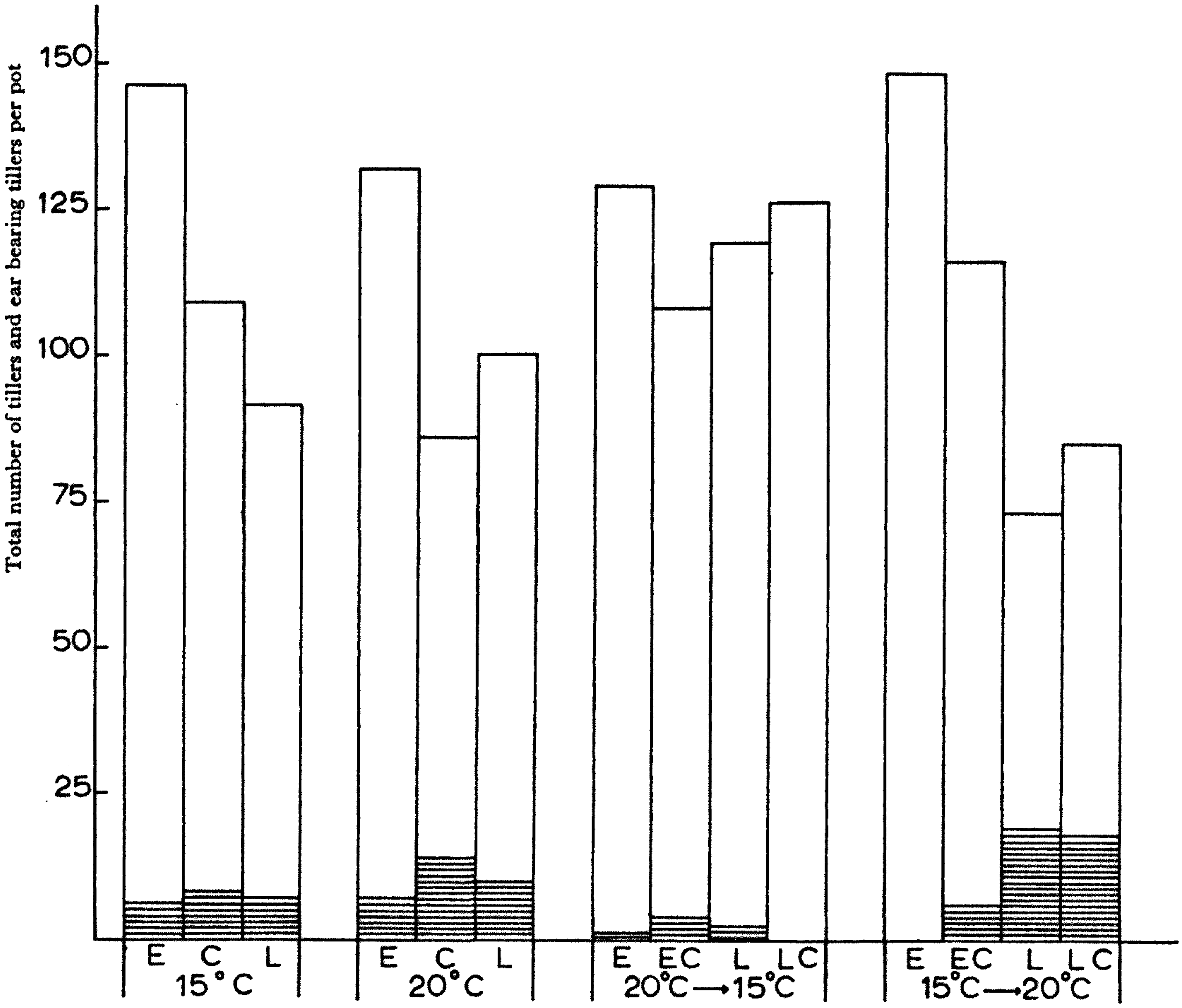
A drop in temperature from 20 °C to 15 °C after treatments decreased the dry matter production to a great extent, compared to their respective controls or treated plants grown continuously at 15 °C.

Treatments of plants transferred from 15 °C to 20 °C after spraying showed the general effect of treatment in producing slightly less organic matter than the control.

3.2.1.2. Total number of tillers formed (FIG. 11)

a. *Non-treated plants.* With non-treated plants at constant temperature at 15 °C plants produced more tillers than at 20 °C.

FIG. 11. Influence of temperature on the total number of tillers (unshaded plus shaded area) and total number of ear bearing tillers (shaded area) of *Lolium multiflorum westerwoldicum* due to 2,4-D treatment



For explana tion of the symbols used vide FIG. 10.

A drop from 20 °C to 15 °C at 1–2 V.P. and 90 % V.P.–10 % D.R. stages stimulated tiller formation to the level of the 15 °C plants or to a higher degree.

A rise in temperature to 20 °C at the stage 1–2 V.P. did not increase the number of tillers above the 15 °C level. At the stage Floral–Em. Infl., however, the number of tillers became greatly reduced in relation to the 15 °C plants.

b. *Treated plants.* Under constant temperature conditions of both 15 °C and 20 °C, 2,4-D treatment at 1–2 V.P. caused a considerable increase in the number of tillers. At 20 °C at the more advanced stage of the growing point 90% V.P.–10% D.R. the increase is much less pronounced and at 15 °C at still a later stage (Floral–Em. Infl.), there is even a decrease in the number of tillers produced.

A drop in temperature after treatment at the stages 1–2 V.P. and 90% V.P.–10% D.R. stimulated plants grown and treated at 20 °C.

A rise in temperature at 1–2 V.P. gave an increase in tiller formation like observed in plants grown and treated constant 15 °C. Treatments at Floral–Em. Infl. stage followed by a rise in temperature showed a decrease in tillering compared to non-treated plants grown at 15 °C constantly.

3.2.1.3. Total number of ear-bearing tillers formed (FIG. 11)

a. *Non-treated plants.* Under constant temperature conditions, the total number of ear-bearing tillers was higher at 20 °C than at 15 °C. A drop in temperature at 1–2 V.P. and 90% V.P.–10% D.R. stages decreased the number below the level of constant 15 °C plants. A rise in temperature at 1–2 V.P. showed a similar decrease but at Floral–Em. Infl. stage the value was higher than observed in the constant 20 °C plants.

b. *Treated plants.* Under constant temperature conditions, treatment showed the tendency to decrease the number of ear-bearing tillers as compared to the control. At 20 °C this tendency is more pronounced than at 15 °C.

A drop in temperature after treatment showed the value to be much below those of untreated or treated 15 °C or 20 °C plants. In comparison with their respective controls, treatments at 1–2 V.P. stage showed the tendency to decrease whereas at 90% V.P.–10% D.R. stage to increase.

A rise in temperature at 1–2 V.P. did not produce any ear-heads but at the Floral–Em. Infl. stage the stimulation observed after subjecting the respective non-treated plants to temperature rise was maintained.

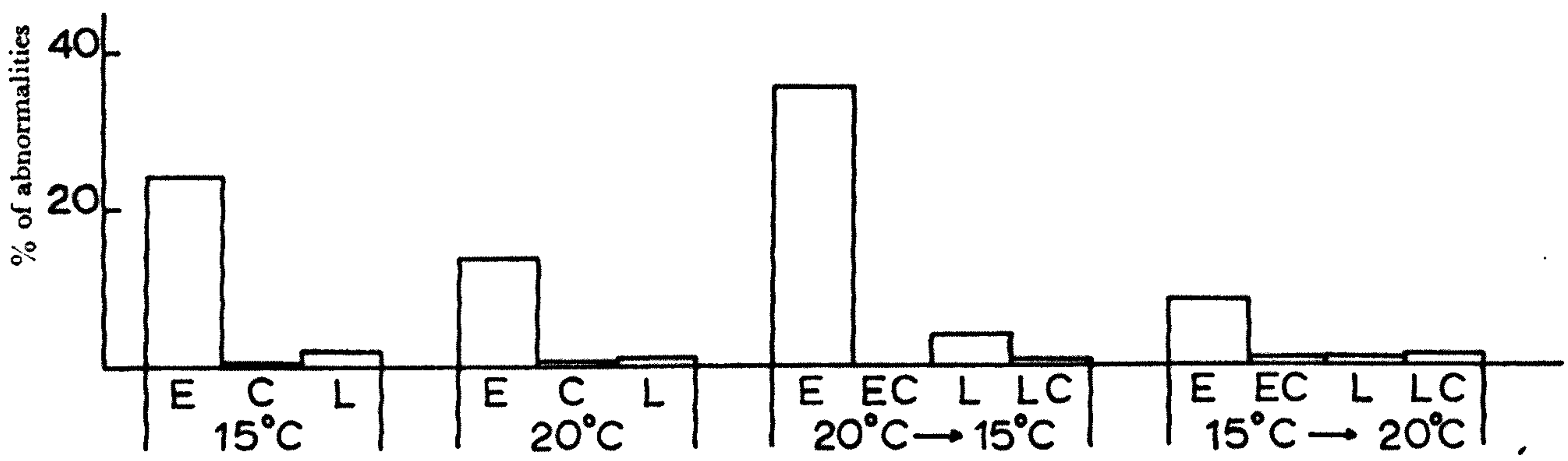
3.2.1.4. Percentage of affected tillers (FIG. 12)

Under constant temperature conditions only treatments at the 1–2 V.P. stage caused high percentages of abnormal tillers; at 15 °C the value was greater than at 20 °C.

A drop in temperature caused a considerable increase in the number of affected tillers, with a greater influence at the early growth than at later one.

A rise in temperature after treatment decreased the percentage of abnormalities at 1–2 V.P. stage and approached nil at Floral–Em. Infl. stage.

FIG. 12. Influence of temperature on percentage of abnormalities of *Lolium multiflorum westerwoldicum* due to 2,4-D treatment



For explanation of the symbols used vide FIG. 10.

3.2.2. Poa annua

In TABLES 27, 28 and 29 data on the 4 experiments carried out are presented. The values indicated were obtained by expressing the results of dry matter production (TABLE 27), the number of tillers formed (TABLE 28) and the number of ear-bearing tillers (TABLE 29) as factors of a scale of 10. In each experiment the level of the treatment giving the highest value was taken as 10 and all other treatments were expressed in this factor according to the following scheme:

Range from 0–10 % of the value of the highest level in the experiment concerned .	1
Range from 10–20 % of the same	2
Range from 90–100 % of the same	10

The tables furnish sufficient background material to permit the conclusions presented in paragraphs 3.2.2.1.–3.2.2.3. From a statistical point of view a mathematical analysis of the figures is not allowed. Averaging figures of experiments carried out at the same temperature is impossible, because the experiments were not all carried out under the same environmental conditions (*viz.* in environmental chambers and in the conditioned greenhouse). Moreover, the standard value of the scale (*viz.* 10) is a standard for each experiment individually and not for all experiments combined.

3.2.2.1. Dry matter production

a. *Non-treated plants.* In non-treated plants at constant temperatures, dry matter production was optimal between 15 °C and 25 °C. Plants subjected to a drop in temperature at the stage V.P. generally gave a decrease in dry matter production in relation to plants growing continuously at the initial temperature. A rise in temperature did not result in a production at the level of plants growing continuously at the final temperature; the relation to the production of plants growing at the initial temperature is uncertain, although transfer to temperatures within the optimal range seems to be beneficial.

b. *Treated plants.* Particularly within the optimal range treatments generally reduced dry matter production in the plants kept under constant temperature conditions. At

TABLE 27. The influence of 2,4-D on the production of dry matter in *Poa annua* (Explanation in text)

Initial Temp.	Exp. No.	Stage of Dev.	Object	Final temperature 10°	15°	17°	20°	23°	25°	32°
10°	148	–	Cont.	**4**						
	E.C.	3–4 V.P.	Tr.	**3**						
	191	–	Cont.	**4**						
	E.C.	1–3 V.P.	C.		3		4			
		1–3 V.P.	Tr.	**4**	4		5			
		Sec.	C.		4		4			
		Sec.	Tr.	**4**	4		3			
	252	–	Cont.	**6**						
	E.C.	1–2 V.P.	C.				9			
		1–2 V.P.	Tr.	**6**			8			
		3–4 V.P.-Sec.	C.				6			
		3–4 V.P.-Sec.	Tr.	**6**			6			
15°	191	–	Cont.		**6**					
	E.C.	1–2 V.P.	C.	5			7			
		1–2 V.P.	Tr.	5	**5**		7			
		Em. Infl.	C.	5			6			
		Em. Infl.	Tr.	8	**7**		6			
	251	–	Cont.		**6**					
	G.H.	2–3 V.P.	C.						7	4
		2–3 V.P.	Tr.		**5**				8	2(5 k)
		V.P.–Floral	C.						8	9
		V.P.–Floral	Tr.		**5**				9	5
	252	–	Cont.		**9**					
	E.C.	1–2 V.P.	C.							
		1–2 V.P.	Tr.		**9**					
		Floral	C.							
		Floral	Tr.		**8**					
17°	148	–	Cont.			**8**				
	E.C.	V.P.–Sec.	Tr.			**7**				
20°	191	–	Cont.				**10**			
	E.C.	1–2 V.P.	C.	7	9					
		1–2 V.P.	Tr.	5	6		**6**			
		Em. Infl.	C.	8	8					
		Em. Infl.	Tr.	8	8		**7**			
	252	–	Cont.				**9**			
	E.C.	1–2 V.P.	C.	6						
		1–2 V.P.	Tr.	5			**8**			
		V.P.–Sec.	C.	8						
		V.P.–Sec.	Tr.	10			**8**			
				E.C.		E.C.				
23°	148	–	Cont.					**10**		
	G.H.	V.P.–Sec.	C.	5		8				
		V.P.–Sec.	Tr.	3		7		**9**		
25°	251	–	Cont.						**9**	
	G.H.	1–2 V.P.	C.		6					4
		1–2 V.P.	Tr.		5				**7**	3(2 k)
		Floral	C.		4					6
		Floral	Tr.		6				**6**	1(6 k)
32°	251	–	Cont.							**5**
	G.H.	1–2 V.P.	C.		5				10	
		1–2 V.P.	Tr.		6				7	**3(4 k)**
		1–2 V.P.	C.		5				8	
		1–2 V.P.	Tr.		5				6	**3**

Cont. = non-treated plants growing continuously at the temperature indicated.
C. = non-treated plants transferred to other temperatures at the stage of development indicated.
Tr. = plants treated with 2,4-D at the rate of 2 kg a.e./ha.
k = number of plants killed out of 9 plants treated.
E.C. = experiments carried out in the environmental chambers under artificial light.
G.H. = experiments carried out in the greenhouse under natural day-light conditions.

TABLE 28. The influence of 2,4-D on tiller formation in *Poa annua* (Explanation in text)

Initial Temp.	Exp. No.	Stage of Dev.	Object	Final temperature 10°	15°	17°	20°	23°	25°	32°
10°	148	–	Cont.	**7**						
	E.C.	3–4 V.P.	Tr.	**6**						
	191	–	Cont.	**8**						
	E.C.	1–3 V.P.	C.		6		6			
		1–3 V.P.	Tr.	**10**	6		5			
		Sec.	C.		7		7			
		Sec.	Tr.	**7**	6		7			
	252	–	Cont.	**10**						
	E.C.	1–2 V.P.	C.				8			
		1–2 V.P.	Tr.	**10**			8			
		3–4 V.P.-Sec.	C.				6			
		3–4 V.P.–Sec.	Tr.	**7**			7			
15°	191	–	Cont.		**10**					
	E.C.	1–2 V.P.	C.	8			7			
		1–2 V.P.	Tr.	10	**9**		7			
		Em. Infl.	C.	9			6			
		Em. Infl.	Tr.	8	**7**		6			
	251	–	Cont.		**6**					
	G.H.	2–3 V.P.	C.						5	8
		2–3 V.P.	Tr.		**6**				7	3(5 k)
		V.P.–Floral	C.						7	8
		V.P.–Floral	Tr.		**6**				6	7
	252	–	Cont.		**10**					
	E.C.	1–2 V.P.	C.							
		1–2 V.P.	Tr.		**10**					
		Floral	C.							
		Floral	Tr.		**9**					
17°	148	–	Cont.			**10**				
	G.H.	V.P.–Sec.	Tr.			**8**				
20°	191	–	Cont.				**8**			
	E.C.	1–2 V.P.	C.	9	8					
		1–2 V.P.	Tr.	9	9		**8**			
		Em. Infl.	C.	9	7					
		Em. Infl.	Tr.	8	7		**6**			
	252	–	Cont.				**9**			
	E.C.	1–2 V.P.	C.	9						
		1–2 V.P.	Tr.	8			**8**			
		V.P.–Sec.	C.	10						
		V.P.–Sec.	Tr.	9			**7**			
				E.C.		E.C.				
23°	148	–	Cont.					**10**		
	G.H.	V.P.–Sec.	C.	8		10				
		V.P.–Sec.	Tr.	8		6		**7**		
25°	251	–	Cont.						**7**	
	G.H.	1–2 V.P.	C.		6					6
		1–2 V.P.	Tr.		7				**8**	6(2 k)
		Floral	C.		6					10
		Floral	Tr.		7				**6**	2(6 k)
32°	251	–	Cont.							**10**
	G.H.	1–2 V.P.	C.		6				7	
		1–2 V.P.	Tr.		7				6	**7(4 k)**
		1–2 V.P.	C.		7				10	
		1–2 V.P.	Tr.		8				8	**6**

For the explanation of the abbreviations used vide TABLE 27.

TABLE 29. The influence of 2,4-D on the number of ear-bearing tillers in *Poa annua* (Explanation in text)

Initial Temp.	Exp. No.	Stage of Dev.	Object	Final temperature 10°	15°	17°	20°	23°	25°	32°
10°	148	–	Cont.	**Nil**						
	E.C.	3–4 V.P.	Tr.	**1**						
	191	–	Cont.	**2**						
	E.C.	1–3 V.P.	C.		4		5			
		1–3 V.P.	Tr.	**2**	3		5			
		Sec.	C.		2		3			
		Sec.	Tr.	**2**	3		3			
	252	–	Cont.	**1**						
	E.C.	1–2 V.P.	C.				8			
		1–2 V.P.	Tr.	**1**			8			
		3–4 V.P.–Sec.	C.				7			
		3–4 V.P.–Sec.	Tr.	**2**			7			
15°	191	–	Cont.		**7**					
	E.C.	1–2 V.P.	C.	2			7			
		1–2 V.P.	Tr.	2	**5**		7			
		Em. Infl.	C.	4			6			
		Em. Infl.	Tr.	6	**6**		6			
	251	–	Cont.		**3**					
	G.H.	2–3 V.P.	C.						5	7
		2–3 V.P.	Tr.		**3**				6	5(5 k)
		V.P.–Floral	C.						7	8
		V.P.–Floral	Tr.		**4**				6	6
	252	–	Cont.		**7**					
	E.C.	1–2 V.P.	C.							
		1–2 V.P.	Tr.		**9**					
		Floral	C.							
		Floral	Tr.		**7**					
17°	148	–	Cont.			**9**				
	G.H.	V.P.–Sec.	Tr.			**9**				
20°	191	–	Cont.				**10**			
	E.C.	1–2 V.P.	C.	2	7					
		1–2 V.P.	Tr.	2	5		**7**			
		Em. Infl.	C.	6	7					
		Em. Infl.	Tr.	7	7		**7**			
	252	–	Cont.				**10**			
	E.C.	1–2 V.P.	C.	2						
		1–2 V.P.	Tr.	1			**8**			
		V.P.–Sec.	C.	5						
		V.P.–Sec.	Tr.	4			**8**			
				E.C.		E.C.				
23°	148	–	Cont.					**8**		
	G.H.	V.P.–Sec.	C.	2		10				
		V.P.–Sec.	Tr.	1		8		**8**		
25°	251	–	Cont.						**9**	
	G.H.	1–2 V.P.	C.		3					2
		1–2 V.P.	Tr.		3				**7**	6(2 k)
		Floral	C.		3					8
		Floral	Tr.		4				**5**	2(6 k)
32°	251	–	Cont.							**5**
	G.H.	1–2 V.P.	C.		3				8	
		1–2 V.P.	Tr.		3				6	**6(4 k)**
		1–2 V.P.	C.		4				10	
		1–2 V.P.	Tr.		3				6	**4**

For the explanation of the abbreviations used vide TABLE 27.

32 °C several of the plants treated at the early stage (1–2 V.P.) died after the treatment.

A drop in temperature after treatment mostly did not influence the intensity of reduction observed in plants remaining at the original temperature after treatment.

A rise in temperature after treatment gave inconclusive effects, although transfer to a temperature above the optimal e.g. 32 °C at early growth stages appeared to be particularly harmful.

3.2.2.2. Total number of tillers formed

a. *Non-treated plants.* In environmental chambers temperature in between 15 °C and 25 °C seems to be optimal for tiller production, the same as with the dry matter production; in the greenhouse plants growing at 32 °C showed a very high number of tillers.

A drop in temperature generally resulted in a slight decrease in the total number of tillers.

A rise in temperature caused a decrease in the total number of tillers. In the experiment in environmental chambers the influence was particularly pronounced at stages V.P.–Sec., Floral and Em. Infl.

b. *Treated plants.* Under constant temperature conditions treatments at the reproductive stage of development caused a decrease in the number of tillers. Treated plants transferred to temperatures below the optimal range showed a similar number of tillers as the non-treated controls.

A rise in temperature within or below the optimal range, in general, increased the total number of tillers and thus reversed the reaction of non-treated plants.

3.2.2.3. Number of ear-bearing tillers

a. *Non-treated plants.* Under constant temperature conditions the optimum range for the number of ear-bearing tillers was between 15 °C and 20 °C.

A drop in temperature from within to below the optimal range caused a decrease in the number of ear-bearing tillers, being more pronounced in transferences at the early vegetative stage. Early vegetative transfer from 32 °C to 25 °C resulted in increased inflorescence numbers.

A transfer to a temperature in the optimal range resulted in a higher number of ear-bearing tillers than in plants continuously grown at optimal temperature. In the greenhouse a transfer from 25 °C to 32 °C caused a very reduced inflorescence emergence.

b. *Treated plants.* Under constant temperature conditions, at higher temperatures at all growth stages investigated, treatments with 2,4-D reduced the number of ear-bearing tillers. Some diverging results occurred, however, at 10 °C and 15 °C and the effects of treatment were less conclusive.

A drop in temperature at early and late stages of development showed the same tendency as in non-treated plants, although generally a slightly lower number was observed.

A rise in temperature after treatment resulted in a higher number of ear-bearing tillers than observed in treated plants kept at the original temperature continuously.

3.2.2.4. Percentage of affected tillers

These percentages are given in TABLE 30.

a. *Non-treated plants.* Under the experimental conditions in some cases control plants as well showed abnormalities. This occurred particularly when plants were transferred to lower temperatures.

TABLE 30. Percentages of leaf- and stem-abnormalities in *Poa annua* owing to the treatment with 2,4-D (Explanation in text)

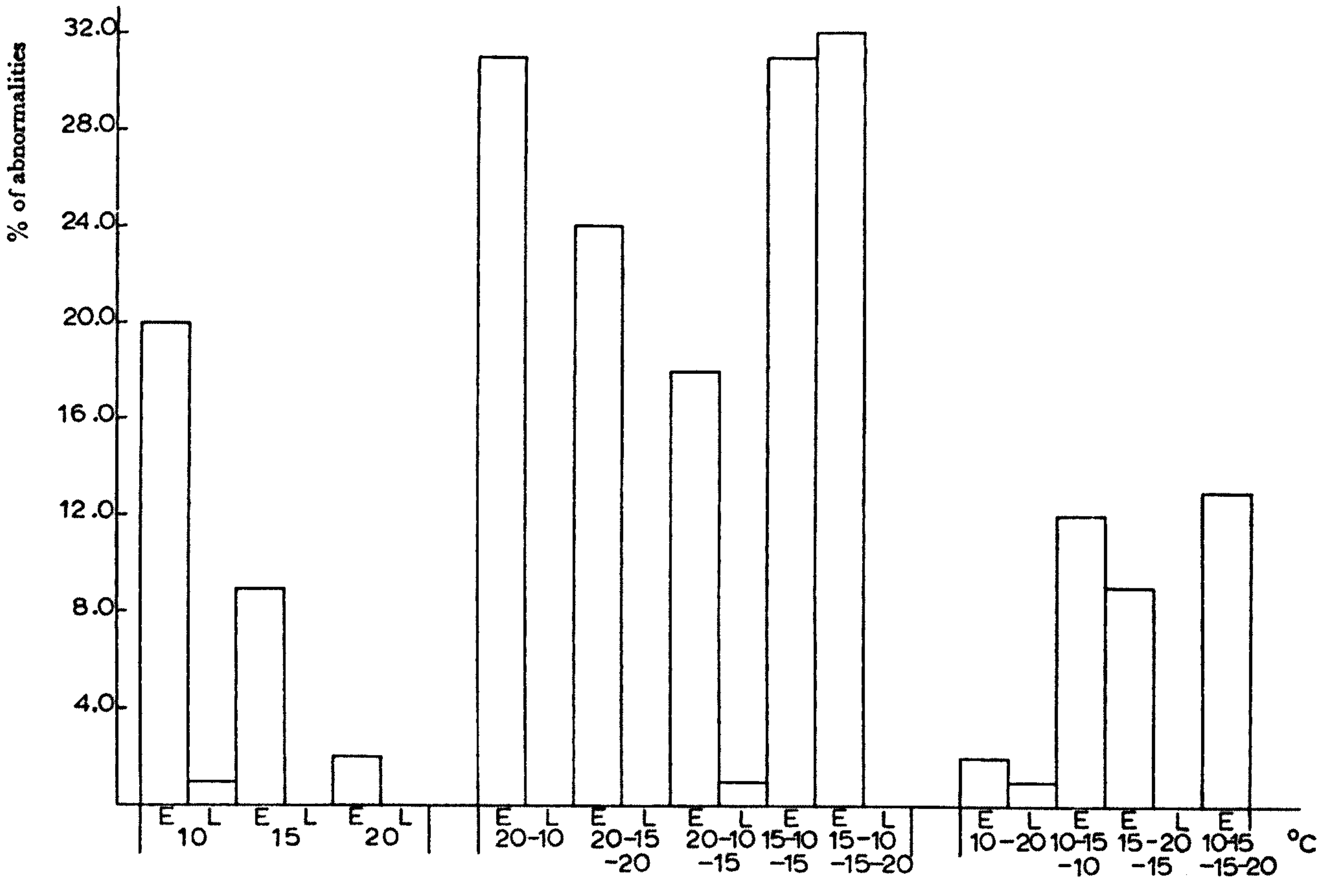
Initial Temp.	Exp. No.	Stage of Dev.	Object	Final temperature 10°	15°	17°	20°	23°	25°	32°
10°	148 E.C.	3–4 V.P.	Tr.	**39**						
	191	1–3 V.P.	C.		Nil		2			
	E.C.	1–3 V.P.	Tr.	**35**	27		8			
		Sec.	C.		Nil		Nil			
		Sec.	Tr.	**Nil**	Nil		7			
	252	1–2 V.P.	Tr.	**20**			2			
	E.C.	3–4 V.P.–Sec.	Tr.	**1**			1			
15°	191	1–2 V.P.	C	32			Nil			
	E.C.	1–2 V.P.	Tr.	40	**29**		29			
		Em. Infl.	C.	Nil			11			
		Em. Infl.	Tr.	Nil	**Nil**		3			
	251	2–3 V.P.	Tr.		**33**				13	8(5 k)
	G.H.	V.P.–Floral	C.							9
		V.P.–Floral	Tr.		**Nil**				1	6
	252	1–2 V.P.	Tr.		**9**					
	E.C.	Floral	Tr.		**Nil**					
17°	148 G.H.	V.P.–Sec.	Tr.			**8**				
20°	191	1–2 V.P.	C.	32	Nil					
	E.C.	1–2 V.P.	Tr.	53	27		**13**			
		Em. Infl.	C.	Nil	Nil					
		Em. Infl.	Tr.	Nil	Nil		**6**			
	252	1–2 V.P.	Tr.	31			**2**			
	E.C.	V.P.–Sec.	Tr.	Nil			Nil			
23°	148 G.H.	V.P.–Sec.	Tr.	E.C. 47		E.C. 21		**15**		
25°	251	1–2 V.P.	C.		3					1
	G.H.	1–2 V.P.	Tr.		27				6	6(2 k)
		Floral	C.		Nil					1
		Floral	Tr.		6				**1**	Nil(6k)
32°	251 G.H.	1–2 V.P.	C.		Nil				1	
		1–2 V.P.	Tr.		25				12	**2(4 k)**
		1–2 V.P.	C.		4				Nil	
		1–2 V.P.	Tr.		11				16	**3**

For the explanation of the abbreviations used vide TABLE 27.

b. *Treated plants.* Under constant temperature conditions the lower the temperature, the higher the percentage of abnormal tillers with the 1–2 V.P. stage. At later growth stages from the onset of reproductive stage onwards, plants are more resistant. 20 °C seems to be the temperature at which the smallest abnormality percentage occurred at this stage. A 32 °C treatment at the 1–2 V.P. stage resulted in nearly 50 per cent death of the plants, thus keeping the percentage low.

A drop in temperature to 10 °C after treatments at the 1–2 V.P. stage showed a much higher percentage of abnormalities than shown by plants treated and remaining at the initial temperature or at 10 °C. Plants transferred to 15 °C, however, showed an increased susceptibility in relation to plants treated and remaining at the initial temperature, but not to plants treated and remaining continuously at 15 °C. The effect of a drop in temperature in later growth stages was very low or absent and did not differ much from the abnormality percentage of the plants remaining continuously at the initial temperature conditions.

FIG. 13. Influence of temperature on percentage of abnormalities of *Poa annua* due to 2,4-D treatment



Explanation of the symbols

In the first block from the left, 10 °C, 15 °C and 20 °C denote the respective constant temperature conditions during the experiment. Symbols used under each temperature regime are as follows:

	10 °C	15 °C	20 °C
E	1–2 V.P.	1–2 V.P.	1–2 V.P.
L	3–4 V.P. (Sec. visible)	Floral	V.P.-Sec.

In the second and third block, any subsequent drops or rises in temperature are denoted by a dash. Between initial and final temperature conditions, treated plants remained for a week in the intermediate temperature conditions. The stages of growth of the plant at the moment of spraying correspond to those mentioned under constant temperature conditions.

A rise in temperature at the vegetative growth stage to the optimal region and generally within the optimal region showed a tendency to decrease the percentage in relation to the percentage observed at the original temperature of the plants, grown continuously at the original temperature. Transference in the V.P. and Floral stages to temperatures above the optimal range resulted in killing many plants. At Em. Infl. as well a rise in temperature to 20 °C caused a very low percentage of abnormalities.

Results on experiments concerning the influence of short rises or drops in temperature on the effect of treatments are not presented in the table. As to abnormality percentage (FIG. 13) even a temporary drop in temperature at early growth stage caused highest percentage of abnormalities, whereas a rise in temperature decreased the value.

4. DISCUSSION

4.1. Introduction

For the practical use of auxin herbicides in seed-growing the effect of the products on the weeds and their influence on seed production and seed quality are factors of major importance. The Tables 7, 12, 17, 22 and 26 summarize dutch experiments on the influence of some auxin herbicides on the seed production of some grasses. It is apparent that within the frame of all field research necessary to support the introduction in the Netherlands of these crops only a limited number of experiments on weed control have been carried out. Weed-control studies had to be undertaken first of all in *Poa pratensis* and *Festuca rubra*, crops covering extensive areas. These studies with herbicides did not exclusively concern the use of MCPA, 2,4-D and 2,4,5-TP but also included a study of several contact herbicides and of more recently developed products, e.g. MCPP (α-(2-methyl-4-chlorophenoxy)-propionic acid), and mixtures of MCPA and trichlorobenzoic acid (Evers, 1959). In addition, and recently, studies on the control of grass weeds *(Poa annua, Alopecurus myosuroides)* had to be included in the experimental work.

Due to reasons mentioned before only for some grasses *(Poa pratensis, Poa trivialis,* and *Festuca rubra)* a reasonable amount of data published on their respective susceptibilities to MCPA, 2,4-D and 2,4,5-TP is present. For other grasses data from dutch field experiments are still scarce (e.g. *Festuca pratensis, Lolium perenne)* or lacking *(Dactylis glomerata, Festuca ovina,* etc.). This situation has contributed towards the starting of the studies reported in this publication in order to allow a comparison to be made with data published elsewhere and to contribute towards a better understanding of the principles involved in the reaction of grasses to auxin herbicides.

4.2. Influence of auxin herbicides on leaf and stem morphology

As to leaf and stem abnormalities caused by auxin herbicides, it is evident from Tables 5, 10, 15 and 19 that in our experiments grasses showed their greatest susceptibility at the moment when the developmental stage of the majority of the growing points was vegetative (Table 31). Also in the experiments of environmental chambers we notice the highest percentages of abnormal tillers (mainly owing to the abnormal leaf- and stem-development) after applications at the vegetative growth stages (Table 30, fig. 12). These observations are completely in agreement with data published by Jeater (1958). This research worker found that treatments of *Phleum pratense* and *Festuca pratensis* with about 1.5 kg of 2,4-D or MCPA caused the highest percentage of leaf abnormalities if they were carried out at the vegetative stage of the growing points. Jeater did not observe malformed leaves in *Lolium perenne* and *Dactylis glomerata*, but observations of the present author (not recorded in this publication) are not in agreement with these findings. Leaf abnormalities can also be observed with *Lolium perenne* and *Dactylis glomerata* after treatments with dosages of 2,4-D or MCPA similar to those applied by JEATER. Of course, when considering these divergencies in the eyperimental results the possibility of different levels of susceptibility in different strains has to be taken into account.

TABLE 31. Percentage of leaf and stem abnormalities at the stage of growth with which the highest percentage occurred

Grass	Growth stage	MCPA 1 kg	MCPA 2 kg	2,4-D 1 kg	2,4-D 2 kg	2,4,5-TP 1 kg	2,4,5-TP 2 kg
Poa trivialis	3 V.P. (March)	22.5	30.0	50.8	61.8		
Poa pratensis	2 V.P. (Oct.)			5.8	4.2		
	2 V.P. (Nov.)			5.8	5.0		
Festuca pratensis	2 V.P. (Oct.)	5.8	16.7	14.1	19.2	0	0
(April sown)	2–3 V.P. (Nov.)	7.5	5.8	15.8	18.3	0	0.8
	3 V.P. (March)	0	0.8	2.5	1.7	0	0
(July sown)	2–3 V.P. (Oct.)	11.7	15.8	20.8	25.8	0	0
	2–3 V.P. (Nov.)	2.5	5.0	14.2	18.3	0.8	0
	3 V.P. (March)	0	0	0	2.5	0	0.8
Festuca rubra	1–2 V.P. (Oct.)			12.5	17.5		
	2–3 V.P. (Nov.)			18.3	11.7		

It can also be concluded from our data that at the specific vegetative primordial growth stages mentioned in TABLE 5, 10, 15 and 19 tillers do not react the same way at different times. In late summer or early autumn the percentage of leaf abnormalities caused is quite low or almost nil, whereas at the same growth stage in winter the percentage is highest. This difference may be explained in two ways. As our system to assess the growth stage only gives an average indication, it is quite possible that during the period in which a specific growth stage occurs differences in the physiological nature of the growing points, determining their susceptibility to auxin herbicides, are not expressed in their outside morphology and consequently are not recognized. Hence the difference in effect between treatments at similar growth stages.

On the other hand, our experiments in environmental chambers indicate that different climatic conditions at the moments of treatments may also account for the difference in behaviour. With constantly low temperature conditions (10 °C) prior to and after spraying *Poa annua* and *Lolium multiflorum westerwoldicum* showed higher percentage of abnormal tillers than with 15° and 20 °C. Also a drop in the temperature after a treatment caused a higher percentage of abnormal tillers. Raised temperatures after treatment, however, decreased the frequency of malformations. Although climatic conditions in the field in autumn are not completely comparable to those maintained in the environmental chambers, these data may give an indication on the effect of temperature on leaf damage.

From the grasses investigated *Poa pratensis* proved to be the least, *Festuca* species intermediately and *Poa trivialis* the most susceptible to auxin herbicides. It is difficult to relate these diverging degrees of susceptibility to any basic difference in the grasses. It is hard to imagine that the stoloniferous growth habit of *Poa trivialis* and consequently possibly a closer contact between the spraying liquid and the susceptible growing points should be the only factor involved in causing the differences with the rhizomatous *Poa pratensis* and *Festuca* species. Other data on the relation between the growth habit of grasses and the effect of auxin herbicides are not available.

JEATER (1958) concluded that grasses with convolute leaves (like *Phleum pratense* and *Festuca pratensis)* more readily produced abnormal leaf growth than grasses with conduplicate leaves (like *Dactylis glomerata, Lolium perenne)*. As to leaf structure *Poa* species belong to the conduplicate type, but the great susceptibility of *Poa trivialis* compared to e.g. *Festuca pratensis* clearly shows that, if at all, the leaf structure is certainly not the only factor involved in determining the susceptibility of the plant to leaf malformations.

The uneven distribution of the spraying liquid over the plants treated („selective wetting") and differences in the rate of penetration and translocation of the active ingredients into different plants may explain the divergent susceptibility of plant species to the products (ROBBINS, CRAFTS and RAYNOR, 1952). A discussion on the importance of these factors to explain the differences mentioned in TABLE 31, and also on possible differences at the cellular level concerning the effect of herbicides cannot be given owing to absence of research data. Such differences, therefore, are presumed to be of importance only for the explanation of the different levels of susceptibility observed.

A striking difference between *Poa trivialis* and other grasses is that the moment at which the highest percentage of abnormalities is observed in *Poa trivialis* comes after winter, while with other grasses mentioned in TABLE 31, this moment occurs in late autumn.

Although no data on leaf and stem abnormalities are available for *Lolium perenne*, it is presumed to react in the same way as *Poa trivialis*. Anticipating the discussion on inflorescence abnormalities, this supposition has been justified by the observation that the highest percentage of structural abnormalities in the inflorescence with *Lolium perenne* and *Poa trivialis* is caused by spraying after the winter season, whereas in all other grasses studied this moment occurs in late autumn. In view of the close correlation between the frequency of leaf and stem abnormality percentages and inflorescence abnormality percentages in all grasses, *Lolium perenne* is assumed to show the maximum leaf and stem abnormalities after post-winter spraying as well. Thus we come to two distinctly different groups of grass species, one of which shows its highest susceptibility to auxin herbicides in late autumn, while the other shows the same characteristic shortly after winter.

The late sowing date of *Lolium perenne* and *Poa trivialis* probably is not the cause of the divergent reaction of these grasses. *Festuca pratensis* can be sown later than *Dactylis glomerata*, *Festuca rubra* and *Poa pratensis*, but whether it is sown early in spring or in summer the moment of its highest susceptibility to auxin herbicides still occurs in the same period, viz. in late autumn.

It is questionable as to how far the number of leaves influenced is an indication of the residual effect of the herbicide treatments. From several investigations it is known that the morphological effects of herbicides are temporary and may be succeeded by the formation of quite normal leaves (GIFFORD, 1953).

Although no data can be furnished as to possible residual presence of morphologically active quantities of auxin herbicides either in the plant or in the soil, it is not likely that this residual effect is of much importance. It is to be expected that it causes the same trend in all species and the different behaviour of *Lolium perenne* and *Poa trivialis* does not agree with the expectation.

Owing to a factor, which could be called 'dilution', an increased percentage of abnormalities could occur in due time. It is well known that treatments of grass plants with auxin herbicides do not prevent any further tillering. Our experiments with *Lolium multiflorum westerwoldicum* and *Poa annua* clearly show that due to the treatment tillering may even have been more intensive than in the control plants. Consequently, it may be expected that after treatments in the field, plants will add new tillers that are not or only slightly affected, to those that have been already affected by herbicides. Later on these tillers too produce inflorescences. In this way the contribution of malformed tillers to the total number of shooting tillers is decreased. This 'dilution'-effect will only take place so long as the new tillers can be induced to flower. Later in the discussion it will be seen that for flower induction in many grass species periods of cold temperature often combined with critical day-length are a prerequisite many times. In general, it may be said that the cold temperature requirement is not usually met with anymore after winter and tillers formed in early spring will no longer contribute to the inflorescences produced in the same year.

From this it may be concluded that the increase in percentage due to decreased „dilution" will continue until the moment that the physiological requirement for induction of the reproductive stage is no longer met, i.e. after winter. This is in agreement with the maximum abnormality percentage observed in *Poa trivialis* and (as we presume) in *Lolium perenne*. This is not the case in the other grasses investigated, in which highest percentages of leaf and stem abnormalities have been found in late autumn. The explanation of this difference has to be found in the response of the groups of grasses to growing conditions in autumn and winter.

As the divergent behaviour of the two groups occurs during the autumn and winter months, data on the morphological development of grasses during this period could be helpful in the explanation of the difference. The low temperature and day-light conditions prevailing during these months will certainly be reflected in the vegetative shoot and root growth and in the intensity of tillering of the grasses. On these points the author was not able to trace sufficient information concerning the individual grasses, in order to establish possible differences between them. The experiments with *Lolium multiflorum westerwoldicum* and *Poa annua* show, that as to dry matter production these grasses differ in their response to temperature. The low temperatures included in the experiments did not involve real cold temperatures comparable to those observed in the field in autumn. However, it is important to notice that dry matter production is closely related to tiller formation and consequently we may expect that grasses continuing growth at low temperature conditions will also develop more tillers.

There are indications that in different grasses formation of new tillers is specifically related to the number of expanded leaves present at the parent shoot (WHYTE, MOIR and COOPER, 1959). Accordingly the intensity of tillering is strongly determined by the growing conditions. Not many data can be found on this intensity under low temperature conditions, but for *Festuca pratensis, Lolium perenne* and *Poa trivialis* it is assumed that in late autumn and early winter tiller formation continues [*Festuca pratensis* (LANGER, 1956), *Lolium perenne* and *Poa trivialis* (SONNEVELD, personal communication)].

SPRAGUE (1943) who studied the top-root growth ratios, did not include grasses from our different groups. There are, however, some data on the influence of temperature on root formation with different grasses. According to STUCKEY (1941) *Poa trivialis* and *Lolium perenne* demonstrate during late autumn a considerably higher rate of root formation than *Poa pratensis*, *Dactylis glomerata* and some other species.

Although it may not be concluded from these studies that in top growth the two groups of species behave differently as well, the demonstration of growth at rather low temperatures is in agreement with the assumed continued tillering of *Poa trivialis* and *Lolium perenne* in late autumn.

Although it can not be concluded from the foregoing paragraphs, it is quite possible that under temperature conditions prevailing during autumn and early winter *Poa trivialis* and *Lolium perenne* continue tiller formation while other grasses stop growth.

From the observations of BOMMER (1959) and our data mentioned in TABLE 32 it appears that in different grasses the reproductive stage in the development of the growing point is observed at different moments. This moment is related to specific day-lengths in spring time, to which these grasses respond by inflorescence development. From TABLE 32 it appears that our data correspond reasonably well to those of BOMMER. The apparent anomaly observed between BOMMER's data and ours in *Dactylis glomerata* may be simply a varietal question.

TABLE 32. Initiation of the reproductive stage in grasses in relation to day-length in spring

Grass species	Period of initiation according to BOMMER (1959)	No initiation of repr. stage observed in own experiments until:
Poa pratensis	January–mid February	4 Febr.
Festuca rubra	Mid February–Mid March	(4 Febr. 50% V.P.) Not observed in January
Festuca pratensis	Mid March–Mid April	4 March
Dactylis glomerata	Mid February–Mid March	19 March
Lolium perenne (early varieties)	Mid March–Mid April	26 March (1958)–3 April (1959)
Poa trivialis	—	19 March (1958)–4 March (1959)

All grasses under study have specific short day and cold temperature requirements for flower initiation (c.f. BOMMER, 1959). It can be stated that all tillers formed upto the moment of specific day-length response in spring will form inflorescences provided their cold temperature requirement has been met. In *Poa trivialis* and *Lolium perenne* the spraying of auxin herbicides in March will result in a high percentage of abnormal tillers (leaves), because the day-length requirement has not been fulfilled yet. Although the tillers are vernalized, according to the outside morphology of the growing points they are still in the vegetative stage. With *Poa pratensis* and *Festuca rubra* day-length requirement in early spring is sooner fulfilled and apparently our treatments occurred after the moment of initiation of inflorescences.

Dactylis glomerata and *Festuca pratensis* take an intermediate position between the two groups mentioned in the preceding paragraph, regarding their moments of flower initiation. There are no data on the behaviour of *Dactylis glomerata* and further research on this species is needed in order to establish its correct place.

With *Festuca pratensis* the presumed continued tiller formation during late autumn and winter and the cold temperature requirement permit to predict a rather high abnormality percentage after winter, until the day-length requirement has been fulfilled. Still we notice highest percentage of leaf abnormalities in pre-winter applications. With our present limited knowledge of this grass species and of the different behaviour of different strains the susceptibility picture of *Festuca pratensis* is hard to understand.

SLAATS and STRYCKERS (1955) reported on the susceptibility of *Phleum pratense* to 2,4-D and MCPA. They found a highest abnormality percentage with applications in October. This observation does not confirm the conclusion drawn from the observations on *Poa trivialis* and *Lolium perenne* that the highest percentage of leaf abnormalities could be observed with post-winter sprayings with grasses showing their day-length response late in spring. The explanation of this behaviour of *Phleum pratense* could be that inflorescence initiation in this grass does not occur after cold winter temperature but after exposure to long day (c.f. BOMMER, 1959). Spraying in October influences a number of shoots from which part has already been initiated to flower early in autumn. The remainder of the shoots will change to the reproductive stage late in spring with a favourable day-length. Due to this phenomenon the figures mentioned do not refer to material similar to our experimental grasses and consequently the results are hard to compare.

A comparison of the effects caused by different auxin herbicides used in this study shows that in field experiments 2,4-D has caused a higher percentage of abnormalities than MCPA. This is in general agreement with the fact that most plants respond more strongly to 2,4-D than to MCPA (AUDUS, 1959), although in our case a direct comparison between the two chemicals is not allowed, because different salts of the acids were used. The malformations caused by the two chemicals were alike and from these it may be concluded that they have a similar physiological activity.

In literature not much attention has been paid to the striking differences in the action of the phenoxyacetic acids and the phenoxypropionic acids. In our studies 2,4,5-TP did not cause any onion-leaf abnormality, although an ester form of this chemical was used, which is commonly accepted to be far more phytomorphologically effective than the salt form (WOODFORD, HOLLY and MCGREADY, 1958). From other personal observations it can be concluded that 2,4,5-TP is either not so actively translocated to the growing points as the phenoxyacetic acids, or is far less active at the cellular level in causing physiological disturbances. The chemical seems to influence mainly those parts of plants with which it comes into almost immediate contact and being biochemically susceptible to it.

4.3. INFLUENCE OF AUXIN HERBICIDES ON INFLORESCENCE STRUCTURE

From the tables on inflorescence abnormalities it is apparent that in all cases the stage of development at which highest susceptibility to 2,4-D and MCPA occurs, corresponds to the stage of highest susceptibility with regard to leaf malformations. The correlation between the degree of occurrence of the two types of malformations is also expressed by the simultaneous low figures for the inflorescence abnormalities caused by 2,4,5-TP. Only in case of *Lolium perenne* this correlation has not been observed, to which we will revert later on.

Many inflorescence abnormalities occur owing to the fact that the emerging inflorescence, when expanding has to pierce the malformed leaves of the shoot. However, also in the structure proper of the inflorescence, abnormalities were observed, e.g. irregularly alternating spikelets or bunching of spikelets, although at the moment of spraying no initiation of the reproductive stage of development could be observed with the microscope in the growing points studied. The microscopical observations made, do not allow to determine whether a protuberance on the growing points in its earliest recognizable stages will finally develop into a leaf or whether after initiation of the reproductive stage it will develop into a vegetative part of the inflorescence. This situation occurs particularly in the stage of development called 'Streckungsstadium' by BOMMER (1959). If plants are treated at this growth stage, later on inflorescence abnormalities are to be expected if the development of the axillary tissues of the initiated leaves into parts of the inflorescence already proceeded, although not to a degree recognizable in the outside morphology of the growing point.

Apart from causing flower sterility at and after the reproductive stage the influence of 2,4-D and MCPA on the structure of the inflorescence is very slight. Apparently, the structural parts of the inflorescences at this stage are too far developed for the chemicals at the concentrations studied to cause great morphological disturbances.

As to different grasses, the highest susceptibility was observed in *Lolium perenne* and *Poa trivialis*, *Festuca pratensis* was intermediate and *Festuca rubra* and *Poa pratensis* were the most resistant. Here again we observe the same sequence as found with the susceptibility of grasses to leaf malformation. As indicated previously, abnormal leaf structures have a great influence on the expansion of the inflorescence and there being a close correlation between the two types of abnormalities is not surprising.

Our results are in agreement with those of JEATER (1958). In his experiments JEATER observed a considerable number of abnormal inflorescences in non-treated plants of *Lolium perenne* and some in *Poa pratensis*. These teratological abnormalities were also found in our experiments.

In our tables on inflorescence abnormalities no distinction has been made between abnormalities resulting from malformations in the leaves and stems and abnormalities in the inflorescence structure proper. Therefore, it can not be concluded at what time of the year plants are the most susceptible with regard to the latter type of abnormality. It can be presumed, however, that in all grasses studied this moment will occur after winter, when the initiation of the reproductive stage has taken place.

The degree of susceptibility indicated in the previous paragraphs seems to be inversely correlated with the earliness of flower initiation as indicated in TABLE 31. In *Poa pratensis* and *Festuca rubra* both demonstrating a high degree of resistance, flower initiation occurs rather early while in *Lolium perenne*, being the most susceptible grass species investigated, flower initiation is very late. The longer the period of time is between the fulfillment of cold requirement and day-length requirement, the greater the abnormality percentage in the inflorescences will be if the plants are sprayed at the late vegetative stage.

Apart from differences in the physiological resistance of the different species to a specific auxin herbicide, this continued development of tiller apices after the fulfillment of cold requirement will lead to a more uniform mass of tillers. Consequently grasses with an early fulfillment of daylength requirement may appear to be more resistant to auxin treatments than species like *Lolium perenne*.

Some irregular results in our tables and observations on field plots point out the importance of a closer study of the influence of weather conditions on the intensity of malformations caused by auxin herbicides. The impression has been gained that cool temperatures after spraying may intensify the reaction of the grass plants.

The experiments in the environmental chambers with *Lolium multiflorum westerwoldicum* and *Poa annua* demonstrate that if cooler temperatures follow treatment the percentage of affected tillers increases in relation to the controls. A rise in temperature after treatments at different growth stages generally decreases the percentage of affected tillers. The speed at which a population of shoots is passing a specific susceptible growing stage apparently influences its level of susceptibility to auxin herbicides. We have to admit, however, that the influence of temperature on the persistence of the chemical in the plant and the soil cannot be completely excluded from the explanation of the observation and may also be of importance. In the lower temperature range this persistence and consequently physiologically dangerous presence of the herbicide will be greater than at a higher temperature.

In his studies with rice KAUFMAN (1953) subjected his plants to high temperatures after treatment in order to stimulate malformation. Our data on *Lolium multiflorum westerwoldicum* and *Poa annua* show that these grasses behave differently. FIG. 13 giving the effects of temporary changes in temperatures indicate that as to number of affected tillers produced the 10 °C environment is rather detrimental to both grasses.

In our experiments drops to freezing temperatures were not included. These may occur in the field, however, in late winter and early spring due to night frosts. The effects of these temporary temperature changes on the susceptibility of the grass require investigation in all crops concerned.

The diverging behaviour of 2,4,5-TP in relation to MCPA and 2,4-D is apparent in *Festuca rubra, Lolium perenne* and *Poa trivialis*. At the stage Floral or at the very early Emergence of Inflorescence stages 2,4,5-TP causes a relatively high percentage of abnormalities while the action of the other chemicals is hardly noticed at these moments. In *Poa pratensis* and *Festuca pratensis* also 2,4,5-TP hardly had any effect at these stages on the morphology of the inflorescence.

According to expectations all inflorescence abnormalities observed are a direct result from the influence of 2,4,5-TP on the inflorescence structure. Leaf abnormalities in these stages were not observed.

No explanation can be given of the levels of susceptibility observed. No grouping of grasses is possible here and inherent differences in the physiological nature of grasses must contribute to the differences in reaction.

4.4. INFLUENCE ON SEED YIELD

From the outset it must be stated that field experiments in grass-seed crops are not easily carried out and that due to several influences differences between small plots must be rather great in order to make them statistically reliable.

As was mentioned at the beginning of the discussion, in many grass-seed crops data on the influence of auxin herbicides are scanty in the Netherlands. Moreover, not all stages of growth in all crops have been included in the experiments. From all grasses only data on *Festuca rubra, Poa pratensis* and *Poa trivialis* are sufficient in quantity to allow generalizations.

In the preceding discussion on leaf, stem and inflorescence abnormalities, as to the degree and to the moment of occurrence of susceptible stages during the development of the grass plants, differences were observed between the grasses studied. It is of interest to relate these observations with the seed production of treated grasses.

From the TABLES on yield 7, 12, 17, 22 and 26 it can be concluded that in several cases the optimal negative influence on seed yield is not related to optimal effects on leaves and inflorescence structure.

In autumn 2,4,5-TP definitely does not influence the development and seed formation of well established plants. The effects of 2,4-D and MCPA cannot be ascertained with certainty but the few data available point to a slightly depressing action.

In *Poa trivialis*, sown in autumn, a yield depression was observed at very early moments of spraying, which was certainly due to a killing effect of the chemicals on the young seedlings.

During the development after winter until shooting *Festuca rubra* seems to be more resistant to 2,4-D and MCPA than the *Poa* species. This could be due to an intrinsic higher resistance of *Festuca rubra* to auxin herbicides.

The susceptibility of the *Poa* species to 2,4-D and MCPA during the early stages of regrowth is such that any of the chemicals investigated, but especially 2,4,5-TP may cause yield decreases.

During the entire process of inflorescence emergence and flowering the three grasses have in common a more or less pronounced decrease in seed yield due to applications of 2,4-D, MCPA and 2,4,5-TP. Although no data are available as to the influence of the treatments on the total number of ear bearing tillers, according to field observations the shooting of individual tillers may have been affected. In case of 2,4,5-TP, this effect may have been even to such an extent that some emerging inflorescences will not have participated in the total number of seed producing tillers finally harvested.

Our field data, however, do not furnish material to seperate this detrimental effect of the auxin herbicides on the total number of seed producing inflorescences from the effect on the seed production of the individual inflorescences. The relation may be rather complex, however. The experiments in environmental chambers indicate that treatment of plants with 2,4-D at developmental stages just before shooting may decrease the number of tillers formed. If, however, after treatment temperature rises occur, the depressing effect of the temperature change on the number of tillers and the stimulation of shooting is counteracted by the herbicide. The data show that under normal conditions during the process of shooting part of the tillers succumb, probably due to the intensive growth of the most strongly developing inflorescences. Auxin herbicides, in retarding this process influence the number of inflorescences finally produced and consequently also the yield.

Further research is required to analyse the influence of auxin herbicides on the seed production by individual plants and tillers.

The data on *Festuca pratensis* do not permit a well-founded conclusion to be made on the susceptibility of this species to auxin herbicides, but figures mentioned in TABLE 17 are in agreement with the conclusion drawn from *Poa pratensis* and *trivialis*, and *Festuca rubra*.

The single experiment on resistance of *Lolium perenne* to autumn applications of 2,4,5-TP shows a rather great resistance. From these observations and from other data presented we may conclude that from the auxin herbicides investigated influence of 2,4,5-TP is the least in relation to seed production of grass species treated in the vegetative growth stage when the plants are well established.

4.5. INFLUENCE ON THOUSAND-KERNEL WEIGHT AND GERMINATION PERCENTAGE

Yield reductions by the auxin herbicides generally were not accompanied by statistically reliable influences on the 1000-kernel weight, although many times the tendency to an increase could be noticed. The data obtained indicate, that if yield reductions occurred they must have been mainly caused by reductions in the number of kernels. This can take place either by a reduction in the number of ear-bearing tillers or by a partial flower sterility. From the discussion on seed yield it is apparent that our data are not sufficient to obtain a further insight into this complex relationship. A general remark may be made; assuming that the photosynthetically active leaf area of grass plants is not influenced by the auxin herbicides, slight increases in 1000-kernel weight can always be expected if sterility of flowers occurs.

From the data collected the influence of auxin herbicides on germination percentage is very hard to be assessed. Stages just before shooting and emergence of inflorescence frequently give decreased germination percentage or a tendency in this direction.

From the data on 1000-kernel weight we may conclude that the average seed weight is not greatly influenced by the treatments. This does not mean, however, that no influence on the germination percentage is to be expected. A mixture of seeds is composed of seeds of different sizes and weights and first of all more knowledge is needed concerning the influence of the germination percentage of these different fractions on the germination percentage of the mixture as a whole. With our data we cannot separate, however, a possible influence of an auxin herbicide on the physiological nature of the individual seeds from an influence on the composition of the seed mixture.

In our data there is a slight indication allowing to conclude that *Festuca rubra* and *Poa pratensis* are more resistant to the influences on their germination percentage than the other grasses investigated, in particular *Poa trivialis*. Data are too scarce, however, to support the conclusion that also in this connection the relation between the earliness of the grass and its susceptibility to herbicides is of importance.

4.6. COMPARISON WITH CEREALS

It has been realized before that the reaction of grasses to auxin herbicides is not fundamentally different from the reactions demonstrated by cereals (JEATER, 1958). This is not surprising, considering the rather great similarity in the major physiological and morphological processes within the Gramineae. It seems, however, that due to the high intensity of tillering in perennial grasses the reaction to herbicides is more complicated than that in the annual cereals.

The reaction of cereals to the applications of auxin herbicides has been studied in detail. Most attention has been paid to the formative effects and the influence on yield of 2,4-D and MCPA. 2,4,5-TP has not been included to a great extent in more fundamentally directed studies.

Also in cereals the stages of development in which flower initiation and development occur are known to be particularly susceptible to auxin herbicides. In the varieties of winter cereals sown in autumn, the conditions under which flower initiation occurs are very similar to those observed in the perennial grasses included in our experiments. There is a specific requirement for low temperatures and short days in order to introduce in the growing point at a longer day length (and consequently at a higher temperature) in spring the development of the reproductive growing stage. In spring cereals there is no requirement for low temperatures but optimum inflorescence development occurs at a length of day, specific for each variety.

The extensive body of data on the influence of 2,4-D and MCPA is well represented by the publications of BLACKMAN and ROBERTS (1950), LONGCHAMP *et al.* (1952a, b) and FRIESEN and OLSON (1953). In spring barley FRIESEN and OLSON found highest formation of abnormal leaves at very early growth stages (1–2 leaves). At this moment the plants are not sufficiently developed yet to reflect in the growing point the effect of day length. Strongest effects on the inflorescence are caused a little later (2–3 leaf stage) and still further in the development a rather resistant period occurs in which no formative effects can be observed. Flower sterility may occur due to treatments in stages of development indicated in our studies as Floral and Em. Infl.

The reaction of tillers is not different from the reaction of the main shoot. When the stage of development of the main shoot is taken as the reference scale for time of application of the chemical, the point of highest susceptibility of the tillers is at a little later stage and also of a smaller intensity than that of the main shoot. This observation is of importance for the susceptibility of grasses to treatments at similar growth stages. In grasses the population of tillers is much greater and probably also less uniform than in cereals. If we take as a measure of the development of the growth stage of the plants the average development of the growing point of several of the most advanced tillers, we may expect in grasses a less pronounced susceptibility than in cereals. Every influence on the grass plants that will tend to make the stage of development of the individual tillers more uniform will increase the percentage of abnormalities caused by applications of auxin herbicides. This could also be the explanation of the trend in susceptibility percentages observed in grasses in relation to the moment of flower initiation. In *Lolium perenne* and *Poa trivialis* individual tillers have more time to become well established and uniform than in *Poa pratensis* and *Festuca rubra*.

Data of BLACKMAN and ROBERTS (1950) are confirmed by observations made by FRIESEN and OLSON (1953). The observed kill of seedlings after applications in a very early stage of germination is in agreement with the high susceptibility of *Poa trivialis* observed in our own experiments.

Like observed differences between various grass species different species of cereals vary in their susceptibility to auxin herbicides (cf. AUDUS, 1959). Oats is the most susceptible to herbicide treatment. Wheat is highly resistant in comparison to oats and barley is intermediate in its reaction. It is also known that susceptibility of oats is due to its slow development between the stages of ear initiation and of inflorescence emergence.

LONGCHAMP *et al.* (1952a, b) studied the behaviour of a great number of winter wheat varieties and the general picture of susceptibility is not different from the one presented by other authors. Varietal differences in susceptibility have been observed in oats (DERSCHEID *et al.*, 1953) and barley (OLSON *et al.*, 1951) and in spring wheat (BLACKMAN, 1950). Differences between the varieties as to uptake, translocation and activity at the cellular level of 2,4-D and MCPA are supposed to be the principal causes of divergence in susceptibility. No special study has been made, however, of the relation of the earliness of the varieties of winter cereals as to reproductive development and shooting behaviour. Consequently no comparison is possible with our data on grasses.

SUMMARY AND CONCLUSIONS

The very rapid development of the grass-seed production in the Netherlands stimulated the undertaking of the research work discussed in this publication. It was necessary to gain an insight into the resistance of grass-seed crops against weed control chemicals within a short period. The investigations undertaken with weed control chemicals have been laid out in farmer's plot in collaboration with the Research and Advisory Institute for Field Crops and Grassland Husbandry (P.A.W.) and the Institute for Biological and Chemical Research on Field Crops and Herbage (I.B.S.), Wageningen.

To achieve the above mentioned purpose of gaining a better insight into the problem, the testing of chemicals which were already in use or promising, i.e. MCPA, 2,4-D and 2,4,5-TP, has been of primary importance. However, dosages, application conditions and times of application have been chosen which generally have not been used in practice. A number of environmental chamber experiments were undertaken at the I.B.S. in order to determine the influence of weather conditions on the reaction of grasses to auxin herbicides.

For the investigation, it was nesessary to determine the stage of development of the grass-plant at the moment of applying the auxin herbicides. For this purpose it was most useful to follow the method used in the investigations on cereals and grass-seed crops by JEATER, who studied the morphological development of the growing point of the treated sprouts. To use this method in the investigation on different grasses at the same time, it was necessary to follow a rather general classification, which took into account the differences between the grasses for the determination of the different stages of development. The methods of sampling the crop and the determination of the stage of development as described in Chapter 2, gave a fully satisfactory basis for the investigation. The morphological method, however, does not give sufficient insight for the stage of development of the growing point during the first phases of passing over from the vegetative to the reproductive stage.

As it has been put forward in other publications, also in our investigations it appeared that the reaction of grasses on the investigated chemicals in principle does not differ from the reaction of cereals.

In the vegetative stages of development of the growing point of individual sprouts 2,4-D and MCPA can cause malformations in the initiated leaves with both groups of grasses. 2,4,5-TP differs in this respect from the other chemicals mentioned by not causing any leaf and stem abnormalities during these stages of development. Malformations of leaf and stem which have been caused during the vegetative growing stage will only show up when the plants start tillering.

Because of the strong tillering, however, the reaction of grasses on auxin herbicides is more complicated than that of cereals. The effect of sprayings on the plants is primarily assessed by the number of influenced sprouts in the harvested haulms. On this basis the time of the highest susceptibility of the grasses under study is not the same. In *Poa pratensis* and *Festuca rubra*, normally sown in spring, it occurs in the months October–November, while in *Poa trivialis* and *Lolium perenne*, mostly sown during the period between July and October, the highest susceptibility occurs in early spring. *Festuca pratensis* and *Dactylis glomerata* take up an intermediate position, which is not fully understood and needs further study.

The reasons of the differences may be found in the intensity of tillering of the mentioned grasses under cold weather conditions. With the first two grasses this should be less intensive in the late autumn and winter than with *Poa trivialis* and *Lolium perenne*. As a result, there is less dilution in the number of affected tillers with *Poa pratensis* and *Festuca rubra* owing to sprouts developing after treatment. On the other hand, the differences in resistance are connected with the observed differences in day-length requirement for the ear initiation of the grasses mentioned. All mentioned grasses require a period of cold and/or short days during their vegetative stage in order to change into the stage of ear initiation with a longer daylength, but in *Poa pratensis* and *Festuca rubra* this long-day requirement in spring is fulfilled earlier than in *Poa trivialis* and *Lolium perenne*. Before this moment occurs, the last two grasses can still be influenced in their leaf development in early spring.

The intensity of leaf abnormalities is more or less correlated with this earliness of ear initiation so that *Poa pratensis* and *Festuca rubra* showed much more resistance than *Poa trivialis* and *Lolium perenne*. It is, therefore, suggested that the more time available between the moment at which the winter requirement of the plant is fulfilled and the moment at which the day-length requirement of the plant is fulfilled owing to which it becomes reproductive, the more susceptible the plant will be to auxin herbicides in relation to the abnormal development of leaf and stem, when applied during the late vegetative stage.

In MCPA and 2,4-D abnormalities during inflorescence emergence are strongly connected with the intensity of leaf malformations, because the emergence of malformed leaves leads to structural abnormalities in the inflorescence. Influence on the actual morphology of the inflorescence in our investigation could not be separated from the indirect structural changes due to leaf abnormalities. It is to be expected here that in different grasses the same differences in resistance can be observed as in the leaf abnormalities, but the time of the highest susceptibility in relation to the latter will occur in spring only.

A comparison with 2,4-D and MCPA shows that 2,4,5-TP has a strong disturbing influence on the structure of the inflorescence mostly in the more developed growth stages during shooting and inflorescence emergence.

A comparison of our results with the yield experiments of P.A.W. and I.B.S. showed that, except for 2,4,5-TP, the moments of the highest susceptibility of leaf and stem growth and of structure of the inflorescence cannot be correlated with the moments of the highest influence on seed yield. Generally, it can be said from all mentioned grasses that from the moment when the young plants have reached a certain development (3–4 tillers) in autumn the mentioned weed-control chemicals only have little or no influence at all on the seed yield. This certainly is the case for 2,4,5-TP which does not cause any yield reduction after treatment in autumn with the normal practical dosage for weed control (0.6–1 kg a.e./ha).

Though the number of yield observations is small it can be concluded that all mentioned chemicals, when used after winter, can cause yield depression in all mentioned grasses. In view of the small number of experiments and the fact that no studies on resistance of different strains or selections of the mentioned grass species against auxin herbicides have been carried out, it must be said that the use of these products in spring is risky.

The influence of the chemicals on 1000-kernel weight is not significant. With yield depression mostly a tendency to higher weight was observed.

The germination percentage was badly influenced only after treatment in the late developmental stages of the inflorescence. Though the observations done are not completely significant, the impression was gained that also in respect to the germination percentage those grasses which change early to inflorescence initiation are less susceptible than *Lolium perenne* and *Poa trivialis.*

Greenhouse experiments with *Poa annua* and *Lolium multiflorum westerwoldicum* lead to the conclusion that a drop in temperature after treatment with 2,4-D causes a higher number of affected haulms.

The investigation has shown that in future when using the mentioned auxin herbicides for plants not yet investigated, an insight into the resistance can already be expected by studying the conditions under which these grasses change into ear initiation. Besides that insight into the process of the tillering of varieties can be of help. It is unfortunate, however, there are only few fundamental results available on the mentioned factors. It is known that within grass varieties there are great differences between certain strains or selections with respect to the morphological reaction of the plants on soil and climate factors. In connection with that the finding of correlations with the susceptibility for herbicides will entail time consuming studies.

SAMENVATTING EN CONCLUSIES

ONDERZOEKINGEN OVER DE INVLOED VAN ENKELE HERBICIDE GROEISTOFFEN OP VOOR DE ZAADWINNING GETEELDE GRASSEN

De urgentie van de in deze publikatie besproken onderzoekingen is voortgekomen uit de zeer snelle uitbreiding van de graszaadteelt in Nederland gedurende de laatste jaren. De noodzaak bestond om op korte termijn een inzicht te krijgen in de resistentie tegen onkruidbestrijdingsmiddelen bij verschillende voor het winnen van zaad als landbouwgewas geteelde grassen. Bij het verrichte onderzoek kon worden uitgegaan van de door het Proefstation voor de Akker- en Weidebouw en het Instituut voor Biologisch en Scheikundig Onderzoek van Landbouwgewassen aangelegde onkruidbestrijdingsproefvelden op praktijkpercelen.

Het eigen onderzoek kreeg primair als taak enkele al in de praktijk gangbare of veelbelovende middelen, nl. MCPA, 2,4-D en 2,4,5-TP, verder te beproeven en hierbij eventueel uit te gaan van doseringen, toepassingsomstandigheden en tijdstippen welke in de praktijk niet gebruikelijk zijn. Bij een aantal proeven over de invloed van weersomstandigheden op de reactie van grassen op groeistoffen kon daarbij worden gebruik gemaakt van de klimaatkamers en geconditioneerde kassen van het I.B.S.

Voor het onderzoek was het noodzakelijk de ontwikkelingstoestand van de grasplanten op het moment van toepassing van de onkruidbestrijdingsmiddelen nauwkeurig vast te leggen. Hierbij bleek het meest doeltreffend de voor het onderzoek in granen en ook door JEATER reeds bij het onderzoek in de graszaadteelt geïntroduceerde bestudering van de morfologische ontwikkeling van het groeipunt van de behandelde spruiten. Om deze methode bij het onderzoek van verschillende grassen tegelijkertijd te kunnen gebruiken was het noodzakelijk bij het vaststellen van de verschillende ontwikkelingsstadia een vrij ruwe indeling te volgen, welke rekening hield met de tussen de grassen bestaande verschillen. De voor de bemonstering van het gewas en vaststelling van de ontwikkelingstoestand gevolgde, in hoofdstuk 2 omschreven methode, gaf voor het onderzoek een voldoend bevredigende basis. Over de ontwikkelingstoestand van het groeipunt tijdens de eerste fasen van de overgang van het vegetatieve naar het reproduktieve stadium blijkt de morfologische methode echter geen voldoende inzicht te geven.

In overeenstemming met hetgeen in andere publikaties al duidelijk naar voren is gekomen, blijkt ook uit onze proefnemingen de reactie van grassen op de onderzochte stoffen in principe niet te verschillen van die bij granen.

Tijdens de vegetatieve ontwikkelingsstadia van het groeipunt van de individuele spruiten blijken bij beide groepen van gewassen door de inwerking van 2,4-D en MCPA misvormingen in de bladaanleg op te kunnen treden. 2,4,5-TP verschilt in dit opzicht van de genoemde middelen, door tijdens deze ontwikkelingsstadia geen blad- en stengelmisvormingen te veroorzaken. Misvormingen in blad en stengel veroorzaakt tijdens het vegetatieve groeistadium uiten zich pas als de spruiten gaan schieten.

Door de sterke uitstoeling van de grassen is de reactie van deze gewassen op de groeistoffen echter gecompliceerder dan die van granen. Het effect van de bespuitingen op het gewas wordt primair bepaald door het aandeel van de beïnvloede spruiten in het uiteindelijk bij de oogst van het gewas aanwezige aantal halmen. Indien dit als vergelijkingsbasis wordt genomen, blijkt het tijdstip van sterkste beïnvloedbaarheid bij de onderzochte grassen niet gelijk te liggen. Het treedt bij de gewoonlijk in het voorjaar gezaaide *Poa pratensis* en *Festuca rubra* op in de maanden oktober–november, terwijl bij *Poa trivialis* en *Lolium perenne*, die meest in de periode tussen juli en oktober uitgezaaid worden, de hoogste gevoeligheid in het vroege voorjaar optreedt. *Festuca pratensis* en *Dactylis glomerata* nemen een niet geheel verklaarde middenpositie in, welke nader onderzoek vraagt.

De verklaring van deze verschillen wordt enerzijds gezocht in de intensiteit van uitstoeling van de genoemde grassen onder koude weersomstandigheden. Deze zou bij de eerste twee grassen in de naherfst en winter minder intensief zijn dan bij *Poa trivialis* en *Lolium perenne*. Dientengevolge treedt bij *Poa pratensis* en *Festuca rubra* een minder sterke „verdunning" van het aantal door de groeistof beïnvloede spruiten op door de na het moment van toepassing aangelegde spruiten. Anderzijds worden de verschillen in resistentie in verband gebracht met de bij de genoemde grassen geconstateerde verschillen in daglengtebehoefte bij de ontwikkeling van de aaraanleg. Alle genoemde grassen hebben gedurende de vegetatieve toestand behoefte aan een periode van koude en/of korte dagen, om later bij een langere daglengte tot aaraanleg te kunnen overgaan, doch bij *Poa pratensis* en *Festuca rubra* is deze behoefte aan lange dag in het voorjaar eerder vervuld dan bij *Poa trivialis* en *Lolium perenne*.

Vóórdat dit moment aanwezig is zijn de laatste twee grassen in het vroege voorjaar dus nog in hun bladontwikkeling te beïnvloeden.

De intensiteit van bladmisvorming blijkt min of meer gecorreleerd te zijn met deze „vroegheid" van de aaraanleg, zodat *Poa pratensis* en *Festuca rubra* zich veel resistenter tonen dan *Poa trivialis* en *Lolium perenne*. Hierbij wordt de stelling geponeerd, dat hoe meer tijd er beschikbaar is tussen het moment waarop aan de koudebehoefte van het gewas voldaan is en het moment waarop de daglengtebehoefte van het gewas vervuld wordt en het daardoor reproduktief wordt, hoe gevoeliger het gewas zal blijken te zijn voor misvormingen in de blad- en stengelontwikkeling bij toepassing van groeistoffen tijdens het late vegetatieve stadium.

Bij MCPA en 2,4-D hangen afwijkingen in de bloeiwijze nauw samen met de intensiteit van bladmisvormingen, omdat het doorbreken van misvormde bladeren bij de bloeiwijzen tot structurele misvormingen leidt. Een eventuele invloed op de eigenlijke morfologie van de bloeiwijze was bij ons onderzoek niet te scheiden van de indirect door bladafwijkingen veroorzaakte structurele veranderingen. Verwacht wordt dat hier bij de verschillende grassen dezelfde verschillen in resistentie geconstateerd kunnen worden als bij de bladafwijkingen, doch dat de tijdstippen van hoogste gevoeligheid alle in het voorjaar zullen liggen.

Een vergelijking met 2,4-D en MCPA toont aan, dat 2,4,5-TP vooral in de gevorderde stadia van ontwikkeling tijdens het schieten van het gewas en het te voorschijn komen van de bloeiwijze een sterk storende invloed heeft op de structuur hiervan.

Een vergelijking van onze gegevens met de opbrengstproeven van het P.A.W. en het

I.B.S. wees uit dat, behalve voor 2,4,5-TP, de momenten van sterkste beïnvloeding van blad- en stengelgroei van de structuur van de bloeiwijze niet gecorreleerd zijn met de momenten waarop de sterkste invloed op de zaadopbrengst te constateren valt. Generaliserend kan gezegd worden, dat bij toepassing in de herfst bij alle onderzochte grassen vanaf het moment dat de jonge planten een zekere ontwikkeling bereikt hebben (3–4 zijspruiten bezitten) door de onderzochte middelen een betrekkelijk geringe tot geen invloed op de uiteindelijke zaadopbrengst uitgeoefend wordt. Dit is zeker het geval voor 2,4,5-TP, waarvan de voor de onkruidbestrijding in de herfst gebruikelijke praktijkdoseringen van 0,6–1 kg zuurequivalent per ha geen opbrengstverlagingen veroorzaken.

Het geringe aantal opbrengstgegevens rechtvaardigt toch de conclusie, dat alle onderzochte middelen in alle onderzochte grassen bij toepassingen na de winter opbrengstdalingen kunnen veroorzaken. Aangezien het aantal van de uitgevoerde proeven beperkt is en geen studie is gemaakt van de resistentie van verschillende rassen of selecties van de genoemde grassoorten tegen groeistoffen, moet in het huidige stadium van onderzoek het gebruik van deze produkten in het voorjaar als riskant beschouwd worden.

De invloed van de middelen op het duizend-korrelgewicht bleek niet betrouwbaar te zijn. Bij opbrengstverminderingen viel in het algemeen een tendens tot vermeerdering van het duizend-korrelgewicht waar te nemen.

De kiemkracht van de zaden bleek alleen bij toepassingen in de late ontwikkelingsstadia van de bloeiwijze nadelig te worden beïnvloed. Hoewel de beschikbare gegevens geen geheel betrouwbare conclusie toelaten, werd de indruk verkregen dat ook wat betreft de kiemkracht de vroeg tot bloeiaanleg overgaande grassen minder gevoelig zijn voor beïnvloeding dan *Lolium perenne* en *Poa trivialis*.

Kasproeven met *Poa annua* en *Lolium multiflorum westerwoldicum* leiden tot de conclusie, dat het optreden van temperatuursdalingen na toepassing van 2,4-D tot een verhoging van het uiteindelijk optredende aantal beïnvloede halmen leidt.

Het onderzoek heeft aangetoond dat in de toekomst bij de toepassing van de onderzochte herbiciden in nog niet bij het onderzoek betrokken grassen een bestudering van de omstandigheden, waaronder deze grassen tot aaraanleg overgaan al inzicht zal kunnen geven over de te verwachten resistentie. Daarnaast kan een inzicht in het uitstoelingsproces van de soorten verhelderend werken. Het is echter te betreuren, dat er over de genoemde punten betrekkelijk weinig fundamentele gegevens ter beschikking staan. Het is bekend, dat er binnen een grassoort op het gebied van de morfologische reactie van de planten op bodem- en klimaatsfactoren tussen bepaalde rassen of selecties aanzienlijke verschillen bestaan. Het opsporen van correlaties met de gevoeligheid voor herbiciden zal in verband hiermede tijdrovende studies vergen.

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