FACTORS AFFECTING THE PERFORMANCE OF FOLIAR-APPLIED BIOCIDES

BY
FRANCES ELIZABETH TAYLOR

ABSTRACT

In the field the susceptibility of Chenopodium album L. to the post emergence herbicide Basagran is dependent on the prevailing weather conditions. Study on the influence of environmental conditions in governing the plant's response to the herbicide, revealed that pre- and post-treatments of light, temperature and humidity greatly affect the susceptibility of C. album to Basagran.

Detailed studies, using T.L.C., G.L.C. and scanning electron microscopy, showed that the leaf surface of C. album is well endowed with wax platelets composed principally of long chained aldehydes. Differences in susceptibility of C. album observed as a result of changing environmental conditions could not be correlated with any change in the deposition, chemical composition, ultrastructure or wettability of the epicuticular wax.

The adaxial and abaxial leaf surface of C. album was, however, found to possess stomata bearing large antechambers which appeared virtually devoid of wax platelets. From in vitro studies these stomata were shown to open in the presence of Basagran in a light induced response over a temperature range of 9 - 29°C. The relevance of these findings is discussed in relation to the penetration of Basagran into C. album leaves.

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SUMMARY

1. The influence of the herbicide Basagran on *Chenopodium album* was investigated under glass, in the field and under controlled environmental conditions. Herbicide performance was found to be dependent on:-

   a) The concentration of the active ingredient, bentazone. Optimum concentration for the control of *C. album* under glass was 0.3 l/280 l H_2O/ha, whereas in the field 3 l/280 l H_2O/ha proved more reliable.

   b) the presence of additives, particularly the oil adjuvant, Actipron. Optimum concentration was found to be in the region of 0.5 % ($^{v}/_{v}$).

   c) the method of application. The fine, even spray as delivered by the Shandon Chromatography sprayer and the Binks Bullows "Spraybee", ensured optimum herbicide dispersal.

   d) plant size. The susceptibility of *C. album* was found to be inversely proportional to plant height.

   e) the time of day of application. The limited number of experiments conducted indicate a trend towards greater effectiveness of the herbicide when applied early morning or late afternoon.

   f) the prevailing environmental conditions. Detailed experiments under controlled environments revealed that the susceptibility of *C. album* was greatly dependent on pre- and post-treatment conditions, with greatest susceptibility observed following low pre- and high post-treatment conditions of temperature and light intensity. Greater susceptibility was observed under low relative humidity conditions, and preliminary experiments

have indicated that plants maintained under high soil moisture conditions are more susceptible than plants maintained under water stress.

2. The differences in susceptibility observed as a result of environment could not be correlated with any change in the deposition, chemical composition or, indeed, ultrastructure of the epicuticular wax of C. album.

3. The epicuticular wax of C. album is composed principally of long-chain aldehydes and alcohols, which form a dense covering over both leaf surfaces.

4. Contact angles of 128° and 142° on adaxial and abaxial leaf surfaces respectively, indicate that this wax is an effective barrier to the foliar penetration of polar molecules such as Basagran.

5. Although the epicuticular wax forms a dense covering on both adaxial and abaxial leaf surfaces, the area in the vicinity of the stomata is less dense, and the stomatal antechamber appears virtually devoid of wax platelets.

6. In vitro studies have indicated that Basagran induces rapid stomatal opening in isolated epidermal peels of C. album as a light-dependent response over the temperature range 9 - 29°C.

C O N T E N T S

CHAPTER 5 : STOMATAL CHARACTERISTICS OF C. ALBUM

INTRODUCTION

HERBICIDES, A GENERAL REVIEW

The demand for effective weed control has intensified in recent years, largely as a result of the development of a more intensive and mechanised system of crop production. This has led to a decline in the use of the more conventional means of weed control, such as cultivation, hoeing and hand pulling, and an intensification in the use of chemical weed control which is well suited to the increased pace of modern agricultural methods.

A herbicide can be simply defined as a chemical capable of killing or inhibiting the growth of plants (Klingman & Ashton 1975). The use of chemicals for the destruction of unwanted vegetation was probably first introduced some hundreds of years ago, when salt and various industrial by-products such as smelters' wastes were applied to roadsides. However, the first true herbicides to be exploited were inorganic chemicals, such as the metal sulphates, the effects of which became apparent in the late nineteenth century, and have since been reviewed by Bissey & Butler (1930).

Further investigations revealed the herbicidal activity of arsenic components (Bolley 1901) and sulphuric acid (Rabaté 1911). Many commercial fertilizers such as sodium nitrate and ammonium sulphate (Rademacher 1940) also proved effective weedkillers when applied in large doses. Chlorates also displayed herbicidal properties and it was probably Åslander (1926) who first demonstrated that sodium chlorate was effective for killing deep-rooted perennial weeds by soil application.

It was not until the early 1930's that organic chemicals were exploited as herbicides. The first organic herbicide, introduced by Truffaut and Pastac in 1935, was DNOC (sodium salt of 2-methyl-4,6-

dinitrophenol). Many other nitrophenols followed, such as dinoseb (Crafts 1945), which became valuable in the control of weeds in cereal crops.

During the second World War the phenoxyacetic acids gained particular importance when Hamner & Tukey (1944) reported the herbicidal properties of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T). These compounds were found to be structurally related to the auxin-type plant hormones and mimicked some of their actions.

Since the 2nd World War other chemicals have proved effective for weed control, including some derivatives of benzoic acid, such as 2,3,6-trichlorobenzoic acid (2,3,6-TBA) (Zimmerman & Hitchcock 1951), and various halo-aliphatic acids, the most important being trichloroacetic acid (TCA) (McCall & Zahnley 1949) and 2,2-dichloropropionic acid (dalapon), introduced by the DOW Chemical Company in 1953 for the control of annual and perennial weed grasses of arable land.

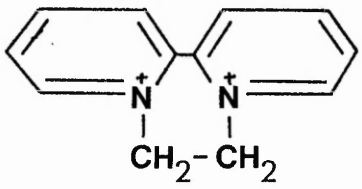
Amides constitute a moderately-sized family of herbicides, having the important property of inhibiting seed germination and seedling growth. Most important members include (naptalam (N-1-naphth-yl-phthalamic acid) (Hoffman & Smith 1949) and allidochlor (NN-diallyl-chloroacetamide) (Hamm & Speziale 1956). Related to the amides are the anilides used for controlling annual grasses and broad-leaved weeds in maize, brassicas, etc., important members being alachlor and propachlor used pre-emergence and pentanochlor and propanil used post-emergence.

Carbamates form a large group of useful herbicides, the aryl carbamates controlling grasses in peas, beet etc., an important member being propham (isopropyl-N-phenylcarbamate), (Templeman & Sexton 1945). Thio- and dithio carbamates have proved toxic to germinating seeds and

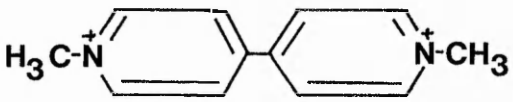
of this group diallate (2,3-dichloroallyl-di-isopropyl-thiocarbamate) and triallate are the important members, especially useful for the control of wild oats (Hannah 1959).

Other important groups of chemicals include the nitriles, such as ioxynil and bromoxynil (Wain 1964) for the control of broad-leaved weeds in cereals, and substituted ureas, such as monuron (Bucha and Todd 1951), effective for the control of annual and perennial grasses.

The bipyridylium quaternary ammonium salts have also proved highly active compounds, resulting in rapid kill of plants, the most important members being diquat and paraquat, the formulae of which are presented below:-



Diquat



Paraquat

Both these compounds are rapidly absorbed by leaves and adhere strongly to soil particles. They are used widely for weed control before crop-emergence, stubble cleaning and killing of old grassland before ploughing (Calderbank 1968).

The derivatives of pyridine form a large family of herbicides, including picloram (3-amino-3,5,6-trichloropicolinic acid, (Hamaker, Johnston, Martin & Redemann 1963), pyridazines including maleic hydrazide (Schoene & Hoffman 1949), and the pyrimidines, important members being bromacil, terbacil and lenacil.

Another important family of herbicides is the triazines, important members being simazine (2-chloro-4,6-bis (ethylamino) -1,3,5-triazine) and atrazine, best known and characterized by their high toxicity to a range of mono- and dicotyledons and are used widely for weed control in

deep-rooted crops such as citrus and deciduous fruits (Gysin 1960).

A number of unclassified herbicides have recently been introduced, one of the most important being glyphosate, developed by Monsanto. This is a phosphonate derivative of the amino acid, glycine, and has proved highly effective for the post-emergence, non-selective control of annual and perennial weeds.

Although there is a large diversity in the type of active chemical group responsible for herbicidal action, the herbicides can be broadly classified according to their modes of action, although many appear to have more than one principal mode of action.

Of the seventy or so herbicides recommended by the Agricultural Chemical Approval Scheme (1979), over thirty are thought to have a primary effect on photosynthesis. This group of herbicides is thought to act either by inhibiting photosynthetic electron transport, characterized by the substituted ureas, diuron, linuron, and monuron (Hoffman 1971) and nitriles such as ioxynil (Smith, Paton & Robertson 1966) or by diverting the reductive energy of the chloroplast to generate toxic free radicals (Halliwell 1978). Characteristic of these herbicides are the bipyridyliums, paraquat and diquat. Early experiments with these showed a rapid phytotoxic action in the light and a retardation in the presence of an electron transport inhibitor such as monuron (Mees 1960).

The next most numerous grouping includes the auxin-type growth regulators, of which the most important are 2,4-D, MCPA and 2,4,5-T. At low concentrations these mimic the action of the natural auxin, IAA. Effects include elongation, epinasty, secondary root production, callus growth and the stunting of young leaves (Dodge 1976). Other groups exhibiting growth regulatory mechanisms include some benzoic acid derivatives (Zimmerman & Hitchcock 1951) and pyridine derivatives such

as picloram (Foy & Penner 1965).

Other small groups of herbicides may interact with a range of biochemical systems. These may act as respiratory uncouplers, such as DNOC, 2,4-dinitrophenol and dinoseb (Mellor & Salisbury 1965), or lipid biosynthesis inhibitors, such as TCA and dalapon. These interfere with the synthesis of unsaturated fatty acids, resulting in reduced leaf surface wax production (Pfeiffer, Dewey & Brunskill 1957).

Ideally, a herbicide should kill the weed without injury to the crop plant. A selective herbicide kills or retards growth of one or more plants, whereas another plant is tolerant. Ideally the weed is killed, but sometimes it is only necessary to retard its growth long enough for the crop to become dominant. Holly (1970) defined various degrees of selectivity, which may arise due to differences in retention or penetration between species, variation in position of roots resulting in differences in availability of the herbicide, differential translocation or metabolism and differential detoxification.

The first selective herbicides, iron and copper salts and dilute sulphuric acid were effective by differential wetting; the droplets bounced off the leaves of cereal crops, but wet the broad leaves of cruciferous weeds (Brian 1976).

With the appearance of many new herbicides in the past 25 years, new types of selectivity have been found. Selectivity may be related, for example, to the depth at which the herbicide remains active in the soil. Simazine, for example, developed in the Geigy Laboratories by Gysin (1960), was found to be active only in the surface soil layer due to its lack of movement. It therefore became useful for weed control in deep-rooted crops such as fruit trees.

Another important property of the triazines, simazine and atrazine, is their metabolism in maize, where they are degraded non-enzymically to the inactive hydroxy derivative (Castelfranco, Foy and Deutsch 1961). Hence they have found extensive uses for controlling annual grasses and broad-leaved weeds in maize.

Selectivity may also be provided by the differential accumulation and metabolism of the chemical within the plant. Thus, Hogue and Warren (1968) suggested that this was the mechanism of selectivity of linuron between tomato (susceptible) and parsnip (tolerant). They demonstrated that tomato translocated the compound throughout the foliar portion of the plant, whereas parsnip metabolized the small amount that reached the ariel parts.

Thus, the vast arsenal of herbicides now commercially available provides chemicals of widely differing physical and biological properties, enabling weed control to become more and more specialized and hopefully more effective in terms of enhanced crop production.

However, a pre-requisite to phytotoxic action of post-emergence herbicides is the ability of the chemical to effectively penetrate the leaf surface in order to reach its primary site of action.

THE FOLIAR PENETRATION OF HERBICIDES

The plant cuticle

The prime barrier to the foliar penetration of herbicidal sprays is the plant cuticle. The cuticle is a thin, non-cellular lipoidal membrane covering plant surfaces. It extends over specialized structures and frequently covers the guard, mesophyll and epidermal cells adjoining sub-stomatal chambers and intercellular air spaces in leaves (Norris and Bukovac 1968).

The chief structural component of the cuticle is cutin, a polyester of long-chain fatty- and hydroxy-fatty acids, the principal constituents being 10, 16-dihydroxyhexadecanoic acid, 10, 18-dihydroxyoctadecanoic acid and 9, 10, 18-trihydroxyoctadecanoic acid (Baker and Holloway 1970; Holloway and Deas 1971). Although the exact structure of the cutin polymer is unknown, ester linkages between the hydroxyl and carboxyl groups dominate, and some peroxide and ether linkages may also be present (Kolattukudy and Walton 1973). The cutin layer contains quantities of embedded wax which is highly oriented and appears strongly birefringent when viewed under plane-polarised light (Norris and Bukovac 1968). Cutin is semi-lipophilic in nature, due to a portion of the polar groups remaining free during polymerisation, and can therefore swell in the presence of water. The compound strongly absorbs ultraviolet light, which helps to protect the photosynthetic mechanism of the plant, especially at high altitudes. Cutin also has a pronounced negative charge which results in selective cation permeability (Crafts and Foy 1962).

Between the cutin matrix and the epidermal cell wall is a region rich in pectins, which is believed to act as a cement between epidermis and cuticle. These consist of long-chain polygalacturonic acid molecules having side carboxyl groups. The acids are water-soluble and are

primarily responsible for the water-holding capacities of the walls (Crafts and Foy 1962).

The external surface of the plant cuticle is frequently covered with deposits of epicuticular wax, often in a specific and characteristic pattern. The quantity of wax present varies widely. In a number of weed species it ranged from less than 10 to about 20μg cm^{-2} (Baker and Bukovac 1971). These surface deposits have been studied in detail by Amelunxen, Morgenroth and Picksale (1967) using the scanning electron microscope. These workers suggested six classifications of deposits as follows:- 1) a simple granular wax crust, 2) wax rods and filaments, 3) wax plates and scales, 4) wax layers and crusts, 5) aggregate wax coatings, and 6) liquid or viscous wax coatings.

The distribution of wax on leaf surfaces varies considerably between individual plant species, and indeed, different types of wax may result in different distribution patterns. On leaves which contain crystalline surface structures, for example, the distribution of these structures is generally quite uniform (Holly 1964). Variation may, however, occur in the vicinity of the stomata, where the wax is often more sparsely distributed. For example, Wortmann (1965) noticed that the rodlet and plate wax structures found on the surface of Brassica napus leaves were almost absent around the stomata. This type of distribution is not, however, universal. Electron micrographs on the lower surface of Prosopis juliflora leaflets clearly demonstrate a density of wax structures on the guard cells approximately equivalent to that in adjacent epidermal cells (Hull 1964).

The chemistry of plant cuticles has been investigated extensively in recent years, and many of the constituents of cutin and the waxes have been elucidated with the aid of thin-layer chromatography (TLC),

gas-liquid chromatography (GLC) and, more recently, mass-spectrometry.

Although the epicuticular waxes of most plants contain a diversity of complex constituents, certain classes are common to leaf waxes of many plants. In general, the epicuticular waxes are complex mixtures of long-chain alkanes, alcohols (both primary and secondary), ketones, aldehydes, esters and fatty acids (Kolattukudy 1970; Martin and Juniper 1970). These classes can be resolved by TLC methods, and Holloway and Challen (1966) have described in detail the various separation techniques available, together with methods of detection of individual fractions. Often each class is present as an homologous series with a dominant chain length of from C_{21-50}. The fatty acids, primary alcohols and aldehydes generally contain even numbers of carbon atoms, whereas the alkanes, ketones and secondary alcohols contain odd numbers (Kolattukudy and Walton 1973). The acids and alcohols may be found free or combined as esters (Kolattukidy 1965). Cyclic compounds, triterpenoids being most representative, are frequently found in fruit cuticles (Radler and Horn 1965).

Although there are many reports of epicuticular wax analysis of numerous plant species, technical problems have hindered progress in the analysis of the cuticular waxes, and therefore only limited data are available on the chemistry of the cuticular waxes per se. The evidence available does suggest, however, that many cuticular waxes contain higher proportions of polar constituents, such as esters and alcohols, than do the epicuticular waxes (Baker and Bukovac 1971).

The structural relationship between the various cuticular components, i.e. cutin, wax and pectin, has been considered in detail by van Overbeek (1956, Crafts and Foy (1962) and others. These workers found a considerable variation between species, not only in the thickness of

individual layers but also to the extent with which they are integrated with one another.

Herbicide penetration

The foliar penetration of herbicides has received considerable attention and there still appears to be a great deal of controversy over the actual site of entry. The cuticle is generally considered to be the principal site of entry, although under certain conditions the stomatal apparatus may play a significant role (Crafts and Foy 1962; Sargent and Blackman 1962; Franke 1964).

Cuticular penetration:- Crafts (1961) was probably the first to suggest that there may be specific pathways through the cuticle. Since the cuticle is largely lipoidal, it is likely that non-polar molecules will penetrate more readily than polar. The former may move along pathways believed to be associated with wax secretion, while polar compounds may traverse more slowly through pectinaceous pathways (Martin and Juniper 1970).

The macrostructure of the leaf surface and the composition and ultrastructure of the epicuticular wax influence the retention and penetration of aqueous solutions (Silva Fernandes 1965, Holloway 1969), and there is increasing evidence that the surface wax provides the chief barrier to the penetration of water-soluble material (Norris and Bukovac 1968). Removal of the surface wax from isolated tomato fruit cuticle, for example, generally enhanced its permeability to 2, 4-D, but no further increase in permeability occurred after the subsequent removal of the wax occluded within the membrane, the cuticular wax (Bukovac and Norris 1967).

The relative importance of the cuticle in foliar penetration has been ascertained using isolated stomata-free cuticle. Silva Fernandes

(1965), for example, found that diffusion through stomata-free cuticular membranes did not occur if the membrane contained more than 0.1 mg cm^{-2} cutin, but diffusion occurred through membranes containing stomata. One must conclude, therefore, that wax is an important barrier to foliar penetration, but its effectiveness depends not only on its composition, but also on its distribution within and over the surface of the cuticle.

Stomatal penetration:- Early work on stomata-free cuticles suggested that polar molecules did not readily penetrate (e.g. Orgell 1954), and so the role of stomata in foliar penetration was implicated.

Direct evidence for stomatal penetration by aqueous solutions is still, however, rather conflicting. While stomatal entry has been reported for aqueous solutions by Skoss (1955) and Currier, Pickering and Foy (1964), other investigators (e.g. Sargent and Blackman 1962) have concluded that mass movement through the stomatal pore does not readily occur. Schönherr and Bukovac (1972) also concluded that aqueous solutions with a surface tension approaching that of pure water do not pass through the stomatal pore. Penetration has, however, been observed when the surface tension was sufficiently decreased by the addition of a surfactant, and penetration was not observed where the stomata were completely closed (Dybing and Currier 1961).

Stomata are considered to be more important during the initial stages of entry, while cuticular uptake is more important over longer periods (Currier et al 1964). Once inside the pore, however, the solution must still traverse some form of cuticle. The cuticle within the pore is thinner, more permeable and more hydrated than the cuticle covering the outer surface (Currier et al 1964), and there is some evidence to suggest that the cuticle lining the pore may also be more polar (Norris and Bukovac 1968).

Entry may take place, however, via the guard and accessory cells, and not the pore itself (Dybing and Currier 1961). Later work by Franke (1964) reinforced this theory, when he discovered that ^{14}C-labelled sucrose and amino acids applied to leaves of Spinacia oleracea and Viola tricolor accumulated in the cuticular ledges around the stomatal pore, and in the anticlinal walls above accessory cells. He suggested that ectodesmata, abundant in these areas, were the main sites of entry.

The role of ectodesmata:- Much has been written on the involvement of ectodesmata as pathways in foliar penetration (Franke 1964, Hull 1970, Bukovac 1976). Their definition is, however, rather confusing. They were originally believed to be protoplasmic extensions of epidermal cells (e.g. Schumacher and Lambertz 1956), although Franke (1964) later suggested that they were well-defined cell wall structures rich in reducing sugars. Convincing evidence against this theory was, however, put forward by Schönherr and Bukovac (1970). These workers suggested that there were areas in the cuticle that were preferentially permeable to polar compounds and that such areas could play a significant role in foliar penetration by serving as polar bridges across the relatively lipoidal cuticular membrane.

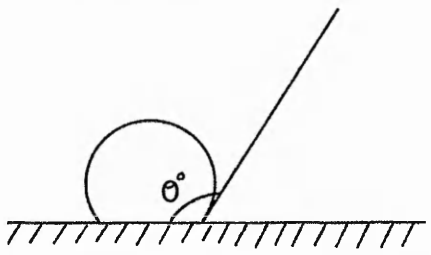
In conclusion, then, the plant cuticle is a highly complex structure, whose chemical composition and physical configuration is undoubtedly species-specific. The structure can be envisaged, not as uniform and static, but one which is in a constant flux of development. It is not surprising, therefore, that penetration studies, involving the use of different plant species, frequently produce conflicting results. Consequently, generalized statements on foliar penetration, for example, based on these studies must be interpreted with extreme caution.

FACTORS AFFECTING FOLIAR PENETRATION

Biotic factors

The properties of the leaf surface:- The physical properties of a leaf surface will determine, to a large extent, the amount of herbicide that is available for penetration. The degree to which a surface can be wetted by a solution, i.e. its wettability, is influenced by a number of factors, including leaf orientation (Holly 1964), the hairiness and corrugation of the surface (Fogg 1948), and the chemical and physical nature of the surface wax (Martin and Juniper 1970). Very pubescent leaves, for example, are less wettable because of the air pockets which prevent contact between the droplets and the leaf surface. Leaves with only a few hairs however, tend to trap droplets (Hull 1970). Wettability is also greatly influenced by the nature of the surface wax. An increase in waxiness decreased the adherence of spray molecules (Mueller, Carr and Loomis 1954), whilst reduction of wax on peas by soil treatment with TCA increased retention, penetration and susceptibility to dinoseb, MCPA and mecoprop (Pfeiffer et al 1959).

The term contact angle, described as the angle between the surface of a leaf and the tangent plane of a water droplet at the circle of contact between air, liquid and leaf, is a useful way of characterising the wettability of leaves (Fogg 1947). The highest contact angle known for water on a smooth surface is 110° on hentriacontane, although contact angles of 140° are not uncommon on plant surfaces (Martin and Juniper 1970). This is due to the trapping of air between repellent projections. For leaves exhibiting a contact angle of greater than 90°, the epicuticular



The contact angle (θ^{o}) of a water droplet on the leaf surface.

wax is the dominant factor governing wettability (Holloway 1969). Indeed, the composition of the epicuticular wax appears to be a factor of greater importance than the degree of waxiness, some fractions of wax having a greater influence on water repellency than others (Martin 1966). The water repellency of cabbage, for example, is ascribed in part by Juniper and Bradley (1958) to wax containing higher quantities of paraffins and ketones, while the non-repellent wax of broad bean consists largely of fatty acid esters. Wettability is also influenced by the physical form of wax, a crystalline form producing strong water-repellency while an amorphous, non-crystalline flat or smooth form allows spreading and retention (Juniper 1959, 1960).

Leaf age and development:- Penetration is also influenced by leaf age and development, absorption being greater in young, expanding leaves (Currier and Dybing 1959; Crafts and Foy 1962). This has often been attributed to the increase with age of cutin, wax deposits and hence cuticular thickness (Schifferstein and Loomis 1959).

Both leaf surfaces are readily penetrated by herbicides, although the greater penetration through the lower leaf surface cannot really be attributed to any one factor (Foy 1964). Usually this surface is rich in stomata and trichomes which may play a role in penetration under some conditions (Hull 1970). Work by Norris and Bukovac (1968), however, showed that the greater penetration of certain growth regulators through the lower surface of pear leaves than the upper could be attributed to the greater degree of molecular orientation of cuticular wax on the lower surface. It has been reported that certain chemical changes occur in the constituents of surface waxes during foliar development. Matsuda (1962), in studying the biosynthesis of wax from the Candelilla plant (Euphorbia antisyphilitica), observed that waxes from young leaves had a lower

percentage of paraffins but a higher percentage of acids and alcohols than did waxes from older tissues. This finding cannot, however, be extended to include all plant species.

Herbicide factors

Formulation:- It has generally been recorded that an inverse relationship exists between polarity and efficiency of penetration of a compound through fatty phases, and van Overbeek (1956) concludes that a suitable balance of polar-apolar groups of the molecule is essential for efficient action. In the case of phenoxyalkyl herbicides, for example, structural changes in either the ring or side chain can markedly alter the absorption properties. Robertson and Kirkwood (1966), for example, demonstrated that the high susceptibility of Vicia faba to MCPA was related to the rapid cuticular penetration of this molecule and its extensive movement throughout the plant, whereas resistance to MCPB was associated with its virtual confinement to the treated leaves.

Additional halogens in a molecule also generally increase its lipophilic characteristics, and Sargent (1965), for example, found that increased chlorination of the phenoxyacetic acids generally favoured penetration in the lipoid phases of the cuticle.

Thus, modification of molecular structure which results in increased lipid solubility will generally enhance foliar penetration (Bukovac 1976).

Surfactants:- A surfactant is a substance introduced into a liquid in order to affect (usually to increase) its spreading, wetting and other properties dependant on its surface tension. In general, the addition of surfactants enhances penetration and the effectiveness of foliar-applied herbicides (Staniforth and Loomis 1949). Surfactants can affect penetration by, a) increasing the area of contact through spreading the solution, b) eliminating air films between the solution and

plant surface, c) acting as cosolvents or solublizing agents in cuticular penetration, d) easing entry through open stomata and subsequent movement through intercellular spaces and, e) acting in a secondary capacity as humectants by retarding the drying out of the solution (Holly 1964).

Generally, as the concentration of the surfactant is increased, the surface tension is lowered to a point beyond which further addition of surfactant is without effect. This is known as the critical micelle concentration (CMC), although usually herbicide effectiveness is often maximal at concentrations ten times the CMC (Foy and Smith 1965). It is believed that above this concentration, the surfactant molecules form micelles or aggregates with their hydrophilic groups towards the inside, and that enhanced penetration is due to the solubilization of the cutin by the aggregates with subsequent removal of the wax (van Overbeek and Blondeau 1954). A more recent theory put forward by Bukovac (1976), however, suggests that the surfactant may interact with the cutin matrix, altering its charge characteristics and swelling properties, and hence, the membrane resistance to the diffusion of a specific chemical.

pH:- The pH of an applied solution is known to affect the ease of cuticular penetration, since it influences the polarity of the cuticle and the penetrant (van Overbeek 1956), although Norris and Bukovac (1972) consider that it is primarily the penetrant that is influenced. pH plays a significant role in the penetration of weak organic acid herbicides such as 2,4-D (Sargent 1965). At low pH these acids are largely undissociated and hence readily partition into the lipoidal phases of the cuticle and the plasmalemma (Simon and Beevers 1952).

Although penetration of the active ingredient is generally greater at low pH, it is sometimes possible to enhance penetration at high pH, by the addition of certain inorganic ions. Ammonium salts, for example,

are known to enhance uptake of NAA (Horsfall and Moore 1962). Similarly, Ladonin (1961) and Likholat (1962) found that 15kg/ha ammonium sulphate in common application with 2,4-D at a rate of 1-2kg/ha significantly increased toxicity of the herbicide towards the weeds, and, at the same time, resulted in increased yields in maize crops. A factor which may well influence the magnitude of pH enhancement obtainable is the inherent phytotoxicity of the active ingredient and the concentration at which it is used (Hull 1970).

Environmental Factors

Temperature:- Since absorption is governed by both metabolic and non-metabolic components, it seems likely that the overall process would be accompanied by fairly high temperature coefficients. The majority of research carried out suggests this (Sargent 1965). Prasad, Foy and Crafts (1967), for example, found a four-fold increase in foliar penetration of ^{14}C-dalapon in bean when the post-treatment temperature was maintained at 43°C as compared to 26°C. Enhanced cuticular penetration resulting from increased temperature is also reported for 2,4-D (Pallas 1960) and phenylmercuric acetate (Silva Fernandes 1965). The effect of post-treatment temperature was also demonstrated by Kelly (1949), who reported that bean plants grown at 15°C showed little effect from 2,4-D until transferred to 25°C, but plants grown at 5°C exhibited a marked affect after transfer to the 15°C temperature. From her results it would appear that a change in temperature following treatment may be as critical as the temperature at the time of treatment.

It seems probable that temperature exerts an indirect effect on absorption rate by influencing the general metabolic rate (Sargent 1965).

Light Intensity:- It has generally been found that light enhances foliar penetration of solutes. This may be due to either a direct effect

on the permeability of the plasmalemma or by affecting the energy mechanisms for uptake associated with photosynthesis (Kylin 1960). Currier and Dybing (1959), however, consider that light promotes absorption by inducing stomatal opening, thus increasing photosynthesis and the export of carbohydrates.

Penetration is enhanced by light, although only relatively low intensities (5-15KLUX) are adequate for maximal response (Sargent and Blackman 1965). Light-enhanced uptake of 2,4-D (Sargent and Blackman 1969) and NAA (Green and Bukovac 1972) can be negated by inhibitors of the Hill reaction, indicating that absorption is dependent on a supply of ATP.

The size and shape of leaves and, indeed, leaf mosaic can greatly be affected by light, and so indirectly affecting penetration by either increasing or reducing the total leaf area available for the retention and hence penetration of herbicides (Muzik 1976).

Humidity and soil moisture:- High humidity, moisture on the external surface and low moisture tension within the plant favour rapid foliar uptake (Clor, Crafts and Yamaguchi 1962). The enhanced penetration under high moisture conditions may be attributed to the swelling of the hydrophilic groups of the cutin matrix pushing the apolar wax units apart (van Overbeek 1956). Apparently, under these conditions the pores would be filled with water and the pectin highly hydrated, promoting absorption of water-soluble herbicides via the apoplast (Crafts 1961, Foy 1964). Increased absorption under high humidity conditions may also be due to enhanced stomatal penetration, since there is evidence to suggest that guard cells swell with increased turgor pressure and the stomatal aperture opens (Pallas 1960).

It is generally conceded that moisture stress inhibits the absorption

of foliar-applied solutes (van Overbeek 1956; Currier and Dybing 1959), although some workers, such as Basler, Wood and Meyer (1961), have shown that in the case of 2,4-D, moisture stress had no effect on absorption, although translocation was severely reduced.

Water stress can reduce cytokinin levels, alter enzyme levels and reduce photosynthesis, as well as resulting in reduced stomatal aperture, some factors of which may result in reduced herbicide efficiency (Hsiao 1973).

Environment and cuticular development:- Reports on environmental factors influencing cuticular development, wax composition and ultra-structure are numerous, and many workers have attempted to correlate changes in cuticular development with leaf wettability and penetrability.

Most of the work carried out on the effects of light intensity on cuticular development suggests a direct relationship between light intensity and cuticle thickness. Skoss (1955), for example, found that ivy (Hedera helix L) leaves had less cuticle, cutin and wax when grown in the shade when compared with leaves grown in full sunlight. Similar relationships have been demonstrated for epicuticular wax. Juniper (1960), for example, found that wax production in pea (Pisum sativum) leaves increased to give a white waxy bloom as light intensity increased over a range of 900-5,000 foot candles.

Cuticular development has also been correlated with changes in photoperiod by Wilkinson (1966), who found that stems grown from cuttings of Tamarix pentandra developed a mean cuticular thickness of 64 µm when on a 14 hour photoperiod. Under shorter or longer photo-periods cuticular thickness was only half as much.

The quality of light is also thought to affect cuticular development. Hull (1958), for example, observed that cuticular development was very

different between greenhouse and field grown seedlings of mesquite (Prosopis juliflora), cuticles of the former being less than 1 μm and those of the latter being as much as 20 μm, and suggested that this may be due to the lack of ultra-violet light under glass.

The effects of temperature on cuticular development are rather confusing in that some workers have shown a direct relationship between temperature and wax production, whereas others have failed to find any such correlation.

Temperature has been shown by many workers to have a marked effect on the ultrastructure of epicuticular wax (e.g. Whitecross and Armstrong 1972), although the chemical composition of epicuticular wax has not been shown to change appreciably under such conditions (e.g. Tribe, Gaunt and Wynn-Parry 1968; Baker 1974).

Whereas some workers have found a direct relationship between cuticle thickness and temperature (e.g. Skoss 1955; Hull 1958), many others have failed to show such a correlation. Donoho, Mitchell and Bukovac (1961), for example, observed variations in foliar absorption of ^{14}C-NAA in apple and pear leaves at different temperatures, but the cuticle thickness of these leaves remained unchanged throughout.

Aridity is generally thought to induce the development of thick cuticles (Muzik 1976). Whether this effect is a function of atmospheric humidity or available soil moisture, however, is not always clearly defined.

Although xeromorphic adaptation correlates well with increased cuticle development, this relationship does not always hold for epicuticular wax production (Schifferstein and Loomis 1957). Daly (1964), on the other hand, has reported variations in surface waxiness between field populations of blue tussock grass (Poa colensoi). He showed by electron microscopy that plants from semi-arid habitats had rod-like

crystalline structures, whereas plants grown in regions of greater rainfall had wax which was in the form of rather sparse, non-crystalline smears.

Water stress has also been suggested by many workers as influencing the development of non-wax cuticular components. Baker (1970), for example, found that the cuticular membrane in many xerophytes had very heavy and pronounced cuticular projections between the anticlinal walls of epidermal cells. These projections were finer and more delicate, however, in deciduous herbs.

In conclusion, then, the factors which may influence the penetration of herbicides through the leaf surface are very wide-ranging. Penetration can be seen to be dependent not only on the physical and chemical properties of the penetrant (i.e. herbicide formulation, surfactant, pH), but also on the characteristics of individual plant species. Probably more significant, however, is the interrelationship between these factors and the environment, which will determine to a large extent the success of any particular herbicide application.

STOMATAL PHYSIOLOGY - A GENERAL REVIEW

The function of stomata in higher plants is to regulate the passage of carbon dioxide and water through the leaf surface. This regulation of gaseous exchange is an important feature of higher plants where the need for water conservation is great. As a result of this, a highly sophisticated sequence of stomatal opening and closure has evolved in order that the carbon dioxide and water levels within the plant can be adequately maintained.

Stomatal physiology and the regulatory mechanisms which control opening have been considered in detail by, amongst others, Zelitch, 1965; Meidner and Mansfield, 1968 and Raschke, 1975. Stomatal opening is the end result of solute accumulation in the guard cells, resulting in increased turgor and opening of the stomatal pore (Hsiao 1976). Early hypotheses explaining the mechanism centred around the "starch-sugar" theory, first introduced by Lloyd in 1908 and more recently updated by Levitt (1967). This hypothesis favours sugar as the key solute, its concentration being determined by starch metabolism, this in turn being triggered by pH changes as a result of fluctuating CO_2 levels. This theory, however, has been severely criticised by authors such as Meidner and Mansfield (1965).

A second mechanism of solute build-up was proposed by Zelitch (1965), who suggested that the glycollic acid produced during photosynthesis at low CO_2 concentrations may be important in stomatal opening. He proposed that glycollate was converted into carbohydrate, thus raising the turgor pressure within the guard cells. At the same time it could also lead to the production of ATP which may provide energy for the opening mechanism. Again this mechanism could not solely account for stomatal movement, since stomata open widely in the dark in response to CO_2 removal (Heath

and Mansfield 1967).

Thus, solute build-up within the guard cells could not explain the opening mechanism entirely, and it was not until the work by Fujino (1967) and Fischer (1968) that substantial evidence was brought forward to implicate the uptake of ions by the guard cells as a means of effecting stomatal opening. In his paper, Fischer (1968) found that stomata in epidermal strips of Vicia faba opened readily in light only when floated on solutions containing K^+. As a result of these publications, further evidence has been accumulated by various workers (e.g. Humble and Hsiao, 1969, 1970; Willmer and Mansfield 1969; Raschke 1975) to establish K^+ transport as a fundamental feature of stomatal movement.

Stomatal movements are known to be affected by environmental conditions and several reviews of these effects have been published (e.g. Zelitch 1965; Meidner and Mansfield 1965). From these reports it appears that stomata respond to changes in temperature, illumination and leaf water content, all of which can be related to their effects on internal CO_2 concentration. These environmental effects can also, however, take place independent of CO_2 concentration. Indeed, the effects of blue light on stomatal opening, independent of CO_2 have been reported by Meidner and Mansfield (1968). The influence of relative humidity on stomatal opening has been reported by Lange, Losche, Schulze and Kappen (1971), who found that subjecting epidermal strips to high relative humidity resulted in stomatal opening, whereas low relative humidity induced closure.

In addition to environmental effects, stomatal movement is also influenced by an endogenous rhythm (Meidner and Mansfield 1965). Rhythms can produce opening in the dark and partial closure in the light, and hence can modify or overrule the response to external factors. Indeed,

Heath and Orchard (1957) reported that the stomata of many species exhibit a "midday closure", especially under field conditions of a high evaporative demand. It may, however, occur as a result of high temperatures which cause an increase in the levels of intercellular CO_2.

The question of hormonal control of stomatal behaviour, which has received relatively little attention (e.g. Zelitch 1965), has recently assumed more importance following the discovery that abscisic acid (ABA), applied to either intact leaves or isolated epidermal peels is a powerful inhibitor of stomatal opening (Jones and Mansfield 1970). Indeed, ABA has been shown to be formed in large amounts when leaves wilt (Wright 1969), and so it is clear that changes in the endogenous levels of this hormone might play an appreciable role in the regulation of stomatal aperture, especially in controlling transpiration under conditions of limited water supply. The control of stomatal movements by ABA has been reviewed by Mansfield (1976), and more recently, Mansfield, Wellburn and Moreira (1978) have implicated the role of another sesquiterpenoid, farnesol, in the alleviation of water stress. These workers believe that farnesol is the agent responsible for altering the permeability of the chloroplast envelope membranes, allowing the release of ABA into the mesophyll cell.

The role of cytokinins in stomatal opening has been implicated by various authors, and Cooper, Digby and Cooper (1972), for example, have demonstrated a significant interaction between ABA and the synthetic cytokinin, kinetin in controlling stomatal movements in barley. More recently, however, Das, Rao and Raghavendra (1976) have nominated the cytokinin benzyl adenine (BA) as another candidate for the control of stomatal opening, since its presence in the incubating medium prevented stomatal closure in epidermal peels by ABA. It is thus considered by

these workers that ABA and BA control stomatal movements and hence control transpiration rate by striking a balance between the two.

The influence of herbicides on stomatal movements has recently received more attention. Work by Pemadasa and Jeyaseelan (1976), for example, has shown that 2,4-D, 2,4,5-T and MCPA induce stomatal closure in epidermal peels of Commelina benghalensis. In the case of 2,4,5-T and MCPA this closure was reversed by flushing the intercellular spaces with CO_2-free air, suggesting that closure was as a result of changes in the CO_2 concentration in the intercellular spaces (by stimulating respiration or by inhibiting photosynthesis). The effects of 2,4-D, however, were not reversed, suggesting that this herbicide exerts a more direct effect on the guard cells, possibly by altering membrane permeability.

It should perhaps be noted here that reports on stomatal behaviour should only be interpreted in relation to the specific plant species studied, since relatively few species have been used.

Stomatal movement, therefore, is governed not only by the underlying endogenous rhythms of the plant, but is also under hormonal and environmental control, all of which probably interact to some extent. The relative importance of each governing factor will obviously depend on the prevailing micro-environment both inside and outside the leaf, and these may in turn influence the degree to which stomatal penetration by herbicides can readily occur.

AREA OF THE INVESTIGATION

Weed control in french bean crops

The acreage in the United Kingdom under french beans (*Phaseolus vulgaris L.*) has increased dramatically over recent years, largely as a result of improved mechanical harvesting methods replacing hand-picking. Although beans are still primarily cultivated in wide rows (40-50 cm apart), more intensive systems are under development, and machinery capable of harvesting crops in rows as close as 13 cm have been developed and tested prior to introduction (King and Handley 1976). Before these intensive systems can be fully exploited, however, more reliable chemical weed control systems must be provided.

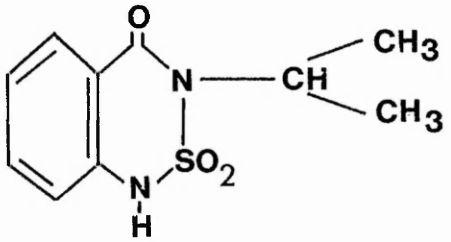
Trials carried out by the Processors and Growers Research Organisation (PGRO) since 1963 indicate that most of the common arable weeds can be found in french bean crops, the predominant species being:- fat hen (*C. album L*), common orache (*Atriplex patula L.*), couch grass (*Agropyron repens L.*), fumitory (*Fumaria officinalis L.*), red dead nettle (*Lamium purpureum L.*), annual meadow grass (*Poa annua L.*), knot grass (*Polygonum aviculare L.*), black bindweed (*Polygonum convulvulus L.*), black nightshade (*Solanum nigrum L.*), chickweed (*Stellaria media L.*), scentless mayweed (*Tripeurospermum maritimum L.*), annual nettle (*Urtica urens L.*) and speedwells (*Veronica* spp.)

French beans are not a competitive crop capable of suppressing weeds, especially when grown in wide rows (King 1976a), with the result that some weeds become especially troublesome, particularly *C. album*. Apart from being a very competitive weed, this species is a late germinator and hence avoids pre-emergence applications of some herbicides (King, 1976b).

Since the mid-1960's a wide range of herbicide programmes has been evaluated. Pre-emergence herbicides such as dinoseb-amine and dinoseb-

acetate were the only treatments available initially, but both lacked persistence and offered no control over late-germinators. In later trials monolinuron showed more persistence and gave good control by root uptake. Its contact action, however, was poor, and so mixtures emerged such as monolinuron and dinoseb-acetate which showed promising results (King 1966). The pre-sowing herbicide, trifluralin (Treflan) was evaluated in the early 1970's (PGRO 1971) and also produced satisfactory results. It was not until the appearance of bentazone, however, that effective post-emergence weed control could be achieved.

Bentazone was first introduced by Badische Anilin and Soda-Fabrik AG (BASF) Limited in 1968 and its herbicidal properties were published by Fischer (1968). Bentazone is the approved common name for 3-isopropyl-1H-2,1,3-benzothiadiazin-(4) 3H-one-2,2-dioxide.



Bentazone

It is a contact herbicide that is selective in agricultural and horticultural crops belonging to the leguminosae and gramineae, and has been developed in many parts of the world for the control of weeds in soybeans (Luib and Van de Weerd 1972) and rice (Mine, Hino, Udea and Matsunaka 1974). Its mode of action has been studied by Mine and Matsunaka (1975), who suggest that photosynthetic inhibition may be the primary mode of action. The selectivity of bentazone may be linked to its rate of detoxification by resistant and susceptible plants (Mine, Miyakado and Matsunaka 1975), since there appears to be no difference in absorption rate between resistant and susceptible plants after foliar application (Zaunbrecher and Rogers 1973).

Susceptibility of french bean to bentazone was first evaluated in 1971 by the PGRO. Trials were carried out with different growth stages of bean, applications being made at the expanded monofoliate, expanded first trifoliate and flower bud stage. Results indicated that crops at the earlier stages of development were more tolerant, and from this the PGRO recommended spraying at the first fully expanded trifoliate stage of development.

The herbicidal action of bentazone is directed against broadleaved weeds and Cyperaceae, and the herbicide has proved to be particularly effective in the control of C. album, S. nigrum and P. persicana, although some important weed species such as P. eviculare, Veronica and Lamium spp. are resistant (May 1974). As a result of this, herbicide combinations were evaluated, and it was found that pre-sowing applications of trifluralin or pre-emergence applications of Ivorin (dinoseb-acetate + monolinuron), followed post-emergence by bentazone produced very good overall weed control (May 1974). Bentazone has also been shown to be active when applied to the soil. Mine and Matsunaka (1975), for example, have shown that susceptible plants are readily killed by root applications of bentazone at concentrations as low as 2-3 ppm.

Characteristics of Chenopodium album

C. album, a member of the Chenopodiaceae, is a very common annual weed found generally in open communities on disturbed ground, rubbish tips and on cultivated land, (Clapham, Tutin and Warburg, 1962). This species thrives on loose, damp, nitrogen-containing, humic loams or sandy soils and extracts large quantities of nutrients from the soil (Hanf 1974). It has proved particularly troublesome in french bean crops, largely due to its tough, wiry stems produced as a result of secondary thickening (Metcalf and Chalk 1950). This feature, characteristic of the Chenopodiaceae, presents difficulty when the beans are mechanically harvested.

C. album germinates predominantly during May and August usually at depths between 0.5 and 3 cm. In dry, well-aerated soils, however, it may germinate at a depth of 8 cm. The leaves are long-stalked with unequal forwardly-directed teeth, oval to lanceolate in shape, (Hanf 1974), and are well-endowed with stomata, which possess no specialised subsidiary cells (Metcalf and Chalk 1950).

C. album is an hermaphrodite, producing small clusters of flowers from July to September. Each plant may produce an average of 3,000 seeds, although as many as 20,000 have been recorded. On ripening, the entire cluster falls off and the residue of the flower surrounds the seeds, (Hanf 1974).

The leaf surface structure of C. album has been investigated by Brian and Cattlin (1968), who describe the leaves as having a silvery bloom consisting of a layer of small, separate spheres of average diameter 80 µm. These globules, which are attached to the leaf by capillary stalks, are thought to be composed principally of silicates, although their exact function is still unknown, since they collapse and disappear as the leaf matures.

The chemical composition of the epicuticular wax of C. album has been studied by Allebone et al (1970, 1972) using GLC and mass-spectrometry techniques. The wax was found to consist largely of alcohols, of which the C_{26} and C_{28} homologueswere the dominant members. Significant quantities of aldehydes were also detected, with a dominant chain length of C_{28}. Hydrocarbons and esters were also present in smaller quantities.

Bentazone as a herbicide for C. album

Due to the late germination of C. album the use of pre-emergence herbicides proved less than satisfactory in its control. It was not

until the introduction of bentazone that effective control of this troublesome weed was achieved.

Bentazone was first evaluated on a commercial scale in 1974 by the PGRO. It was then marketed as Basagran and was used in combination with trifluralin. The addition of the proprietary mineral oil, Actipron (BP Chemicals) was also evaluated. This improved control considerably, but also reduced selectivity of the herbicide, resulting in increased crop damage (PGRO 1974). Further trials carried out in 1975, however, when applications were made following a period of hot, dry weather, showed that bentazone used alone resulted in poor control of C. album. Incorporation of Actipron, however, markedly improved control, this time without any substantial increase in crop damage. Further trials carried out in the dry season of 1976 (King and Handley 1976) again highlighted the poor control of C. album with bentazone, especially when applied at a high volume rate of 560 l H_2O/ha. Control was increased by 40-90%, however, upon the addition of Actipron. From these trials it was suggested that under periods of hot-dry weather, C. album became resistant to bentazone due to the production of a thick, waxy cuticular layer, which impaired herbicide penetration. The improved control observed in the presence of Actipron was considered to be due to the increased coverage afforded by this additive, resulting in a greater degree of penetration through the cuticle.

Aims of this study

This project was initiated as a result of the information provided by BASF and the PGRO on the poor control of C. album with bentazone under prolonged dry weather, and the claim of increased wax production in the cuticle of C. album leaves under such conditions (BASF personal communication; King and Handley 1976; BASF Technical Bulletin 1977). At the time it appeared that this theory lacked experimental proof and left

considerable scope for investigation. The observations had been based on a limited number of field experiments and in fact no studies had been performed on wax analysis. Thus, the principal aim of this project was to establish the potential role of the surface wax of C. album in effecting resistance to herbicide uptake. This required the development of reliable methods of extraction and analysis of the epicuticular wax by TLC and GLC techniques. In addition, it was intended not only to carry out a complete chemical analysis of the surface wax, but also to investigate its ultrastructure by means of scanning electron microscopy. From such detailed work it was hoped to establish the nature and properties of the epicuticular wax layer in C. album. It was also felt that additional information could be obtained from the use of contact angle determinations made on the leaf surface of C. album. These may also reflect properties of the epicuticular wax and thus provide an overall picture as to the influence of the surface wax layer on herbicide retention and penetration.

Since environmental factors are believed to influence not only wax development but also plant resistance to the herbicide, the influence of environmental conditions on wax development, and subsequent herbicide performance, was considered to be a fundamental area of research.

Though this was considered to be the central role of the project, various other areas of research also required investigation. The role of Actipron, for example, in enhancing herbicide performance appeared to be little understood and it was considered desirable to investigate more thoroughly the use of Actipron under different environmental conditions and also to investigate the effectiveness of some additives which have proved effective in other herbicide systems.

Since stomata are believed to play a significant role in herbicide penetration and hence performance, the morphology of C. album stomata was

investigated by means of both light and scanning electron microscopy. In addition, it was also proposed to investigate the influence of environmental conditions on stomatal behaviour in this species by detailed *in vitro* studies.

From the scope of these investigations it was hoped to establish the role of environment in governing plant response and to assess the importance of the epicuticular wax barrier and the role of stomata in the penetration of Basagran into *C. album* leaves.

CHAPTER I

PRELIMINARY GLASSHOUSE AND FIELD STUDIES

1.1 INTRODUCTION

The principal function of this introductory chapter is to provide a general background to the thesis as a whole, by describing some of the exploratory investigations which initiated this study. These preliminary experiments were designed to assess the response of C. album to Basagran and Actipron under glasshouse and field conditions. This response, however, may be influenced by numerous factors relating not only to the properties of C. album, such as plant size, but also to the properties of the herbicide, i.e. concentration, the presence of additives within the formulation and its method of application. Environment has also been shown to play a significant role in determining the response of plants to herbicides (e.g. Muzik and Maudlin 1964), as well as the time of herbicide application (e.g. Weaver and Nyland 1963, King and Handley 1976). It is thus the aim of this chapter to describe how some of these factors may influence the response of C. album to Basagran.

These exploratory investigations also served to establish the basic methodology of herbicide application and to standardise as far as possible the experimental techniques and damage assessment principles adopted throughout the course of this study.

1.2 MATERIALS AND METHODS

1.2.1 Growth of C. album under glass

Seeds of C. album, obtained from B & S Weed Seed Suppliers (Notts.), were placed in petri dishes containing moist seed test paper (Whatman, Grade 182) and allowed to germinate at room temperature for approximately 7 days. The germination capacity of the

seeds was approximately 63%. Seedlings were transferred to 3 inch plastic pots containing John Innes No. 2 compost. Planting was kept to just below the soil surface and three seedlings were transferred to each pot.

All plants were maintained in a glasshouse at a minimum temperature of 18°C. The pots were positioned on sand beds and irrigated from below. Supplementary lighting was provided by a series of warm-white fluorescent tubes for a 16-hour photoperiod. These provided a light intensity of approximately 25 Wm^2 at the leaf surface.

1.2.2 Use of C. album in the field

Sites were selected with a natural high density of C. album. Experiments were conducted on sites at Saxilby (Lincolnshire), Codnor (Derbyshire) and Trent Polytechnic grounds (Nottingham).

1.2.3 Experimental design

1.2.3.1 Glasshouse studies:- Plants were arranged on sand beds in a fully randomised manner, both prior to and following herbicide application. Replication of treatments varied between individual experiments, largely as a result of restricted material/space, but at least 8 plants were selected per treatment.

1.2.3.2 Field studies:- With few exceptions, experiments were conducted on a fully randomised and replicated basis. In all cases blocks and individual plots were separated by hand weeding. Details of plot size and replication for each treatment are provided with the results.

1.2.4 Herbicide application

A range of spraying equipment was tested. The glasshouse and smaller field experiments were conducted using a Shandon

Chromatography sprayer, while in the remaining larger-scale experiments, sprayers of greater capacity were employed. These included a knapsack sprayer, a killaspray unit and a Binks Bullows compressor-operated sprayer.

Plants were sprayed at the recommended field rate of 280 l H_2O/ha. Basagran and additive concentrations were varied relative to this volume.

For field work, each plot was sheltered from the wind by a polythene or cardboard screen prior to being sprayed. The required volume of herbicide was calculated and sprayed over each plot as evenly as possible from one direction. The sprayer nozzle was directed approximately 2 feet from the plants. Following spraying, the plants were examined for retention of spray droplets to ensure an even distribution of the spray.

In the glasshouse, plants received the correct dosage by placing them in a known area, usually 1 m^2, and were sprayed with the calculated volume of herbicide as previously described.

The Basagran formulation used throughout was a 48% ($^w/_v$) aqueous solution of the sodium salt of bentazone (BAS 35107H). Additives used were Actipron (B.P. Chemicals), ammonium sulphate (BDH) and 2-methoxyethanol (BDH).

Whereever possible, plants were sprayed when they had reached the 8th-12th fully expanded leaf stage. Where this was not possible, reference has been made in individual experiments.

1.2.5 Assessment of damage

Herbicide damage to plants was assessed on an individual plant basis on a 0 - 5 scale:-

0 = no effect

1 = slight flecks, no sign of withering

2 = chlorotic areas - upto 25% leaf area withered

3 = " " - " 50% " " "

4 = " " - " 75% " " "

5 = plant death

In some field experiments damage was assessed on a plot basis on a 0 - 10 scale, where 0 represents no damage and 10 is equivalent to total control.

The initial symptoms of damage in C. album are a curling of the leaf edge, followed by general leaf necrosis. Wilting soon becomes evident, culminating in plant death.

Assessments were made on several occasions following herbicide application in order to follow the pattern of plant necrosis under the different treatments administered.

1.2.6 Analysis of results

Due to the relatively small number of plants used per treatment, and also the arbitrary nature of the damage assessment scale, a complete analysis of variance was not attempted on many of the smaller scale experiments. In these experiments standard deviations were calculated.

1.3 RESULTS AND DISCUSSION

1.3.1 The efficacy of spray equipment under glass and in the field

Much of the preliminary work involved an assessment of the range of spraying equipment available and to select from this range a spray system which would prove efficient under glasshouse and field conditions, both in terms of consistency of application and ease and accuracy of use. The sprayers used are listed in descending

order of efficacy as follows:-

a) Shandon Chromatography sprayer

b) Binks Bullows "Spraybee" compressor sprayer

c) Knapsack sprayer

d) Killaspray unit

There was little difference between the Shandon and Binks Bullows sprayers in terms of spray quality. The major disadvantage of the "Spraybee", however, is the fact that an electricity supply is required to operate the compressor pump, and hence, under a field situation, this would be less practical. The Shandon sprayer, on the other hand, is light, easy to handle, and is ideal for spraying relatively small areas. The major drawback with this sprayer is the fact that, since it is operated by an aerosol, the nozzle tends to "Freeze" with prolonged use, particularly so when the canister is full. This, however, is by no means a deterrent to its use. A major advantage of both these spray systems is their low volume capacity. This means that, for small plot experiments, the calculated volume can be measured accurately and dispersed completely within the experimental area.

The Knapsack sprayer produced a relatively fine and even spray, though by no means comparable with the Shandon or Spraybee. Nevertheless, effective coverage was generally achieved. The principal disadvantage of this spray equipment is its large volume capacity, which means that small volumes cannot be accurately dispensed and thus this type of sprayer is limited to larger-scale field trials. The Killaspray unit produced a relatively coarse and uneven spray, which proved inconsistent both under glasshouse and field conditions. Its large volume capacity was a further disadvantage.

When dealing with principally contact herbicides such as Basagran, spray efficacy is of utmost importance in achieving good plant cover. In view of this, the Shandon Chromatography sprayer was considered to be the most effective and convenient, and was thus selected as a standard spray system for the subsequent work described in Chapter 4.

1.3.2 The influence of concentration on the susceptibility of C. album to Basagran and Actipron

Experiments conducted under glass consistently showed that applications of Basagran made at the recommended field rate of 3 l/280 l H_2O/ha were very effective in the control of C. album. Applications of the herbicide at one tenth this rate (0.3 l/ha) also proved effective under glass, albeit over a longer period of time. Under field conditions, however, applications of Basagran at 0.3 l/ha proved ineffective (Appendix 1, Table 1). It is perhaps of significance to note that plants grown under glasshouse conditions are generally more susceptible to herbicide treatments than their counterparts growing in the field. This observation correlates well with that of Hull (1958), who concluded that plants grown under glass developed thinner cuticles due to the lack of ultra-violet light which is unable to penetrate the glass screen. Applications of Basagran at concentrations between these limits (i.e. 0.3 - 3 l/ha) produced some promising results in the field. When applied at a concentration of 2.25 l/ha, for example (Appendix 1, Table 11), control was comparable to that achieved at field rate. However, it appears that, although adequate control can be achieved at concentrations below the recommended field rate, consistency appears to be rather variable. In an experiment conducted at Saxilby (Lincs), for example, applications of Basagran made at 2 l/ha were significantly

less effective ($p = 0.01$) than applications made at 3 l/ha (Appendix 1, Table 111; Appendix 11, Tables 1 , 11). Since these experiments were conducted using either the Shandon or the Spraybee, it is unlikely that these differences in susceptibility are related to different efficacies of spray application. Differences in susceptibility may, however, be related to the size of the weed at application time. Indeed, it is perhaps of significance to note that plants sprayed at the Saxilby site were generally larger (approx. 25 cm) than those sprayed on Trent Polytechnic grounds. Perhaps of equal importance is the fact that the soil at the Saxilby site was of clay type, whereas that of the Trent Polytechnic grounds was very sandy. It may also be pertinent to note that environmental conditions at the time of herbicide application varied considerably, which may well account for the differences in susceptibility. Clearly, more detailed information of this kind is essential in assessing the response of plants to herbicide treatments.

Herbicide performance was enhanced on all occasions both under glass and in the field when the oil adjuvant, Actipron, was incorporated into the formulation. Here, concentration appeared to be of less importance. Under glasshouse conditions, for example, applications of Basagran containing Actipron at the two recommended rates of 1 and 2 l/ha showed no difference in C. album control. This effect was also found to be consistent under field conditions. In the experiment conducted at Saxilby, for example, a range of Actipron concentrations between 0.5 and 2.5 l/ha showed no significant difference in enhancement of herbicide performance (Appendix 1, Table 111; Appendix 11, Tables 1, 11). These observations contrast with those of King (1976), who concluded that Actipron used at the higher recommended rate of 2 l/ha was more reliable in terms of weed

control These trials were conducted in the 1975 and 1976 seasons, where the weather conditions were exceptionally hot and dry for prolonged periods. The investigations in this study were carried out in the 1977 season, which on the whole, proved to be rather cooler and wetter. Clearly, considerations of this kind should be made before decisions on application rate are taken.

1.3.3 The influence of other additives on the susceptibility of C. album to Basagran

Since adequate control of C. album had been achieved in the glasshouse with Basagran at 0.3 l/ha (section 1.3.2), the influence of ammonium sulphate and 2-methoxyethanol was assessed at this rate under glass (Appendix 1, Table IV). Both additives improved the control of C. album by Basagran, comparable to that achieved with Actipron. When assessed under field conditions, Basagran only proved effective at its recommended rate of 3 l/ha (Appendix 1, Table 1). The influence of additives appeared to vary between experiments. In some cases their presence did not appear to enhance herbicide performance to any significant degree (Appendix 1, Table 1), whereas in others a substantial improvement in herbicide performance was achieved in their presence (Appendix 1, Table V). Under field conditions, however, none proved as effective as the recommended additive, Actipron.

As discussed previously, these variations in susceptibility may arise as a result of different field populations or may, indeed, be influenced by the prevailing environmental conditions at the time of application. Where the additives appeared to have little effect, conditions were very warm and dry (Appendix 1, Table 1). Greater effectiveness was observed where plants were sprayed during cooler, more moist conditions (Appendix 1, Table V).

It is interesting to note that ammonium sulphate appeared to enhance herbicide activity both under glass and, in some cases in the field. This effect has previously been reported and some workers have reported increased crop yields as a result (Ladonin 1961; Likholat 1962). Clearly, these reports, together with the findings reported here would favour more intensive investigations into this aspect of weed control.

1.3.4 The effect of plant size on the susceptibility of C. album to Basagran and Actipron

Experiments both in the glasshouse and in the field have consistently shown that the susceptibility of C. album to Basagran is inversely proportional to plant height at the time of herbicide application (Appendix 1, Tables VI, VII). Plants sprayed between 5 and 10 cm in height were highly susceptible, whereas plants at 40 cm in height showed considerably more tolerance to the herbicide. It is perhaps of significance to note that, under glasshouse conditions, susceptibility appeared to be independent of plant size when Actipron was incorporated into the formulation. The full significance of this finding will be further developed in the light of further investigations in the final discussion. This effect was not so apparent, however, in plants in the field (Appendix 1, Table VII). It seems likely that the greater susceptibility of young plants is related to their greater overall rate of metabolic activity which, under more favourable glasshouse conditions, is more pronounced. Indeed, Muzik (1976) has reported that susceptibility is greater in plants of high meristematic activity. Clearly, investigations of this type would yield more meaningful results with the inclusion of quantitative assessments (e.g. fresh/dry weight) and more detailed morphological measurements (e.g. leaf no., internodal length).

1.3.5 The influence of application time on the susceptibility of C. album to Basagran

From the outset of this work it became evident that prevailing environmental conditions at the time of herbicide application may have an important influence on its subsequent performance. It has also been reported (e.g. Weaver and Nylund 1963; King and Handley 1976) that the efficacy of some herbicides varies with application time. In view ofthis, experiments were conducted to assess the influence of time of application on the efficacy of Basagran in the control of C. album.

From two experiments conducted, the data from one of which are presented in Appendix 1, Table VIII, results have consistently indicated a trend towards reduced efficacy following a mid-day application. Applications made early morning and late afternoon tended to be more effective in the control of C. album in the field. This effect has also been reported in personal communications from farmers in the district, who have concluded that applications of Basagran made early morning and late afternoon resulted in improved weed control in french bean crops.

This aspect of weed control requires more thorough investigation in the future with complete replication of experiments, and perhaps a more quantitative assessment of herbicide damage by fresh/dry weight determinations. The effect of Actipron should also be assessed during different application periods.

It must be stressed that the range of experiments described in this introductory chapter are purely exploratory in nature and any conclusions

drawn from them are highly tentative. It could be argued that the assessment of herbicide damage by visual means is very arbitrary, which indeed, it is. Nevertheless, the experienced eye can readily differentiate within such a narrow scale, and it is felt that an extension of the scale to 0 - 10 for individual plant assessments would lead to a greater degree of human error. It must be conceded, however, that a visual assessment together with a quantitative type of assessment (e.g. fresh/dry weight; chlorophyll determination) would perhaps yield more valid results. In these experiments, however, a continual assessment was made of treated plants over a 2-3 week period following herbicide application, which, with the restrictions of plant material and space, was considered to be more informative than to make single destructive quantitative assessments. It would also have been of value to have made more detailed observations of plant development during the treatments period, such as leaf numbers and internodal length, both at the time of herbicide application and at the time of visual assessment. This would perhaps have made visual assessments more meaningful.

In the field it is apparent that environmental conditions play an important role in determining the response of C. album to Basagran, and the results emphasize the need for more detailed recording of temperature, light intensity, relative humidity, soil moisture and soil type. Indeed, although these preliminary experiments have outlined the wide range of factors which may influence herbicide performance, the overriding factor to consider is the influence of environment. However, in order to quantify the influence of environmental components individually, detailed experiments must be conducted in controlled environmental chambers. Thus, the remaining chapters of this thesis are primarily concerned with elucidating the role of different environmental conditions on the response of C. album to Basagran and Actipron.

CHAPTER 2

THE PHYSICAL PROPERTIES OF SPRAY SOLUTIONS IN RELATION TO THE LEAF SURFACE OF C. ALBUM

2.1 INTRODUCTION

The physical characteristics of spray solutions, and of the surfaces to which they are applied, can provide valuable information with regard to spray retention and herbicide performance. The addition of surfactants to herbicide formulations, for example, is known to enhance penetration by a reduction in the surface tension of the solution (Weintraub *et al* 1954; Holly 1964). Penetration is also influenced by the degree of contact of the spray droplet with the leaf surface.

The following experiments are included to demonstrate the relationship between contact angle, surface tension and leaf surface wettability, and how these factors are altered in the presence of additives such as Actipron. These studies were also incorporated into the environmental work (Chapter 4) in an attempt to assess the influence of environment on leaf wettability in relation to herbicide penetration.

2.2 MATERIALS AND METHODS

2.2.1 Surface Tension Measurements

The surface tension of Basagran at the recommended field rate (3l/ha) alone and in combination with a range of Actipron concentrations (0-1.0% $^{v}/_{v}$) was determined by the following method:-

A Cambridge De Noüy tensiometer was used for the investigations. Essentially this instrument measured the minimum force required to detach a circular ring of platinum wire from the surface of a liquid which it meets with a contact angle of zero degrees.

The instrument was calibrated using a 0.5g weight. A shallow beaker was then filled with 10 ml of the test solution and five readings were taken and averaged. The platinum ring was dipped in methanol and flamed between determinations to avoid contamination. Throughout this work an attempt was made to maintain room temperature to a constant level to reduce error.

2.2.2 Contact Angle Measurements

Leaf strips, 5mm width, were cut perpendicular to the midrib. The strip was fixed onto a stage using double-sided adhesive tape. A 2 µl drop of the test solution was formed at the tip of a micro-syringe and carefully placed onto the leaf surface.

Advancing contact angles were determined according to the method reviewed by Furmidge (1965). The image of the droplet was projected onto a clear screen and the advancing contact angle was measured directly. The procedure was repeated with five separate droplets. Contact angles were determined for Basagran (3l/ha) together with a range of Actipron dilutions (0-1.0% $^{v}/_{v}$) on both adaxial and abaxial surfaces of *C. album*.

2.3 RESULTS

2.3.1 Surface Tension Measurements

Surface tension determinations made of Basagran, diluted to a field rate of 31/280l H_2O, plus a range of Actipron concentrations are summarised in Fig. 6a.

Increases in the concentration of Actipron resulted in corresponding decreases in surface tension. Once a surface tension of approximately 32 dynes cm^{-1} had been reached, however, increasing the concentration of Actipron resulted in no further corresponding decreases in surface tension. This point corresponds to an Actipron concentration of 0.5% when in combination with Basagran at field rate.

The reductions in surface tension are not a function of the oil alone, but are associated with the properties of the surfactant or cosolvent within the Actipron formulation. These surfactants are normally added to ensure sufficient dispersal of the active ingredient between oil and water phases. Maximum reductions in surface tension usually occur within the critical micelle concentration (CMC) for the surfactant, which in this case, occurs at an Actipron concentration of 0.5% ($^{v}/_{v}$). It is at this CMC that the surfactant molecules are believed to form micelles or aggregates with their hydrophilic groups towards the inside, thus completely altering the physical characteristics of the solution.

2.3.2 Contact Angle Measurements

Contact angle determinations of Basagran (31/280l H_2O/ha) and a range of Actipron concentrations made on both adaxial and abaxial surfaces of C. album are summarised in Table I.

Table I The effect of Actipron concentration on the contact angle of Basagran made on both adaxial and abaxial surfaces of C. album

Actipron concentration (%)	Contact Angle	
	Adaxial	Abaxial
0	$128^{\circ} \pm 3^{\circ}$	$142^{\circ} \pm 3^{\circ}$
10^{-4}	$124^{\circ} \pm 2^{\circ}$	$138^{\circ} \pm 3^{\circ}$
10^{-3}	$120^{\circ} \pm 3^{\circ}$	$130^{\circ} \pm 4^{\circ}$
10^{-2}	$111^{\circ} \pm 4^{\circ}$	$115^{\circ} \pm 3^{\circ}$
10^{-1}	$107^{\circ} \pm 3^{\circ}$	$112^{\circ} \pm 4^{\circ}$
5×10^{-1}	$27^{\circ} \pm 4^{\circ}$	$43^{\circ} \pm 2^{\circ}$
1.0	$25^{\circ} \pm 2^{\circ}$	$25^{\circ} \pm 3^{\circ}$

Contact angles for Basagran at field rate were found to be 128° and 142° for the adaxial and abaxial surfaces of C. album respectively. The addition of Actipron to the formulation resulted in reduced contact angles on both surfaces. As the concentration was increased, the difference in contact angle between the two surfaces diminished, and at a concentration of 1% Actipron both surfaces displayed equal contact angles of 25°.

The relationship between surface tension and contact angle is well illustrated in Fig. 1b. As the surface tension was lowered, a gradual decrease in contact angle was observed. Once a surface tension approaching 32 dynes cm^{-1} had been reached, however, sharp reductions in contact angle were observed.

FIGURE 1. Physical properties of Basagran and Actipron spray solutions

A. The effect of Actipron concentration on the surface tension of Basagran

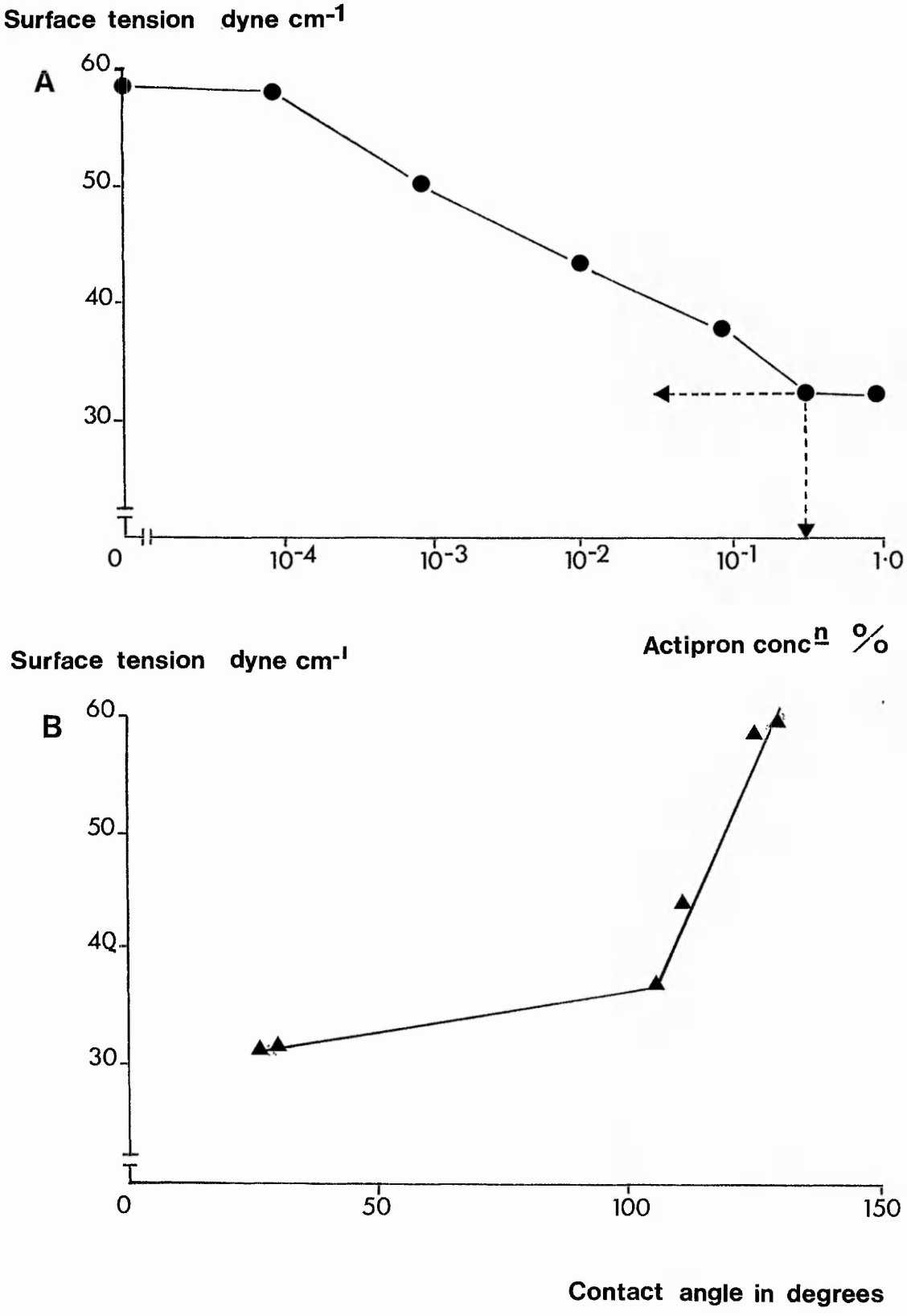
Surface tension determinations were made using Basagran at field rate (3 l/280 l H_2O/ha) plus a range of Actipron concentrations (1 - 10^{-4} % v/v).

Arrows indicate the concentration of Actipron at which maximum reductions in surface tension are observed, equivalent to the CMC for its constituent surfactant.

B. Contact angle vs. surface tension of Basagran and Actipron

Contact angles, determined on the adaxial surface of C. album leaves using Basagran (field rate) and Actipron (1 - 10^{-4} % v/v), were plotted against the surface tension values obtained for these solutions. Maximum reductions in contact angle occur in the region of 32 dynes cm^{-1}.

FIGURE 1


Surface tension dyne cm-1
A
60
50
40
30
0
10-4
10-3
10-2
10-1
1·0
Actipron conc^n %
Surface tension dyne cm-1
B
60
50
40
30
0
50
100
150
Contact angle in degrees

2.4 CONCLUSIONS

From the experiments in this chapter it can be concluded that:-

(1) Maximum reductions in surface tension were achieved when Actipron was used in the Basagran formulation at a concentration of 0.5 % (v/v).

(2) Greater contact angles were determined for Basagran on the abaxial leaf surface of C. album.

(3) The contact angles recorded for Basagran on both abaxial and adaxial leaf surfaces of C. album were substantially reduced when Actipron was incorporated at a level of 0.5% (v/v), equivalent to the CMC for its constituent surfactant.

2.5 DISCUSSION

From the results presented it is apparent that at Actipron concentrations in the region of 0.5%, the physical characteristics of the herbicide solution are completely altered. This is believed to be largely a function of the surfactant present within the Actipron formulation. These are usually present at concentrations in the region of 5%, but unfortunately, detailed information with regards concentration and the chemical composition of both the oil, Actipron and its surfactant are not readily available and so further interpretation of these effects cannot be developed. Nevertheless, it can be concluded that the concentration of Actipron (0.5% v/v) which results in maximum reductions in surface tension and contact angle is equivalent to the CMC for the surfactant. Thus, it is not strictly correct to refer to the 0.5% (v/v) concentration range for Actipron as being its CMC. In this context, this concentration will be subsequently referred to as the surfactant CMC.

It is interesting to note that the recommended field rates for Actipron (1 and 2 l/ha) are equivalent to 0.36% and 0.72% respectively and that these figures closely approximate to the recorded surfactant

CMC at 0.5% (v/v) Actipron. Indeed, determinations carried out on these two field rates in combination with Basagran at 3 l/ha resulted in surface tensions of 32.98 and 32.82 dynes cm^{-1} respectively. The fact that the higher rate of Actipron has proved more reliable in terms of enhanced weed control in the field (King 1976) suggests that surface tension reduction may not be the principal function of the additive. Indeed, Foy and Smith (1965), investigating the activity of dalapon on maize, found that in all surfactants examined minimum surface tension and contact angles occurred at 0.1 - 0.5%, but that maximum herbicidal activity occurred at ten times these levels. Thus, above the low concentrations, herbicidal enhancement was not correlated to surface tension lowering, contact angle or wettability. It has been suggested (Van Overbeek and Blondeau 1954) that at high concentrations, where the surfactant molecules form micelles, the surface tension remains constant but the micelles may solubilise the cutin with subsequent removal of the wax.

The relationship between surface tension and contact angle is an interesting one in that maximum reductions in surface tension and contact angle both occur within the surfactant CMC range. This finding correlates well with previously published data by Schonherr and Bukovac (1972). These authors measured contact angles and surface tension of a range of surfactants and found maximum reductions in contact angle on Zebrina leaves occurred with surfactant solutions of surface tensions approaching 30 dynes cm^{-1}.

The high contact angle recorded on the abaxial surface of C. album (142^{o} compared to 128^{o} on the adaxial surface) suggests that this surface is strongly hydrophobic. The hydrophobic nature of the leaf surface is partly a function of its epicuticular wax. Indeed, the importance of surface wax on leaf wettability has been studied by

Holloway (1969). He suggests that on leaves displaying contact angles below 90° the surface wax is not a prominent feature of the leaf surface. Contact angles greater than 90°, however, suggest that wax plays a significant part in the hydrophobic properties of the surface. It would appear, therefore, that the wax present on the surface of C. album leaves plays a significant role in determining the wettability of the leaf surface.

CHAPTER 3

LEAF SURFACE ANALYSIS OF C. ALBUM

3.1 INTRODUCTION

The plant cuticle, and in particular, deposits of the epicuticular wax, is reported by many workers as being the prime barrier to the foliar penetration of herbicides (e.g. Leece 1976), and many workers believe that differences in wettability and penetration between species are related to epicuticular wax composition and ultrastructure (Silva Fernandes 1965).

It is the aim of this chapter, therefore, to develop and standardise techniques for the analysis of the epicuticular wax of *C. album*, including chemical analysis by TLC and GLC and ultrastructural studies with the aid of a scanning electron microscope. Once standardised, the techniques were also incorporated into the environmental work described in Chapter 4, in order to assess the influence of environment on the chemical composition and physical configuration of the epicuticular wax in *C. album*.

3.2 MATERIALS AND METHODS

3.2.1 The chemical analysis of the epicuticular wax of C. album by TLC and GLC

3.2.1.1 Sample preparation

Epicuticular wax was extracted from leaves of C. album (6-10g) by immersion in chloroform (A.R.) for 30 seconds. Silva Fernandes et al (1964) have shown that this length of time is sufficient to remove all traces of surface wax without contamination from intracellular lipids. The extracts were combined and evaporated to dryness by rotary evaporation and dried to constant weight. The wax extract was taken up in a known volume of chloroform (0.5 - 1.0 ml) ready for analysis.

3.2.1.2 Thin-layer chromatography

For qualitative work the wax sample was spotted onto glass plates (20 x 20cm) coated with 25mm Kieselgel G (Type 60) and the plate was then run in toluene to within about 2cm from the top (45-60 mins). The plate was removed from the solvent tank and all traces of toluene were removed with a hot air drier. The plate was then sprayed with 40% sulphuric acid and heated in an oven maintained at a temperature of 150°C for 15-30 minutes, until brown spots appeared. This revealed the major wax classes present.

For preparative work, samples were banded onto 75mm thick Kieselgel G plates. After running in toluene, the plates were developed by placing in a tank containing a few crystals of iodine. Yellow-brown spots appeared within a

few minutes. This staining technique proved to be a quick and reliable method of detecting all major wax classes, and, being non-destructive, enabled further analyses to be carried out on the fractions.

Following staining, the bands were scraped off the plates and the components eluted with warm (50°C) chloroform. Each fraction was then evaporated to dryness in a stream of nitrogen ready for further analysis.

Identification of the various fractions was assisted by spotting a sample of the leaf surface wax from brussels sprout (Brassica oleracea) leaves onto the plate adjacent to the samples. This wax contains all the major wax constituents and has proved to be an ideal standard.

3.2.1.3 Gas-liquid chromatography

All analyses were carried out on a Perkin-Elmer F33 gas chromatograph fitted with a flame ionisation detector. A 3-foot glass column, internal diameter 3mm, was used, packed with 1.5% Dexsil 300 on chromosorb W (80-100 mesh, AW DMCS).

The major peaks were identified using standards kindly provided by Dr. E. A. Baker (Long Ashton Research Station, Bristol).

Hydrocarbons, esters, aldehydes and ketones were analysed directly by injecting a sample dissolved in chloroform, onto the column. Alcohols, although they can be analysed directly, are better resolved when converted to their trimethylsilyl ether derivatives. This was achieved by reacting the sample with 50-100µl, N, O-bis (TMS)

acetamide for 30 minutes at 50°C. The sample was then injected directly onto the column.

Fatty acids were analysed as their methyl esters, by reaction with diazomethane. Preparation of diazomethane:- 0.4g potassium hydroxide was dissolved in 96% ($^{V}/v$) ethanol (10ml). A solution of 2.14g p-tolyl-sulphonyl methylnitrosamide was made in 30ml dry ethyl ether. The solutions were mixed in a round-bottomed flask for 5 minutes and then a simple distillation head and receiver were attached. The mixture was heated gently and the diazomethane then collected in a round-bottomed flask containing a little dry ether, immersed in an ice bath.

Methylation technique:- The wax sample was dissolved in 0.5-1.0 ml ethyl ether and a few drops of ethanol were added. The diazomethane solution was added dropwise until the yellow colour persisted. The mixture was left at room temperature for 15 minutes,, after which the solvent was evaporated in a stream of nitrogen. The sample was then taken up in chloroform (0.5-1.0ml) ready for analysis.

The acid and alcohol components of the esters were obtained by methanolysis with methanol/acetyl chloride:-

The ester sample was dissolved in 1ml dichloromethane and 2ml methanol/acetyl chloride were added. The solution was refluxed for 3 hours and then 5ml NaCl were added. The esters were extracted with 2 x 5ml portions of hexane. The hexane layer was washed with 4ml potassium bicarbonate (2% $^{W}/v$) and dried over anhydrous sodium sulphate. The solution was evaporated to dryness and the constituent acids and

alcohols were prepared for analysis as before.

Determination of response factors:- In order to correct for detector characteristics, response factors were determined for each wax fraction present. This was achieved by analysing standard samples of known concentration. Peak areas were determined by measuring peak height x width at half height. From this the area produced per concentration unit was determined. All standards were then expressed as factors relative to a selected reference standard.

Calculation of percentage composition of samples:- Having determined the response factors for each fraction, peak areas of individual homologues within each sample were calculated as before and multiplied by the corresponding response factor in order to obtain a corrected area. The percentage composition of homologues was then determined by expressing each corrected area as a percentage of the total corrected area.

3.2.2 Investigation of epicuticular wax ultrastructure by scanning electron microscopy

3.2.2.1 Sample preparation

Adaxial and abaxial sections (about $5mm^2$) were removed from the leaves of C. album and mounted on 1cm diameter aluminium stubs using double-sided adhesive tape. The stubs were coated with gold using a Polaron Mark II E, 5,000 sputter coater. The pressure in the coating unit was reduced to 0.02-0.03 Torr over a period of 2 minutes. Gold was then evaporated from a disc forming a cloud which provided an even coating of the specimen.

3.2.2.2 Scanning electron microscopy

Examination of the specimens was carried out using either a Cambridge Sterioscan Mark IIa electron microscope (courtesy of Long Ashton Research Station, Bristol), or a Cambridge Sterioscan 600 electron microscope (Trent Polytechnic), operated at H.T. voltages of 10kv and 25kv respectively.

3.3 RESULTS AND DISCUSSIONS

3.3.1 Thin-layer chromatography of the surface wax of C. album

The fractionation of the epicuticular wax of C. album and Brassica oleracea by TLC is shown in Fig. 2. The surface wax of C. album is shown to consist of a mixture of hydrocarbons, esters, aldehydes, primary and secondary alcohols and fatty acids.

The methodology for the chemical analysis of the epicuticular wax is essentially similar to that developed by Dr. E. A. Baker (personal communication), with the singular exception of the use of the mobile solvent phase in the TLC work. Although benzene is generally employed by Dr. Baker as the mobile phase, toluene was adopted in these investigations, largely for safety reasons, but also due to the slightly better resolution of the non-polar constituents.

Various other solvent systems were also investigated. Dichloromethane, for example, though recommended by Holloway and Challen (1966) resulted in poor resolution throughout these investigations. A mixture of ethyl acetate/chloroform ($^{1}/_{1}$, $^{v}/_{v}$) resulted in no resolution whatsoever, and toluene/chloroform (7:3) resolved the acid and alcohol components, but not the esters, ketones and hydrocarbons.

The use of iodine as a non-destructive stain was also compared with other techniques outlined by Holloway and Challen (1966). Rhodamine 6G (aqueous) and 2,7-dichlorofluorescein were both investigated, and although the sensitivity of the methanol was perhaps equivalent to that employing iodine, the technique in itself proved rather inconvenient and time-consuming.

FIGURE 2. The separation of the epicuticular waxes of C. album and B. oleracea by thin-layer chromatography

Samples of epicuticular wax, dissolved in chloroform, were spotted onto Kieselgel G plates and developed in toluene.

Fractions were stained by treatment with 40% (v/v) sulphuric acid and charring for 30 mins in an oven at 150°C.

FIGURE 2

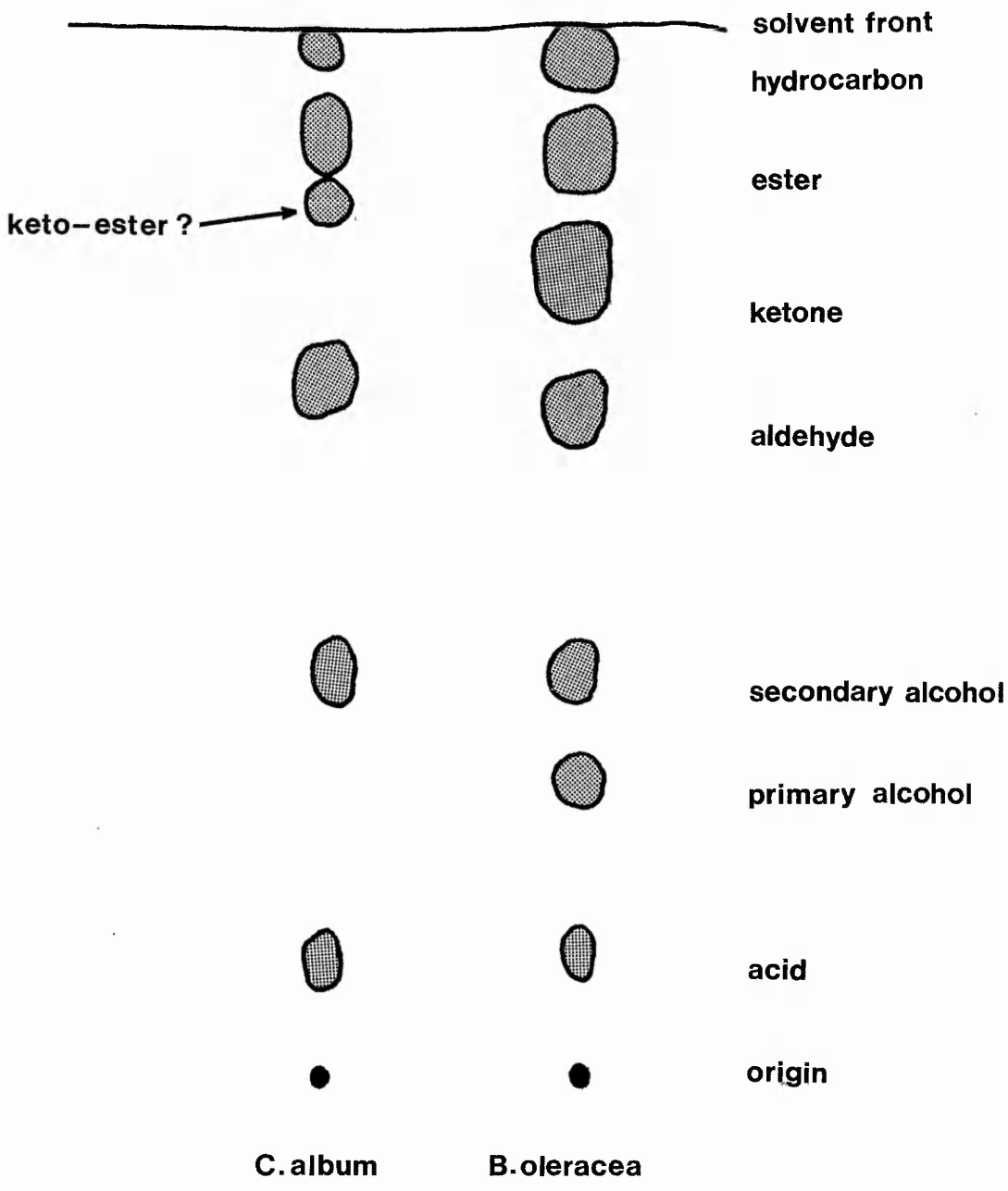

solvent front
hydrocarbon
ester
keto-ester ?
ketone
aldehyde
secondary alcohol
primary alcohol
acid
origin
C. album
B. oleracea

FIGURE 3. Chemical composition of the epicuticular wax of C. album grown under normal glasshouse conditions

GLC analysis using 1.5% Dexsil 300 on Chromosorb W (80-100 mesh, AW DMCS). Temperature programme : 150-350°C at 5°C/min. N_2 = 40 ml/min.

The numbered peaks represent the following constituents:-

1, 2, 3 - C_{25}, C_{27}, C_{29} hydrocarbons

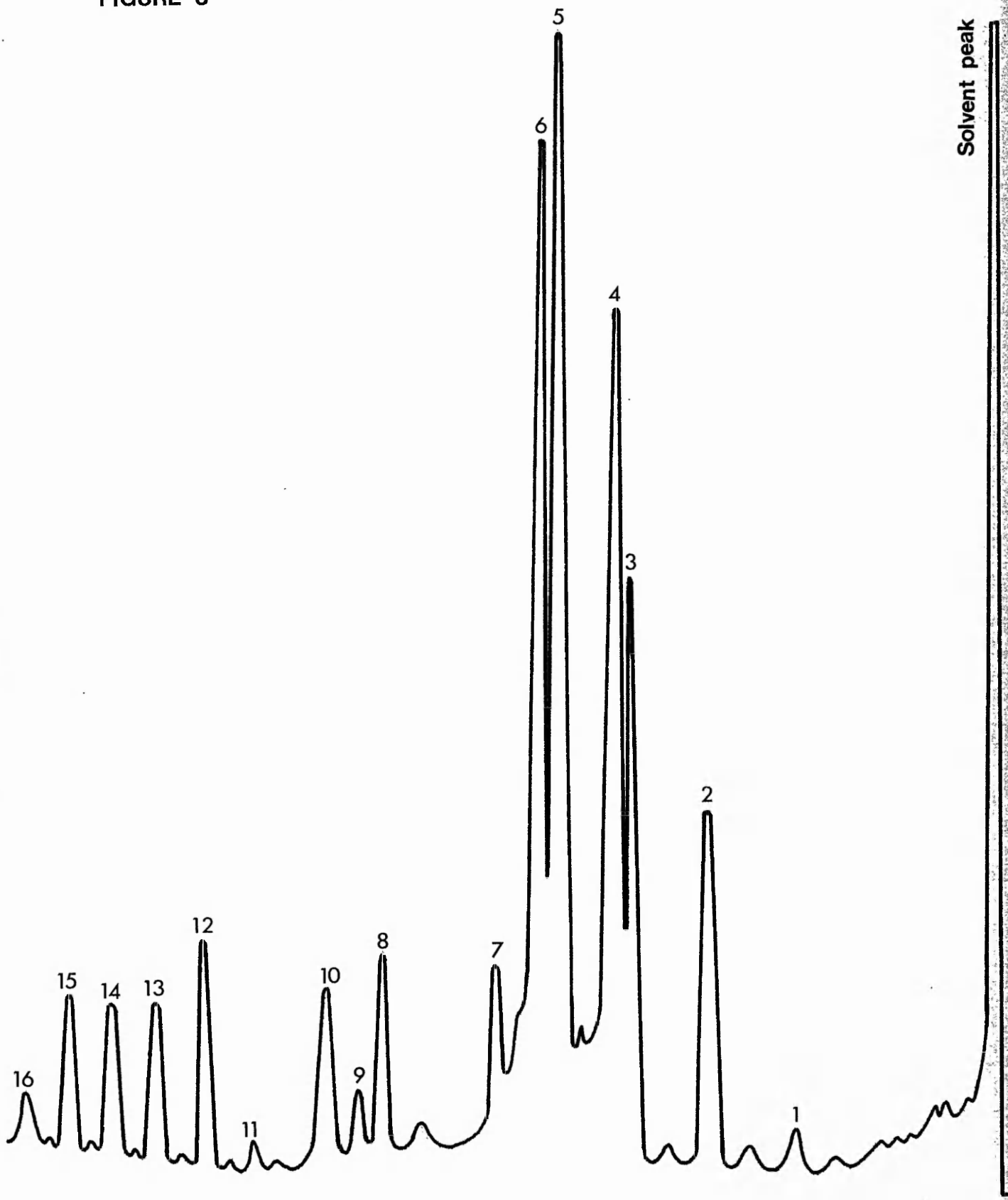
4, 6 - C_{26}, C_{28} alcohols

5, 7 - C_{28}, C_{30} aldehydes

8,9, 10 - keto-esters

11-16 - C_{40}-C_{50} esters

FIGURE 3



3.3.2 Gas-liquid chromatography of the epicuticular wax from C. album

Fig. 3 shows the separation of the epicuticular wax of C. album by GLC using a Dexsil 300 column. This sample is underivatised and hence the fatty acid components are absent due to their low volatility.

The percentage composition of the homologues was determined, results of which are presented in Table 11. Aldehydes and alcohols appeared to be the major constituents, with the C_{28} components being the predominant homologues. Hydrocarbons (C_{27-29}) and esters (C_{42-50}) were also detected in significant quantities. Fatty acids components were not detected in significant amounts in any of the analyses.

Table 11. The Chemical composition of the epicuticular wax of C. album

Homologue	% Composition
C_{27} Hydrocarbon	3.3
C_{29} "	3.3
TOTAL HYDROCARBON	6.6
C_{26} Alcohol	15.3
C_{28} "	29.4
TOTAL ALCOHOL	44.7
C_{28} Aldehyde	30.3
TOTAL ALDEHYDE	30.3
Keto-ester	4.4
C_{40} ester	-
C_{42} "	5.0
C_{44} "	4.2
C_{46} "	1.6
C_{48} "	1.9
C_{50} "	0.6
TOTAL ESTER	13.3

Methylation of the samples proved to be difficult. This may have been due to the method of preparation and storage of diazomethane, since water must be excluded from the solution. The use of N, O-bis (trimethylsilyl) acetamide, however, proved to be a very successful method of derivatising the alcohol fractions and resulted in a smoother baseline. Because of the difficulty in the

preparation of fatty acid derivatives using diazomethane, any fatty acids present were detected by conversion to their TMS derivatives using N,O-bis (TMS) acetamide in more recent analyses.

The ester fraction was confirmed by methanolysis of the sample, which resulted in a breakdown of the esters into their constituent acids and alcohols. There did, however, remain one series of peaks in the C. album sample which was eluted with the ester band, and did not appear to break down on methanolysis. Further TLC plates were prepared and carefully examined for any additional spots which may explain the anomaly. An additional spot was observed below the ester spot (Fig. 2) which, when eluted and analysed by GLC, represented the peaks under scrutiny. It would appear that these peaks were in fact keto-esters, although confirmation by GLC-mass-spectrometric techniques has not been possible to date.

The epicuticular wax of C. album has previously been investigated by Allebone et al (1970) and Allebone and Hamilton (1972). These authors also report high proportions of aldehydes and alcohols present in the wax, although the presence of keto-esters was not reported. It may well be, however, that different clones of C. album were investigated, which may account for these differences in composition.

3.3.3 Scanning Electron Microscopy of the leaf surface of C. album

The surface topography of C. album was investigated at a range of magnifications. Figs. 4 and 5 show both abaxial and adaxial surfaces of C. album grown under normal glasshouse conditions. The epicuticular wax was present in the form of small platelets protruding from the surface. The wax did not, however, appear to

form an homogeneous covering over the leaf surface. The platelets were less dense, for example, over areas of the midrib and over large veins. Significant reductions in the density of wax also occurred in the vicinity of the stomata (Fig. 6). It is also interesting to note that the density of platelets appeared to be greater on the abaxial surface of the leaf, although the actual pattern of the wax deposits remained the same.

The surface of C. album also displayed significant quantities of collapsed spheres, the density of which appeared to be greater on the abaxial surface, and particularly so on young, immature leaves. These spheres have previously been reported by Brian and Cattlin (1968), although their function has not yet been elucidated.

FIGURE 4. The surface topography of C. album leaves - adaxial surface

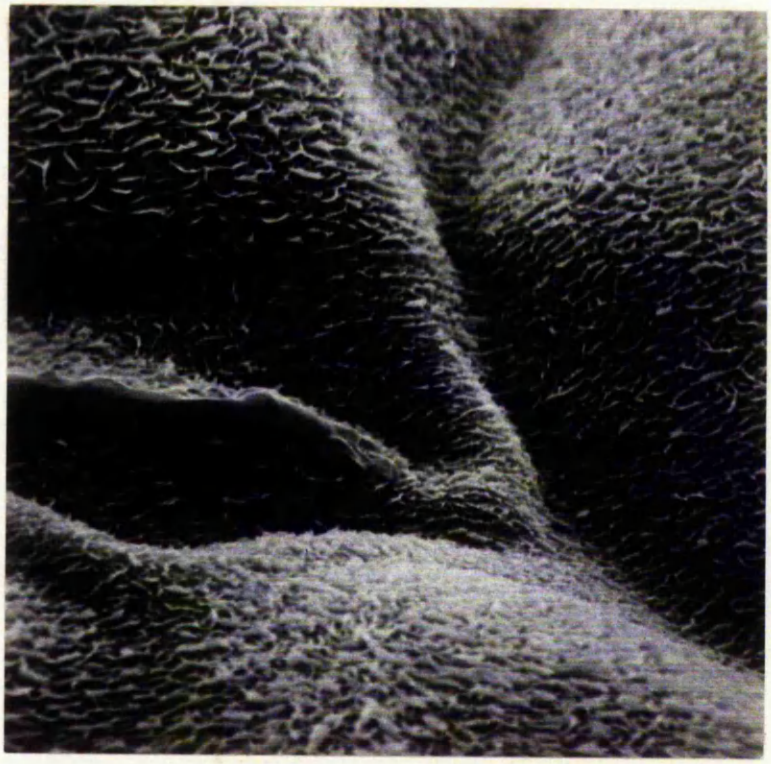
The adaxial surface is densely covered with platelets of epicuticular wax, the average size of individual platelets being 1 x 0.2 um (length x depth).

a) Adaxial surface, Mag 5.45 K

b) Adaxial surface, Mag 11.2K

FIGURE 4

A



B

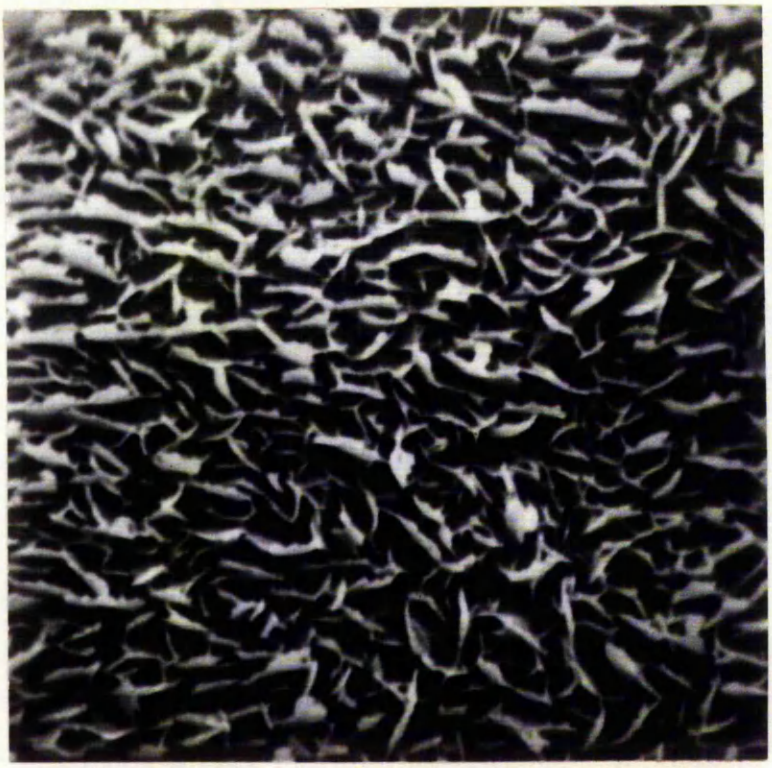


FIGURE 5 . The surface topography of leaves of C. album - abaxial surface

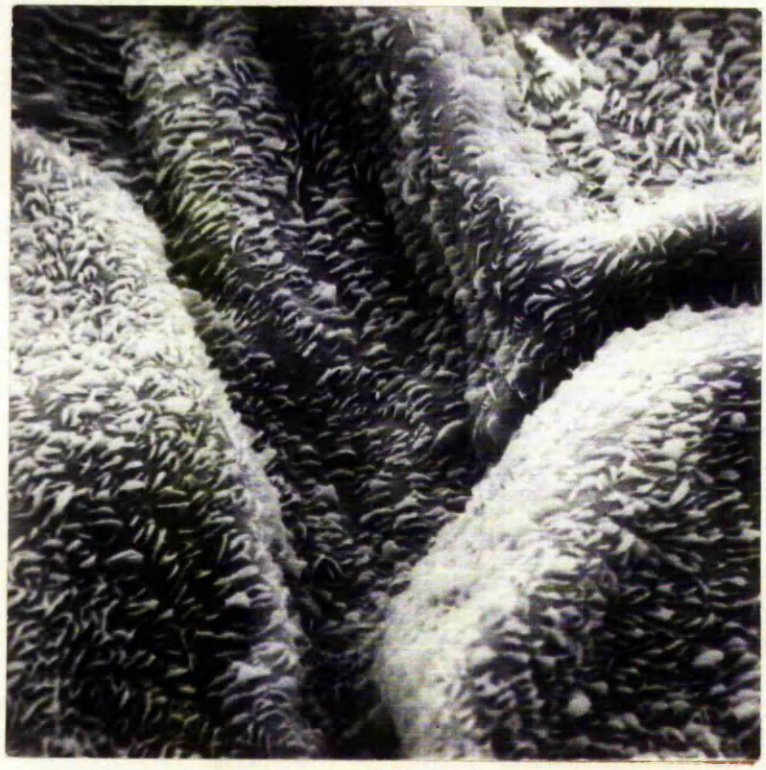
The distribution of epicuticular wax on the abaxial surface is of a greater density than that on the adaxial surface, though the specific pattern remains unchanged.

a) Epicuticular wax, Mag 5.45 K

b) Wax platelets, Mag 11.0 K

FIGURE 5

A



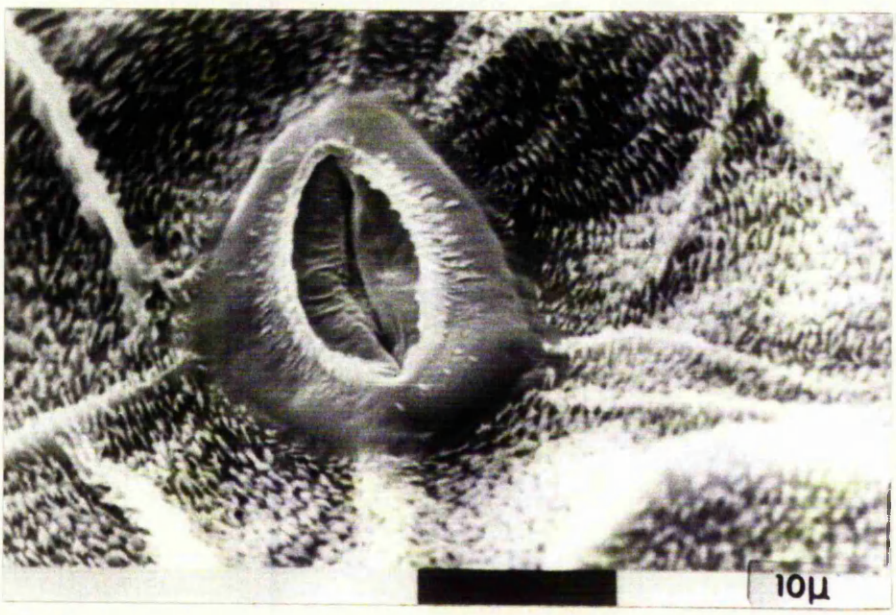
B

FIGURE 6

Stoma on the adaxial surface of C. album. The surrounding epidermal cells are densely covered with wax platelets, while the area in the immediate vicinity of the stoma remains virtually devoid of surface wax projections.

Mag 2.2K.

FIGURE 6



CHAPTER 4

CONTROLLED ENVIRONMENTAL STUDIES

4.1 INTRODUCTION

The influence of environment on herbicide performance is well documented (e.g. Muzik and Maudlin 1964; Hammerton 1967), and from the preliminary investigations under glass and in the field (Chapter 1), it has become apparent that environmental factors play a significant role in determining the response of *C. album* to Basagran.

In order to quantify the effects of individual environmental components, a series of experiments was conducted under controlled environmental conditions. The principal aim of these investigations was to assess the influence of temperature, light intensity, relative humidity and soil moisture on the susceptibility of *C. album* to Basagran and Actipron.

A pre-requisite for herbicidal action, however, is an ability to penetrate the leaf surface. The cuticle, and in particular, the epicuticular wax is believed by many authors (e.g. Leece 1976) to be the principal barrier to foliar penetration. Since it has been shown that the physical and chemical structure of the epicuticular wax of many plant species is dependent on environmental conditions during development (e.g. Hallam 1970, Baker 1974), the opportunity was taken during this series of experiments to determine the influence of environment on epicuticular wax composition and ultra-structure in *C. album*.

In addition to its influence on herbicide penetration, epicuticular wax is also thought to play a significant role in determining leaf wettability, i.e. the amount of herbicide intercepted

by the leaf surface that is subsequently available for penetration (Martin and Juniper 1970). The importance of the epicuticular wax in determining the wettability of C. album leaves was therefore assessed during these investigations.

Thus, although the principal aim of these investigations was to ascertain the influence of environment on the susceptibility of C. album to Basagran and Actipron, it was also hoped to establish the relationship between susceptibility and epicuticular wax composition and deposition in this species.

4.2 MATERIALS AND METHODS

4.2.1 Growth of plants

In all experiments seeds of C. album were germinated at room temperature on moist filter paper prior to transplanting into 3 inch pots with John Innes No. 2 compost. In the experiment on relative humidity, conducted at the National Vegetable Research Station (NVRS), Wellesbourne, seedlings were transplanted into 3 inch pots containing pre-washed medium grade vermiculite. These plants were provided with a constant supply of the following nutrient solution (recipe kindly provided by NVRS):-

Stock Solutions

1.	KNO_3	484.8g/4 l
2.	$MgSO_4 . 7H_2O$	295.2 "
3.	$Ca(NO_3)_2 . 4H_2O$	577.0 "
4.	$NaH_2PO_4 . 2H_2O$	166.4 "
5.	EDTA disodium salt	33.3 "
	$FeCl_3$	14.5 "
6a.	Trace elements	
	$CuSO_4 . 5H_2O$	1.9 "
	H_3BO_3	14.9 "
	$MnSO_4 . 4H_2O$	17.8 "
	$ZnSO_4 . 7H_2O$	2.3 "
	$(NH_4)_6 Mo_7O_{24} . 4H_2O$	0.6 "
6b.	400 ml. 6A made up to 4 l	

Working Solution

400 ml stock solutions 1-5 + 6B made up to 80 l.

4.2.2 Environmental Conditions

4.2.2.1 Temperature

The influence of temperature on the susceptibility of C. album to Basagran was investigated over a temperature range 12-28°C.

As only two environmental cabinets were available, it was only possible to compare two temperatures at one time. Three experiments were designed to compare the following series of temperatures:- a) 12°C vs 28°C, b) 17°C vs 28°C, c) 22°C vs 28°C.

In the three experiments, the cabinets were operated at a relative humidity of 70% ± 10%. Lighting, provided both by fluorescent tubes and tungsten lamps, was set at an average intensity of 96 Wm^2 at the leaf surface for a daily period of 16 hours.

Plants were maintained at day temperatures of 12, 17, 22 and 28°C with corresponding night temperatures of 6, 10, 15 and 17°C.

Seedlings were raised under glasshouse conditions (Chapter 1). Once the plants had reached the 4-6th leaf stage (approx. 5 weeks after sowing), they were transferred to two constant environment cabinets for 8 days prior to treatment.

The environmental cabinets (Series 111) were manufactured by Fisons, Loughborough, Leics. Model 600G3/TTL provided controlled temperature over a range of 0-45°C ± 0.75°C, and light intensity over a range of 7.5 - 96.0 Wm^2, by 12, 4ft x 40 watt warm-white fluorescent tubes (Philips colour 29) and 3 x 40 watt tungsten lamps. Additional control for relative humidity over the range 50-90% ± 3% was

provided by the 600G3/THTL model.

4.2.2.2 Light Intensity

The effect of light intensity was investigated over the range 12-96 Wm^2. Two experiments were conducted to compare the following light intensities:- a) 12 vs 96 Wm^2 b) 37 vs 96 Wm^2.

Cabinets were operated at a constant day temperature of 22°C and a corresponding night temperature of 17°C. Relative humidity was set at 70% ± 10%, light intensity being provided by Warm-White fluorescent tubes plus 3 tungsten lamps over a 16-hour photoperiod.

4.2.2.3 Relative Humidity

The effect of relative humidity at levels of 80% ± 10% and 40% ± 10% were investigated. Both cabinets were maintained at a day temperature of 21°C with a corresponding night temperature of 16°C. Light intensity was provided by a series of warm-white fluorescent tubes at 120 Wm^2 with 6 tungsten lamps, operating over a 16-hour photoperiod.

4.2.2.4 Soil Moisture

Following the main series of experiments, preliminary investigations were initiated into the effect of soil moisture on the susceptibility of C. album to Basagran and Actipron. Cabinets were operated at a day temperature of 21°C with a corresponding night temperature of 17°C. Light intensity was set at 96 Wm^2 for a 16-hour photoperiod, using both fluorescent and tungsten sources. Relative humidity was maintained at 70% ± 10%. In one cabinet, plants were watered daily in the usual manner, whereas watering in the

other cabinet was kept to a minimum for the duration of the experiment.

At the time of herbicide application, soil moisture levels were determined by removing soil samples from control pots and weighing directly. The soil samples were then dried in an oven at 150°C for 48 hours or to constant weight. Soil moisture was expressed as a percentage of the total weight of the original soil sample.

4.2.3 Experimental Design

Each environmental cabinet contained 3 troughs each with a capacity of twelve 3" pots (2 plants/pot), i.e. 36 pots/cabinet. Treatments were arranged on a fully randomised basis, with at least twelve plants selected per treatment.

4.2.4 Herbicide Application

Following the 8-day pre-treatment period under constant conditions, the plants were removed from the cabinets and sprayed, in a similar manner to that described in Chapter 1, with Basagran at 3 l/ha and Actipron at 2 l/ha. All applications were made using a Shandon Chromatography Sprayer. Plants were examined following spraying to ensure efficient coverage by the herbicide. Retention by the leaf surface was noticably improved when Actipron was incorporated into the herbicide formulation, with subsequent spreading of the droplets. Following treatment, half the plants were returned to their original positions within the cabinets, the remaining plants being transferred to the conditions of the other cabinet, thus enabling pre- and post-treatment conditions to be investigated.

4.2.5 Assessment of damage

Plants were assessed over a two-week period for herbicide damage. Scoring was on an individual plant basis on a 0-5 scale (see Chapter 1).

4.2.6 Epicuticular wax analysis

Leaf samples were obtained from plants grown under the varying conditions of temperature, light intensity and relative humidity at the time of herbicide application. The harvesting procedure was standardised by removing the 3rd-8th leaves from each plant (an average of 20 plants/harvest). Epicuticular wax was extracted from both adaxial and abaxial surfaces and the chemical composition was determined according to the TLC and GLC techniques described in Chapter 4. The surface area of the extracted leaves was also determined in order to calculate the quantity of wax produced per unit area of leaf.

Due to the relatively large number of experiments carried out, and also to the size of C. album leaves, calculating the surface area of individual leaves from a large sample would prove rather time-consuming. In view of this, investigations were carried out to determine the correlation between surface area and weight of C. album leaves. This was achieved using a wide range of leaf sizes and by calculating the surface area and weight for each individual leaf. A regression analysis was then carried out, which showed a linear relationship between surface area and weight of C. album leaves. In subsequent experiments the total weight of leaves was thus recorded and converted to surface area by multiplication by the correction factor (i.e. weight of C. album leaves in g. x 2.81 = surface area in cm^2). N.B. this is only an approximate estimate of surface area, since no allowance has been made for leaf veins.

4.2.7 Scanning electron microscopy

Leaf samples which were visibly free from damage by handling, abrasion etc., were removed from plants grown under the different environmental conditions at the time of herbicide application. Care

was taken to remove only fully expanded leaves, usually between the 5th to 8th nodes. This inevitably meant sampling leaves that were already present on the plants before being placed under constant environments for the 8-day period.

Sections were prepared for scanning electron microscopy as described in Chapter 3. Both abaxial and adaxial surfaces were examined.

4.2.8 Contact angle determinations

Leaf samples were selected in a similar manner to those selected for scanning electron microscopy. Contact angles were measured on both abaxial and adaxial leaf surfaces of C. album according to the method described in Chapter 2.

4.3 RESULTS

4.3.1 The effect of environmental conditions on the susceptibility of C. album to Basagran and Actipron

4.3.1.1 Temperature

The effect of temperature on the susceptibility of C. album to Basagran and Actipron is summarised in Fig. 7 . A complete account of damage assessment is provided in Appendix 1, Tables IX-XI.

Greatest herbicide damage was observed when plants received a low temperature pre-treatment followed by a high temperature post-treatment, this response being particularly marked at the two lowest temperature pre-treatments of 12°C and 17°C (Fig. 7 a and b). As the pre- and post-temperature range was reduced, however, this effect diminished (Fig. 7 c). All three experiments revealed little difference in the response of plants maintained at a constant temperature throughout. Transition from a high pre-treatment to a lower post-treatment temperature, however, generally reduced control. The addition of Actipron to the herbicide formulation substantially improved weed control throughout.

4.3.1.2 Light Intensity

The influence of light intensity on the response of C. album to Basagran and Actipron is summarised in Fig. 8 , additional information on damage assessment being provided in Appendix 1, Tables XII and XIII.

Plants receiving a low light intensity pre-treatment of 12 Wm^2 with a high light intensity of 96 Wm^2 following herbicide application showed greater damage than those

FIGURE 7. The effect of temperature on the susceptibility of C. album to Basagran and Actipron

Plants were maintained under constant conditions of relative humidity (70% ± 10%) and light (96 Wm^2) for a 16 hour photoperiod with the following variations in pre- and post-treatment temperature:-

A - 12°C vs 28°C.

Damage assessment 8 days following herbicide application.

B - 17°C vs 28°C

Damage assessment 8 days following herbicide application.

C - 22°C vs 28°C

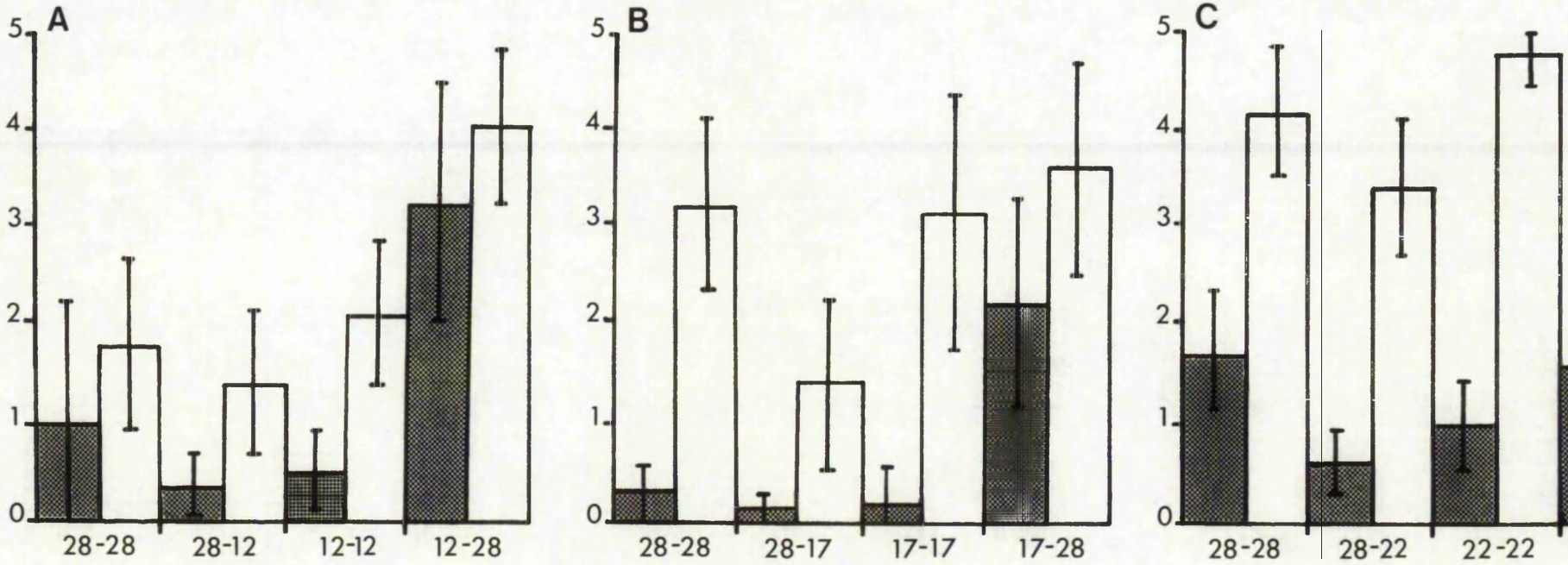
Damage assessment 3 days following herbicide application.

▒ - Basagran at 3 l/ha

□ - " " " + Actipron at 2 l/ha

Bars indicate standard deviation values.

FIGURE 7



Pre- and post-treatment tempera

FIGURE 8. The effect of light intensity on the susceptibility of C. album to Basagran and Actipron

Plants were maintained under constant conditions of temperature (22°C) and relative humidity (70% ± 10%) for a 16 hour photoperiod with the following variation in pre- and post-treatment light intensities:-

A - 12 Wm^2 vs 96 Wm^2

Damage assessment 5 days following herbicide application.

B - 37 Wm^2 vs 96 Wm^2

Damage assessment 6 days following treatment.

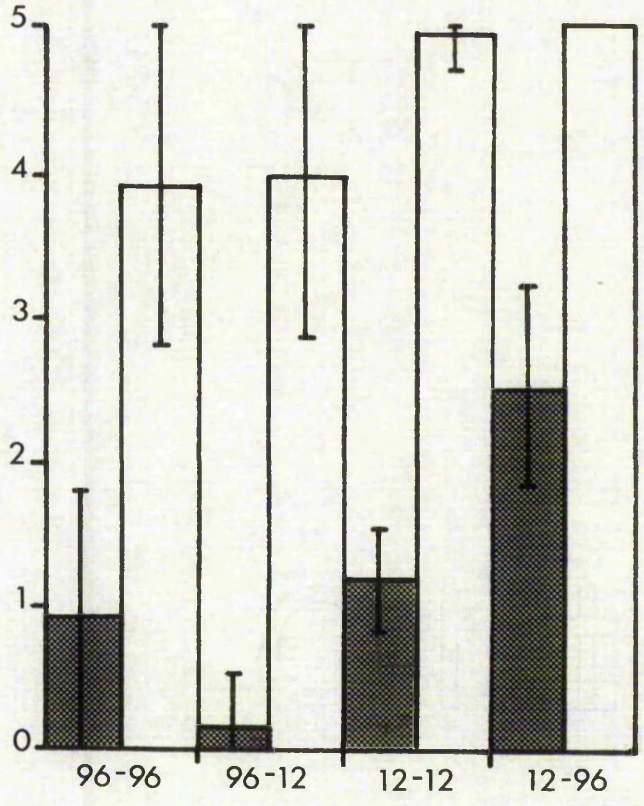
▒ - Basagran at 3 l/ha

□ - " " " + Actipron at 2 l/ha

Bars indicate standard deviation values.

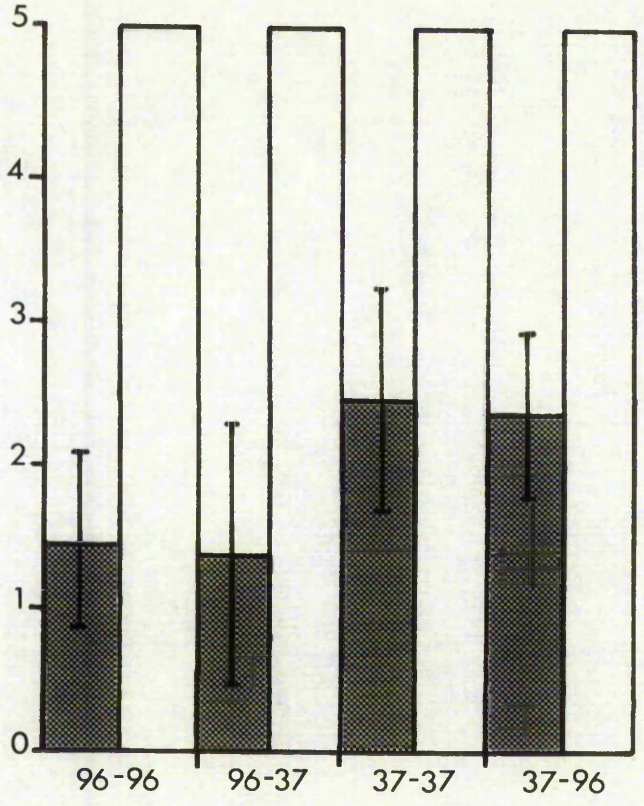
FIGURE 8

A Damage assessment (mean score)



Pre- and post-treatment light intensity WM^2

B Damage assessment (mean score)



Pre- and post-treatment light intensity WM^2

maintained under constant light conditions for the duration of the experiment (Fig. 8a). Transition from the high light intensity pre-treatment to low intensity conditions following herbicide application, however, resulted in little damage. These effects diminished as the range between pre- and post-treatment conditions was reduced (Fig. 8b). Herbicide performance was enhanced in all cases by the inclusion of Actipron.

4.3.1.3 Relative humidity

The susceptibility of C. album to Basagran and Actipron as influenced by relative humidity is summarised in Fig. 9. Additional information on herbicide assessment is provided in Appendix 1, Table XIV.

Susceptibility of C. album to Basagran was greatest when plants were maintained under low (40% ± 10%) humidity conditions for the duration of the experiment. High (80% ± 10%) relative humidity throughout the experiment, however, resulted in poor weed control. Transferring plants to either high or low humidity conditions following treatment did not appreciably affect the response. In all cases, however, greater damage was observed when Basagran was applied in combination with Actipron.

4.3.1.4 Soil Moisture

The influence of soil moisture on the susceptibility of C. album to Basagran and Actipron was assessed using plants maintained under conditions of high and low soil moisture. Throughout the experiment stomatal cuticular lip apertures were determined in an attempt to establish the

FIGURE 9 . The effect of relative humidity on the susceptibility of C. album to Basagran and Actipron

Plants were maintained under constant conditions of temperature ($21^{o}C$) and light intensity (80 Wm^{2}) for a 16 hour photoperiod with different pre- and post-treatment conditions of 40% and 80% ($\pm$ 10%) relative humidity.

Damage assessment 8 days following herbicide application.

▒ - Basagran at 3 l/ha

□ - " " " + Actipron at 2 l/ha

Bars indicate standard deviation values.

FIGURE 10. The effect of soil moisture on the susceptibility of C. album to Basagran and Actipron

Plants were maintained under constant conditions of temperature ($21^{o}C$), light intensity (96 Wm^{2}) and relative humidity (70% $\pm$ 10%) for a 16 hour photoperiod at both high (27.5%) and low (9.35%) soil moisture levels.

Damage assessment 7 days following treatment.

▒ - Basagran at 3 l/ha

□ - " " " + Actipron at 2 l/ha

Bars indicate standard deviation values

FIGURE 9

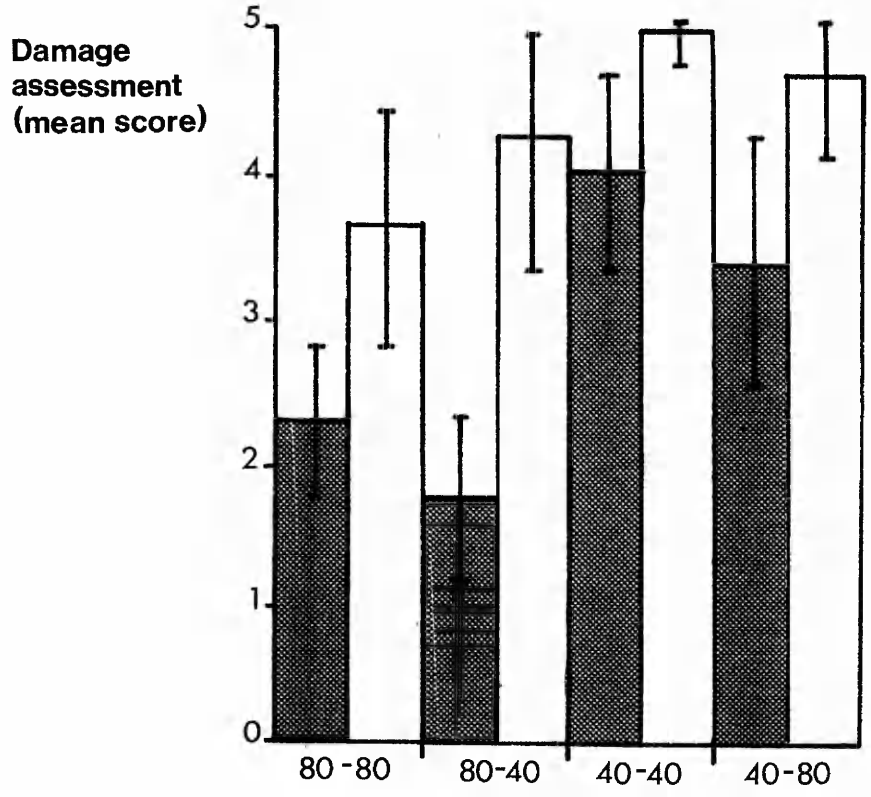
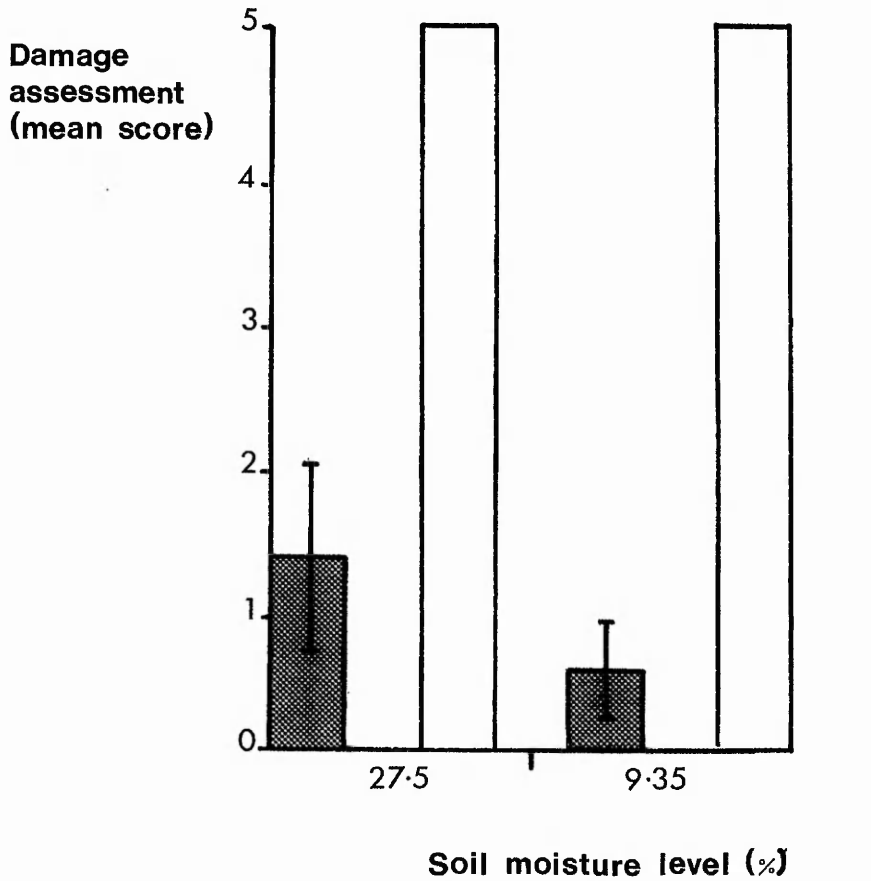


FIGURE 10



water status of the plants prior to herbicide application. Soil moisture levels were also monitored at the time of application. These findings are summarised in Table III.

Table III. The effect of soil moisture on susceptibility of C. album to Basagran and Actipron - Stomatal apertures and soil moisture levels

Moisture regime	Mean moisture content	Mean stomatal aperture
Low soil moisture	9.35% ± 1.9%	5.0 µm ± 0.56
High " "	27.5% ± 0.14%	8.34 µm ± 0.74

Plants maintained under high soil moisture conditions showed slightly more damage than those kept under water stress for the duration of the experiment (Fig. 10). Control was substantially improved, however, when Basagran was applied in combination with Actipron, regardless of water status.

4.3.2 Environmental effects on epicuticular wax composition and ultrastructure in C. album

4.3.2.1 Wax yield

The quantities of epicuticular wax extracted from plants grown under the various environmental regimes are summarised in Table IV. Yields of epicuticular wax varied considerably between individual experiments, even though the growth conditions of each batch of plants were standardised as far as possible.

Table IV. The effect of environment on epicuticular wax production in C. album

Environmental parameter			Epicuticular wax yield
Temperature	a.	$12^{o}C$	0.14 $mg.cm^{2}$
		$28^{o}C$	0.19 "
	b.	$17^{o}C$	0.21 "
		$28^{o}C$	0.18 "
	c.	$22^{o}C$	0.06 "
		$28^{o}C$	0.06 "
Light Intensity	a.	12 Wm^{2}	0.03 "
		96 Wm^{2}	0.07 "
	b.	37 Wm^{2}	0.25 "
		96 Wm^{2}	0.20 "
Relative Humidity		80%	0.222 "
		40%	0.225 "

There appeared to be little difference in the quantity of wax produced as a result of maintaining plants under different regimes of temperature, light intensity and relative humidity.

4.3.2.2 Wax composition

The chemical composition and homologue content

Table V The effect of environmental conditions on epicuticular wax composition in C. album

Environmental Parameter	TEMPERATURE						LIGHT INTENSITY				RELATIVE HUMIDITY	
Homologue	12°C	28°C	17°C	28°C	22°C	28°C	12Wm^2	96Wm^2	37Wm^2	96Wm^2	40%	80%
C_{27} Hydrocarbon	1.9	1.9	5.2	3.3	3.4	2.1	4.0	3.5	3.1	3.6	4.5	3.2
C_{29} "	3.0	6.6	16.2	16.6	3.3	5.4	5.2	4.9	4.0	5.0	4.8	7.1
Other "	-	-	-	-	10.0	2.6	11.1	8.2	7.1	8.0	-	-
TOTAL HYDROCARBON	4.9	8.5	21.4	19.9	16.7	10.1	20.3	16.6	14.2	16.6	9.3	10.3
C_{26} Alcohol	18.6	-	-	-	-	-	14.4	10.3	10.4	8.0	-	5.6
C_{28} "	20.3	33.8	11.4	16.4	10.9	20.6	23.5	33.0	27.9	22.8	31.9	26.2
TOTAL ALCOHOL	38.9	33.8	11.4	16.4	10.9	20.6	37.9	43.3	38.3	30.8	31.9	31.8
C_{28} Aldehyde	47.6	41.8	26.3	29.3	38.1	53.7	22.5	22.5	33.0	36.9	39.9	30.6
C_{30} "	-	-	-	-	19.8	-	-	-	-	-	-	5.0
TOTAL ALDEHYDE	47.6	41.8	26.3	29.3	57.9	53.7	22.5	22.5	33.0	36.9	39.9	35.6
KETO-ESTER	3.1	2.0	6.1	2.7	6.1	4.8	6.0	4.0	4.5	6.6	4.8	2.1
C_{40} Ester	0.1	0.2	0.8	0.7	1.1	0.9	0.6	0.6	0.6	0.4	-	-
C_{42} "	1.6	2.5	6.1	3.5	1.4	1.7	2.0	1.6	1.9	1.5	3.8	3.3
C_{44} "	1.0	4.3	12.2	9.4	1.2	1.2	3.8	2.1	2.3	1.5	4.9	7.9
C_{46} "	1.0	2.5	4.3	4.2	2.7	3.1	1.6	2.0	1.2	1.6	1.8	3.2
C_{48} "	1.0	2.4	7.4	4 1	1.4	3.0	2.8	4.0	1.8	2.4	2.3	3.8
C_{50} "	0.3	1 4	3.5	3.0	-	0.4	1.6	2.8	1.5	0.9	1.0	1.7
TOTAL ESTER	5.0	13.3	34.2	24.9	7 8	10.3	12.4	13.1	9.3	8.3	13.8	19.9

of epicuticular wax extracted from plants maintained under various conditions of temperature, light intensity and relative humidity is summarised in Table V.
It should be noted here that results from separate experiments are not comparable. The quality of wax produced at 12°C, for example, cannot be compared directly with that from 17°C or 22°C. Comparisons can only be made within individual experiments.

Aldehydes and alcohols constituted the major wax fractions, accounting for as much as 60% total wax in many instances, the dominant chain length in both cases being the C_{28} component. Hydrocarbons (C_{27} and C_{29}) and esters (C_{40-50}) were present in significant amounts with small quantities of keto-ester also being detected.

The relative proportions of these wax fractions were found to vary considerably between individual experiments, in spite of the rigorous attempts to reproduce identical growth conditions for all batches of plants used in these analyses. Aldehydes, for example, accounted for as much as 50% total wax in some instances (22°C vs 28°C), whereas in other cases this fraction only accounted for little over 25% total wax present (17°C vs 28°C).

As well as variations between the ratios of wax fractions present, differences in homologue content were also noted, even within individual experiments. Significant quantities of C_{26} alcohol, for example, were detected in wax produced at 12°C, although this homologue was absent in the wax extracted from similar plants maintained at 28°C.

Despite these minor discrepancies, which are so far unaccounted for, there appeared to be no substantial change in the wax composition, either in total composition of wax fractions, or in the relative percentage composition of their homologues, as a result of changes in any of the environmental parameters examined.

4.3 2.3 Wax ultrastructure

Scanning electron micrographs were taken of the adaxial surface of C. album grown under varying conditions of temperature (Figs. 11, 12, 13), light intensity (Figs. 14, 15), relative humidity (Fig. 16) and soil moisture (Fig. 17).

The epicuticular wax is in the form of small platelets, approximately 1 x 0.2 µm (length x depth) extruded from the leaf surface. Within the confines of each individual experiment, there appears to be no obvious differences either in the size and form, or in the density and distribution of wax platelets as a result of changes in any of the environmental parameters examined.

4.3.3 The influence of environmental conditions on leaf wettability in C. album

The advancing contact angle of water on the leaf surface

FIGURE 11. The influence of temperature on epicuticular wax ultrastructure on C. album

Plants were maintained for 8 days under constant conditions of relative humidity (70% ± 10%) and light (96 Wm^2) for a 16 hour photoperiod with temperature variations as follows:-

a) 12°C day, 6°C night

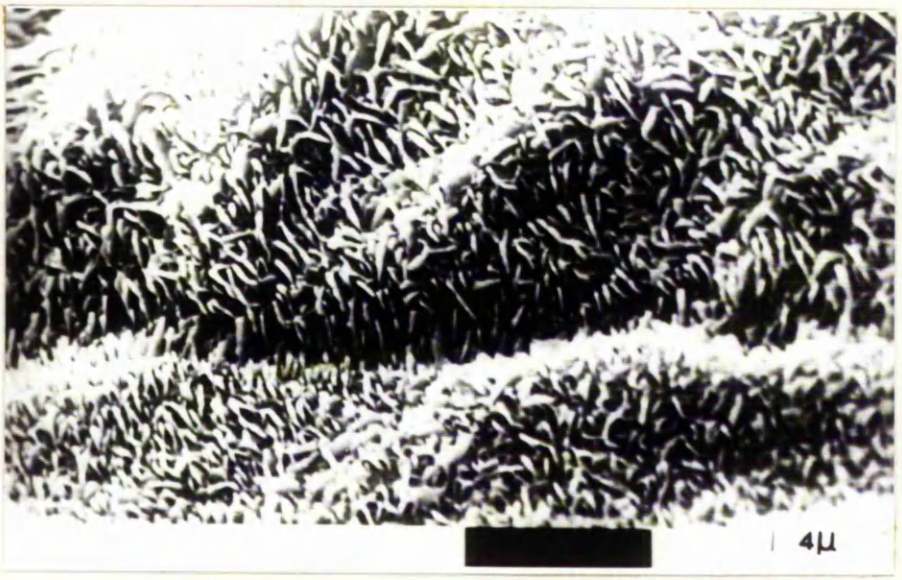
Mag. 5.6 K

b) 28°C day, 17°C night

Mag. 5.6 K

FIGURE 11

A



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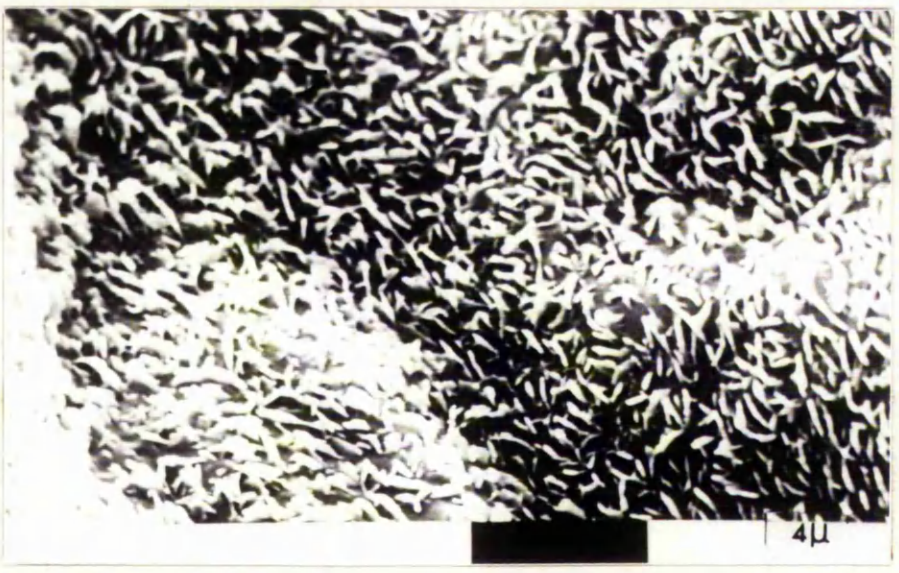


FIGURE 12. The influence of temperature on the epicuticular wax ultrastructure of C. album

Plants received constant conditions (over an 8 day period) of relative humidity (70% ± 10%), light (96 Wm^2) with the following temperatures:-

a) 17°C day, 10°C night

Mag. 5.6 K

b) 28°C day, 17°C night

Mag. 5.6 K

FIGURE 12

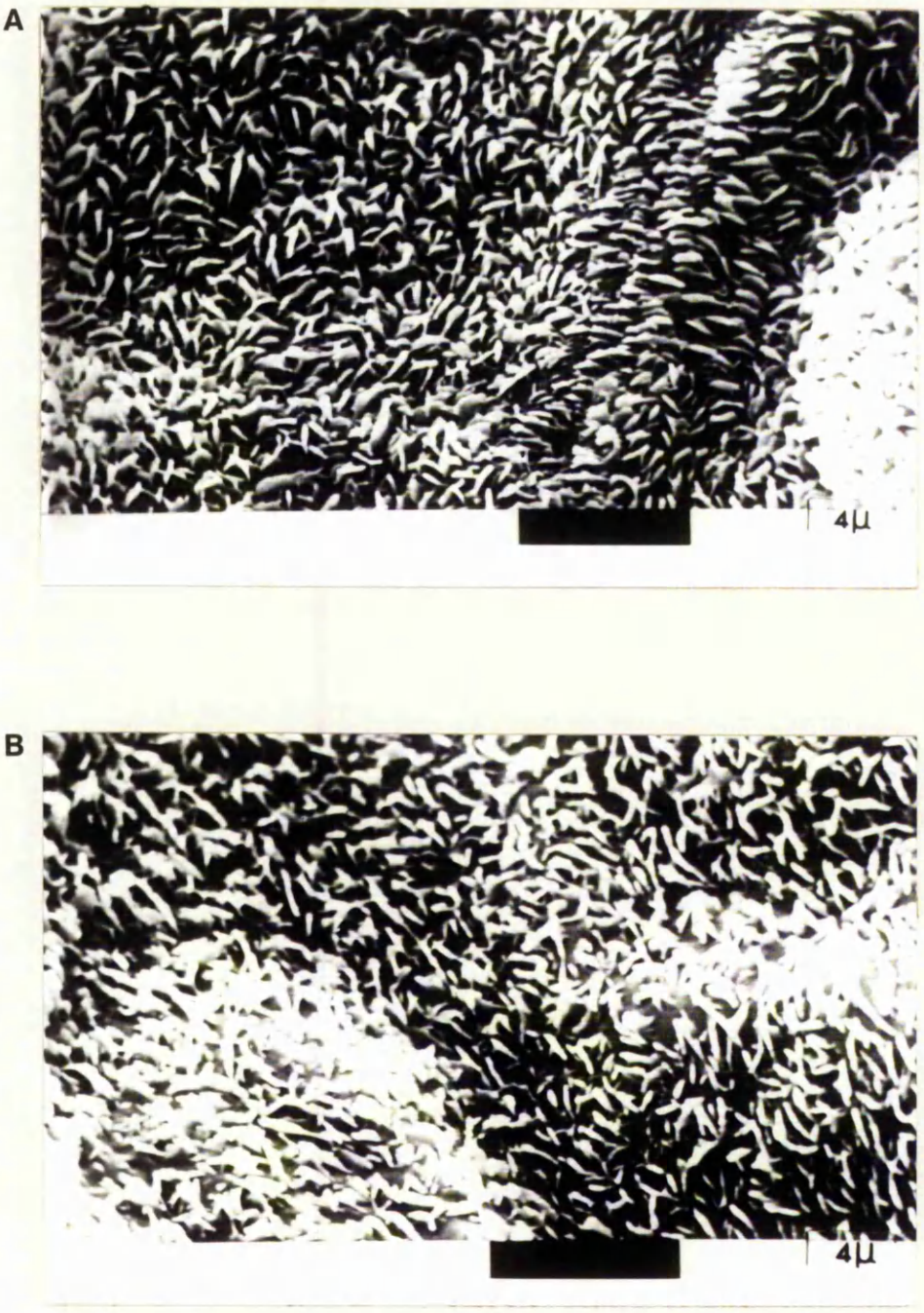


FIGURE 13. The influence of temperature on the ultrastructure of C. album epicuticular wax

C. album plants were maintained for 8 days under controlled conditions of relative humidity (70% ± 10%) and light intensity (96 Wm^2) for a 16 hour photoperiod with the following temperatures:-

a) 22°C day, 15°C night

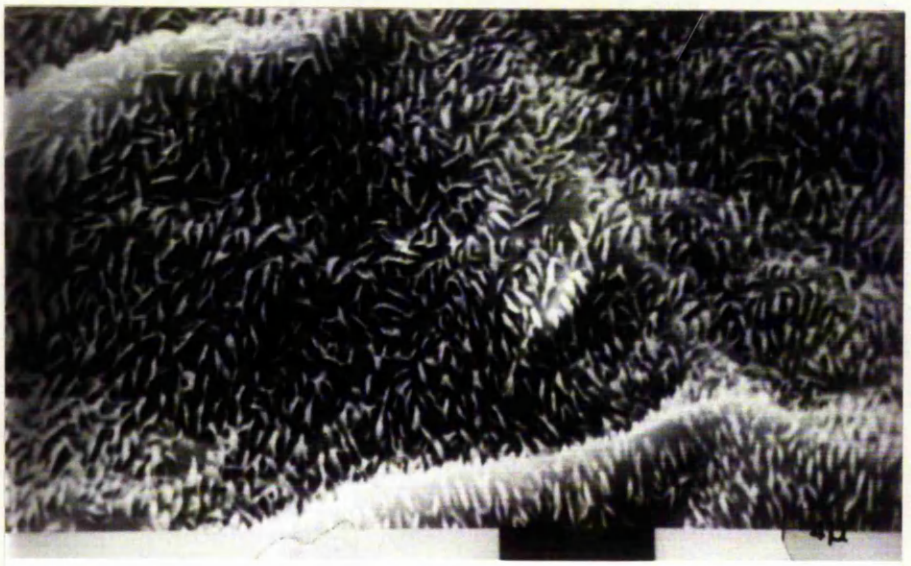
Mag. 5.1 K

b) 28°C day, 17°C night

Mag 6 K

FIGURE 13

A



B

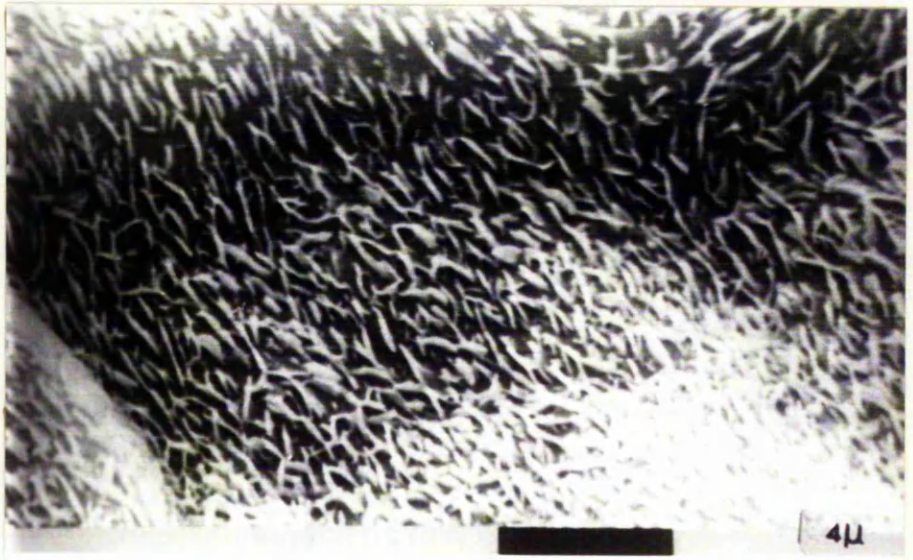


FIGURE 14. The influence of light intensity on epicuticular wax ultrastructure in C. album

Plants were grown under constant conditions (over an 8 day period) of temperature (22°C) and relative humidity (70% ± 10%) with the following variations in light intensity:-

a) 12 Wm^2 for a 16 hour photoperiod

Mag. 5.1 K

b) 96 Wm^2 with a 16 hour photoperiod

Mag. 5.3 K

FIGURE 14

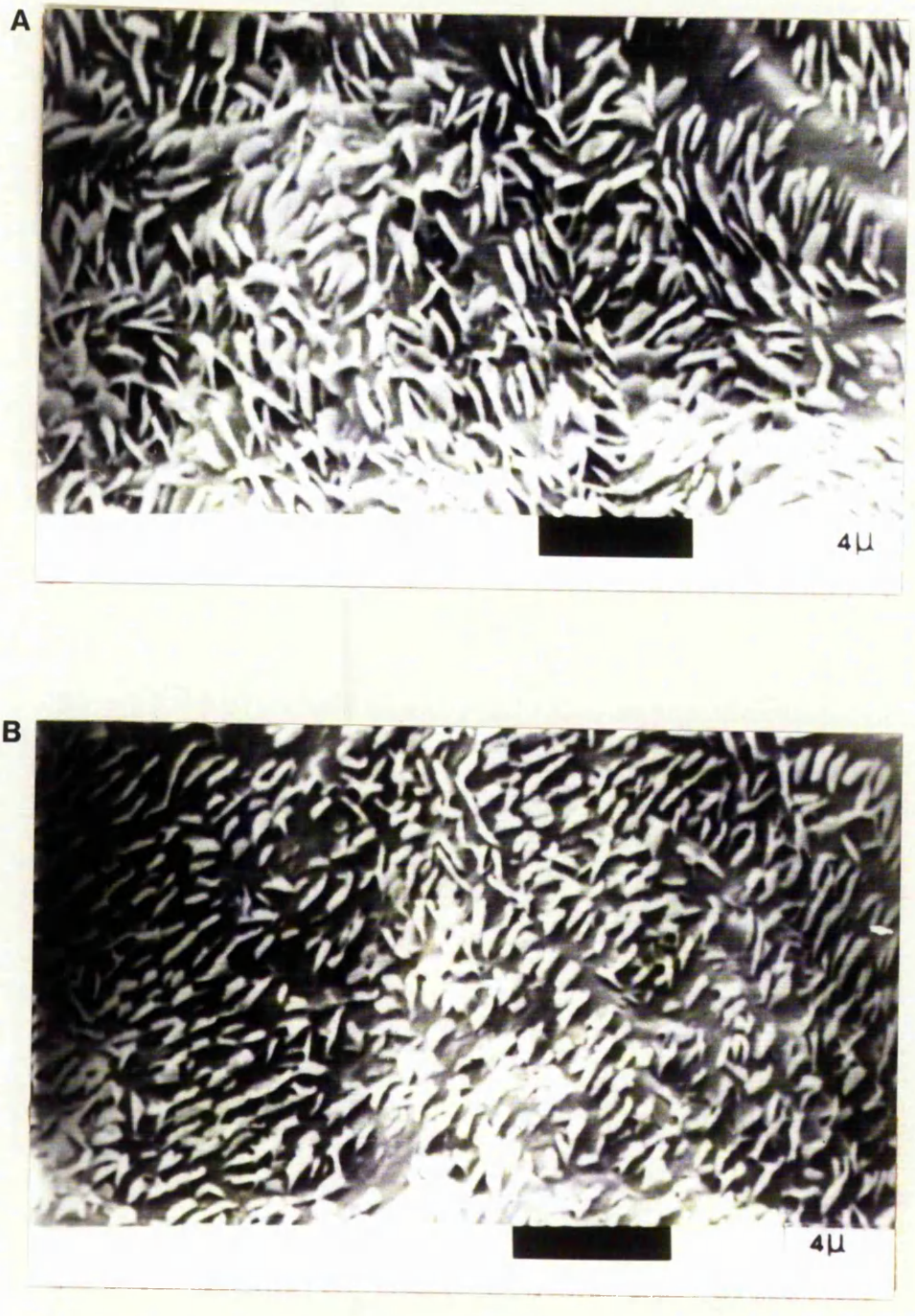


FIGURE 15. The influence of light intensity on the ultra-structure of the epicuticular wax of C. album

Plants received controlled conditions (over an 8 day period) of temperature (22°C) and relative humidity (70% ± 10%) with the following light intensities over an 16 hour photoperiod:-

a) 37 Wm^2

Mag. 5.3 K

b) 96 Wm^2

Mag. 5.6 K

FIGURE 15

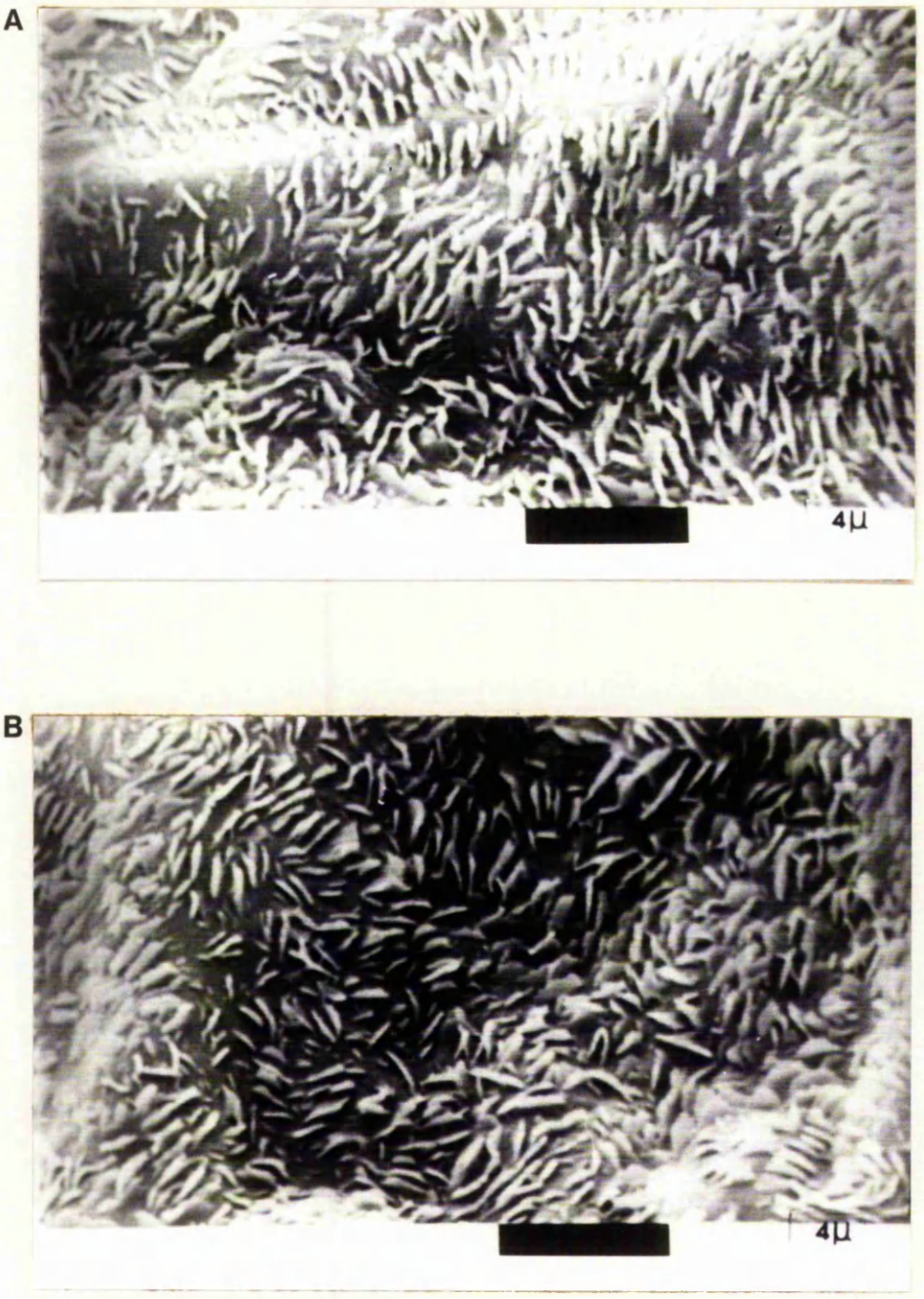


FIGURE 16. The influence of relative humidity on the ultrastructure of C. album epicuticular wax

Plants were grown under constant conditions (over an 8 day period) of temperature (21°C) and light intensity (80 Wm^2) for a 16 hour photoperiod with the following degrees of relative humidity:-

a) 40% ± 10%

Mag. 10.7 K

b) 80% ± 10%

Mag. 11.0 K

FIGURE 16

A

B

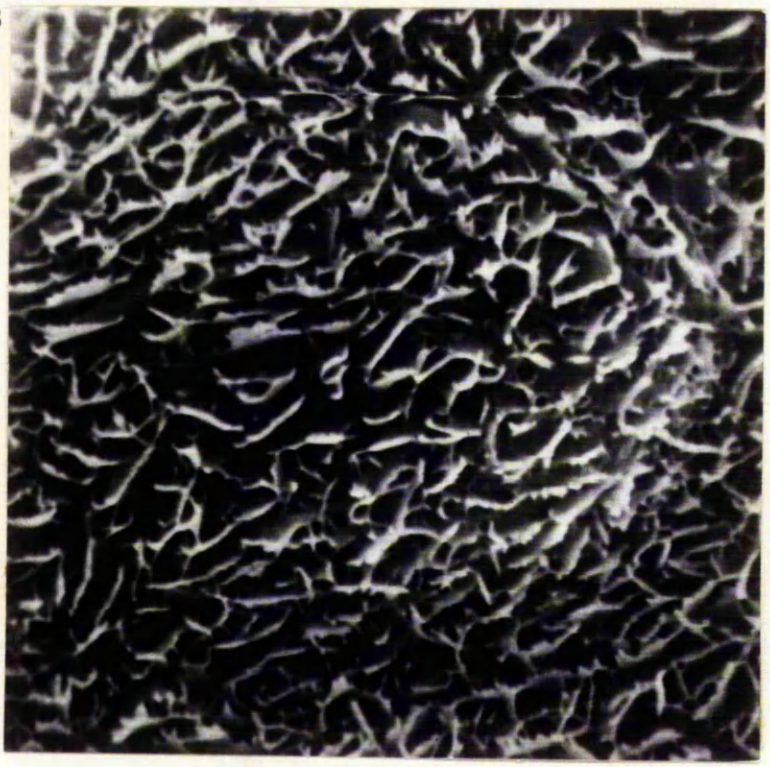


FIGURE 17. The influence of soil moisture on epicuticular wax ultrastructure in C. album

Plants were maintained for 8 days under controlled conditions of temperature (21°C), relative humidity (70% ± 10%) and light (96 Wm^2) for a 16 hour photoperiod with the following degrees of soil moisture:-

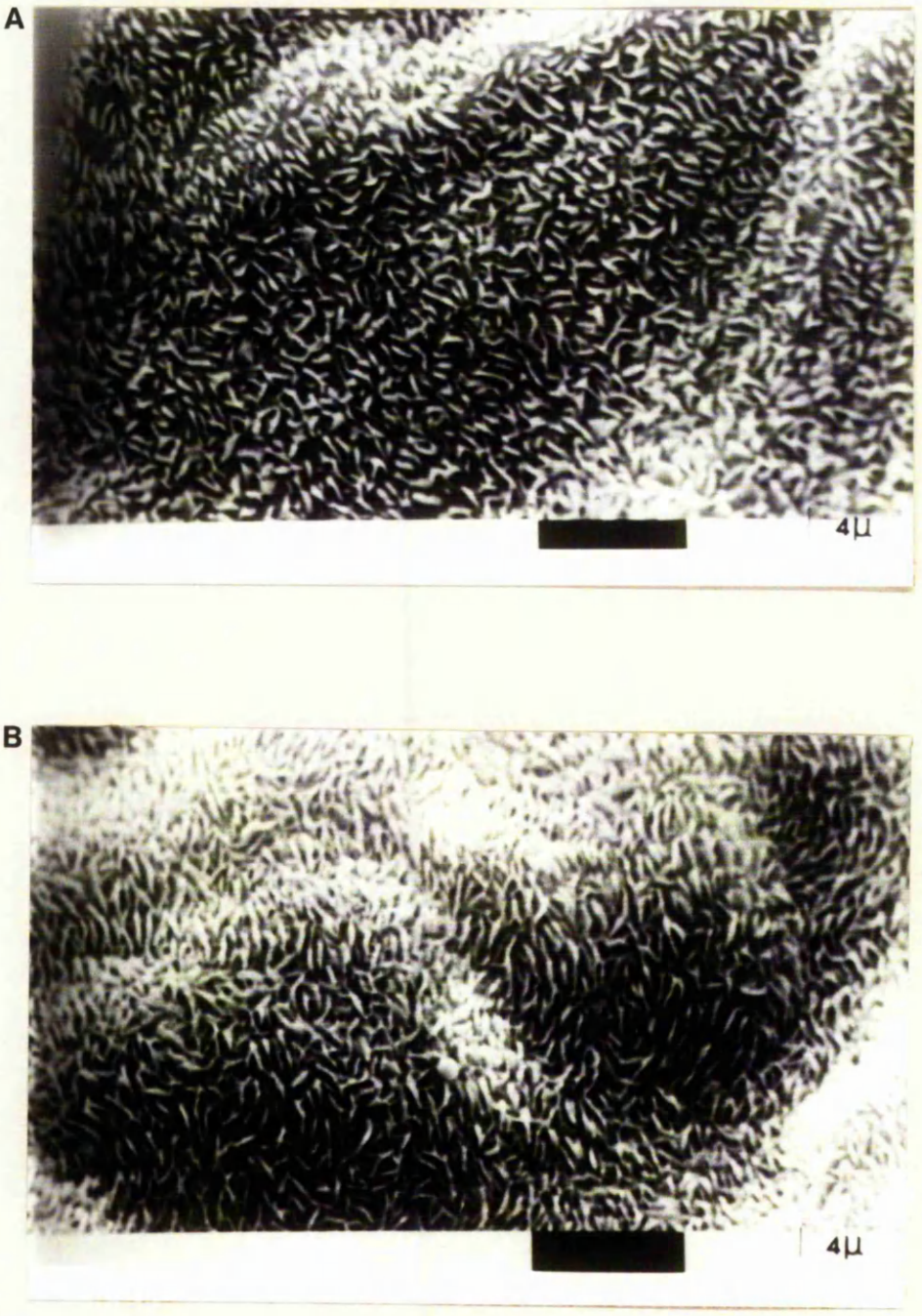
a) 9.35%

Mag. 5.1 K

b) 27.5%

Mag. 5.1 K

FIGURE 17



of C. album was taken as the criterion for assessing leaf wettability in this species.

Contact angles were determined on both adaxial and abaxial leaf surfaces of C. album plants maintained under varying conditions of temperature and light intensity (Table VI).

Greatest contact angles were recorded on the abaxial leaf surface in all cases. Changes in contact angle as a result of environment are most apparent at low regimes of temperature and light intensity. Substantial reductions in contact angle, for example, were recorded on both abaxial and adaxial surfaces of C. album maintained at a temperature of 12°C when compared to similar plants at 28°C. Similarly, a low light intensity of 12 Wm^2 resulted in reduced contact angles on the adaxial surface of C. album when compared to plants maintained at the maximum light intensity of 96 Wm^2. These effects diminished, however, as the temperature/light intensity gap was further reduced.

Table VI . The influence of environment on leaf wettability in C. album

Environmental Parameter		Adaxial surface	Abaxial surface
Temperature	a. 12°C	118° ± 1.25°	129° ± 2.21°
	28°C	136° ± 2.5°	145° ± 4.24°
	b. 17°C	116° ± 1.52°	147° ± 2.46°
	28°C	126° ± 0.95°	148° ± 2.60°
	c. 22°C	128° ± 3.78°	143° ± 0.57°
	28°C	126° ± 0.99°	137° ± 2.08°
Light Intensity	a. 12 Wm^2	115° ± 3.21°	134° ± 4.99°
	96 Wm^2	126° ± 1.25°	131° ± 1.41°
	b. 37 Wm^2	120° ± 3.87°	130° ± 2.50°
	96 Wm^2	122° ± 3.59°	142° ± 3.65°

4.4 CONCLUSIONS

From this series of experiments it can be concluded that, under constant conditions:-

(1) Low temperature and light intensity pre-treatments followed by high post-treatment conditions result in substantial herbicide damage.

(2) Low relative humidity results in greater damage than high relative humidity.

(3) High soil moisture conditions may enhance herbicide performance.

(4) Maintaining plants under constant environmental conditions for 8 days result in no significant changes in wax yield, composition or ultrastructure.

(5) Contact angle appears to be reduced in plants maintained under low temperature and light intensity conditions.

(6) Susceptibility to the herbicide is not correlated with changes in the nature of the surface wax.

4.5 DISCUSSION

It is apparent from these investigations that environmental conditions play a significant role in determining the efficiency of Basagran in the control of C. album. Although susceptibility was found to be directly proportional to temperature and light intensity, the most striking differences in susceptibility were observed in plants receiving low pre-treatment followed by high post-treatment conditions.

These responses may be interpreted by reference to the mode of action of Basagran. This herbicide is a potent inhibitor of photosynthetic electron transport (Mine and Matsunaka 1975), and so it follows that photosynthetic demand will determine, to a large extent, its efficiency. Thus, any factor influencing the general metabolism of the plant will correspondingly affect its photosynthetic demand, and hence susceptibility to the herbicide. Hence, large and sudden increases in temperature and light intensity following application would be expected to cause a rapid surge in metabolic activity within the plant as a result of the sudden increase in available energy. This in turn would increase the overall photosynthetic demand of the plant, and consequently, a block in the electron transport chain under such conditions would result in a build-up of energy unable to be harnessed and death would rapidly ensue as a result of photosynthetic membrane disruption and cell plasmolysis. Indeed, cell plasmolysis has been reported by Potter and Wergin (1975) as one of the initial toxic effects of bentazone in cocklebur (Xanthium pensylvanicum). It is interesting to note, however. that although photosynthetic inhibition was independent of light intensity, cellular damage was light-dependent and plants

treated and left in darkness failed to produce toxicity symptoms. From this it was concluded that cellular damage and subsequent necrosis was due to photo-induced by-products that result from arrested photosynthesis. Thus, if a similar system is present in C. album surges in light intensity might be expected to increase the production of toxic by-products and hence accelerate the development of necrosis in this species.

Although the effects of temperature and light intensity can be interpreted on a physiological basis, the effects of relative humidity are less obvious. Greater damage was incurred by plants maintained under low regimes of relative humidity throughout the experiment. Physiologically, one would expect greater damage at high relative humidity since higher levels of metabolic activity would be expected under favourable moisture conditions. Indeed, it would appear from preliminary experiments that low moisture tension within the plant results in greater susceptibility to the herbicide, although this effect requires confirmation by further studies.

These physiological considerations, however, assume unlimited availability of the herbicide at its site of action within the chloroplast. It is well known, however, that, as well as being a function of leaf structure, herbicide penetration is also influenced by environmental conditions, and so changes in environment may also be expected to influence herbicide efficiency by limiting its rate of penetration through the various components of the leaf, i.e. cuticular membrane, epidermal cell plasmalemma and finally chloroplast envelope. A consideration of these components will be further developed in the final discussion.

The primary barrier to be overcome, however, is the surface layer of wax, since this is the most hydrophobic component of the cuticle and as such constitutes the greatest barrier to the foliar penetration of polar molecules. Changes in the nature of the epicuticular wax, as affected by environment, are considered by many authors (e.g. Leece 1976, 1978) to be a primary factor in determining the ease, and hence efficiency, of foliar penetration.

The present studies, however, infer no relationship between environment and epicuticular wax production in C. album. Maintaining plants under different environmental conditions did not result in any significant change, either in the deposition (Table IV) or indeed, in chemical composition (Table V) of the epicuticular wax in this species. This lack of correlation between environment and wax production is further substantiated by the scanning electron microscope studies of wax ultrastructure, where there was no evidence of change, either in the overall density or in the specific pattern of wax deposition as a result of different pre-treatment conditions of temperature, light intensity, relative humidity or soil moisture.

It is generally conceded that the hydrophobic nature of the wax covering will determine to a large extent the wettability of the leaf surfaces (Holloway 1969; Martin and Juniper 1970). Contact angle determinations, however, revealed that high regimes of temperature and light resulted in greater contact angles on the adaxial surface of C. album (Table VI), which cannot be correlated with wax structure, or indeed, with differences in susceptibility under these conditions. However, as pointed out by Fogg (1947) contact angle can change diurnally, and hence, differences in

wettability may be more related to other plant characteristics, such as leaf hydration which would cause the cuticle to swell or contract, depending on moisture conditions.

A point of criticism which could be raised is that the environmental observations reported here are based on a relatively short incubation period (an 8-day pre-treatment under controlled conditions prior to wax analysis and herbicide treatment), although during this time substantial differences in the susceptibility of C. album to Basagran were observed. The optimum incubation time appears to be largely dependent on the growth characteristics of the plant species under investigation, and in many cases appears to be at the discretion of the researcher. Juniper (1960), for example, investigating the development of epicuticular wax in pea (Pisum sativum), observed changes in wax structure as soon as 24 hours following incubation under controlled conditions. Baker (1974), on the other hand, working with brussels sprout (Brassica oleracea var. Gemmifera), incubated plants in environmental cabinets for 42 days prior to analysis. In view of the present work it would perhaps have been informative to have analysed wax from plants of C. album throughout their life cycle in order to establish the pattern of wax development.

While the present investigations suggest a lack of correlation between epicuticular wax production and environment, it is evident from the results presented that there are wide variations, both in the quantity and quality of wax from plants used in individual experiments (Tables IV and V). It is proposed that these variations arise from the inherent differences in the growth and developmental patterns of the different batches of plants raised under glasshouse conditions. Seedlings raised under such conditions

are subject to seasonal and daily variations in temperature, light, relative humidity and soil conditions, despite the rigorous attempts at standardisation.

The use of controlled environmental cabinets, however, does not necessarily exclude variation in conditions, although they substantially reduce error in some problem areas. Small fluctuations in temperature, relative humidity, carbon dioxide concentration, air flow and even the decay in the quality of light are still common sources of error in environmental cabinets and require quantification and careful monitoring. Simple factors, such as variations in plant height, canopy and, indeed, position within the cabinet are perhaps of equal importance.

Thus, although the investigations conclude that the effect of environment on the susceptibility of C. album to Basagran is not directly related to changes in the nature of the epicuticular wax, it is evident, from SEM studies, that this surface wax is an important feature of the leaf surface. The cuticle in this species, therefore, may not be the principal means by which foliar penetration is effected. The significance of this will be further developed in the final discussion.

CHAPTER 5

STOMATAL CHARACTERISTICS OF C. ALBUM

5.1 INTRODUCTION

The role of stomata in foliar penetration has been implicated by numerous workers (e.g. Crafts & Foy 1962; Schonherr & Bukovac 1972), who consider that under certain conditions the stomata may play a principal role.

Observations in the field (Chapter 1) have indicated a trend towards reduced control of C. album when applications of Basagran were made during the mid-day period. Since the stomata of many plant species follow a common diurnal pattern of movement with, in many cases, a mid-day closure (Heath & Orchard 1957), the stomatal characteristics of C. album were investigated in an attempt to interpret the results obtained in the field.

The influence of Basagran on stomatal movement in epidermal peels of this species was also examined in detail under different conditions of temperature and light intensity, since environmental conditions have also been shown to be highly influential on the activity of the herbicide (Chapter 4).

5.2 MATERIALS AND METHODS

5.2.1 Leaf surface morphology of C. album

The leaf surface of C. album was investigated by scanning electron microscopy. Leaf sections were prepared as described in Chapter 3 and examined using a Cambridge Steroscan 600 electron microscope at an H.T. voltage of 25 kv.

5.2.2 The effect of Basagran on stomatal movement in C. album

In all laboratory experiments fully expanded leaves were selected from 8-10 week old C. album plants grown under glasshouse conditions (Chapter 1) in John Innes No. 2 compost.

Epidermal strips (approximately 0.5 cm^2) were peeled from the abaxial surface at the same time each day (9-10 a.m.). From these, peels with intact epidermal cells were selected, which were microscopically free from contamination by mesophyll cells, and floated immediately, cuticle upwards, in petri dishes containing 20 cm^3 50 mM Hepes (N-2-Hydroxyethylpiperazine-N-2-ethanesulphonic acid) buffer, pH 6.5, and stored in the dark at room temperature until required for use. Incubations were performed in environmental cabinets (Fisons series 111, models 600G3/TTL and 600G3/THTL) and in a temperature-controlled light incubator (Astell).

The Basagran formulation (BAS 35107H) was diluted in Hepes buffer to a concentration of 10^{-2}, which was approximately equivalent to the recommended field rate of 3 l/280 l H_2O/ha. A pure preparation of the active ingredient, bentazone (98% technical grade) was also used in some experiments. In all cases the treatment solutions were prepared in petri dishes to a volume of 20 cm^3.

Parameters examined were incubation time (0-8 hr), temperature (9-29°C), light intensity (0-96 Wm^2) and bentazone concentration. In addition, the effect of short "pulse" treatments of Basagran on stomatal movement in isolated epidermal peels of C. album was also investigated.

5.2.2.1 Stomatal measurement:- As the stomatal pore of C. album was very small (1-2 μm compared with pore widths of 12 μm for Commelina communis L., Willmer and Mansfield, 1968), the maximum distance between the cuticular lips surrounding the pore was taken as the criterion of measurement (see Fig. 18a), since this also reflected changes in guard cell turgor. Apertures were measured by direct microscopic observation under an oil immersion lens using a calibrated eyepiece micrometer. Cuticular lip measurements ranged from 4 μm when the stomatal pore was closed, to 15 μm in mature leaves. Details of experimental replication and number of stomatal readings are included in the results section. In all cases, however, stomatal readings were taken from at least two epidermal peels, care being taken not to include those at the edge of an epidermal strip.

Results were analysed statistically by either calculating standard deviation values or by conducting a complete analysis of variance.

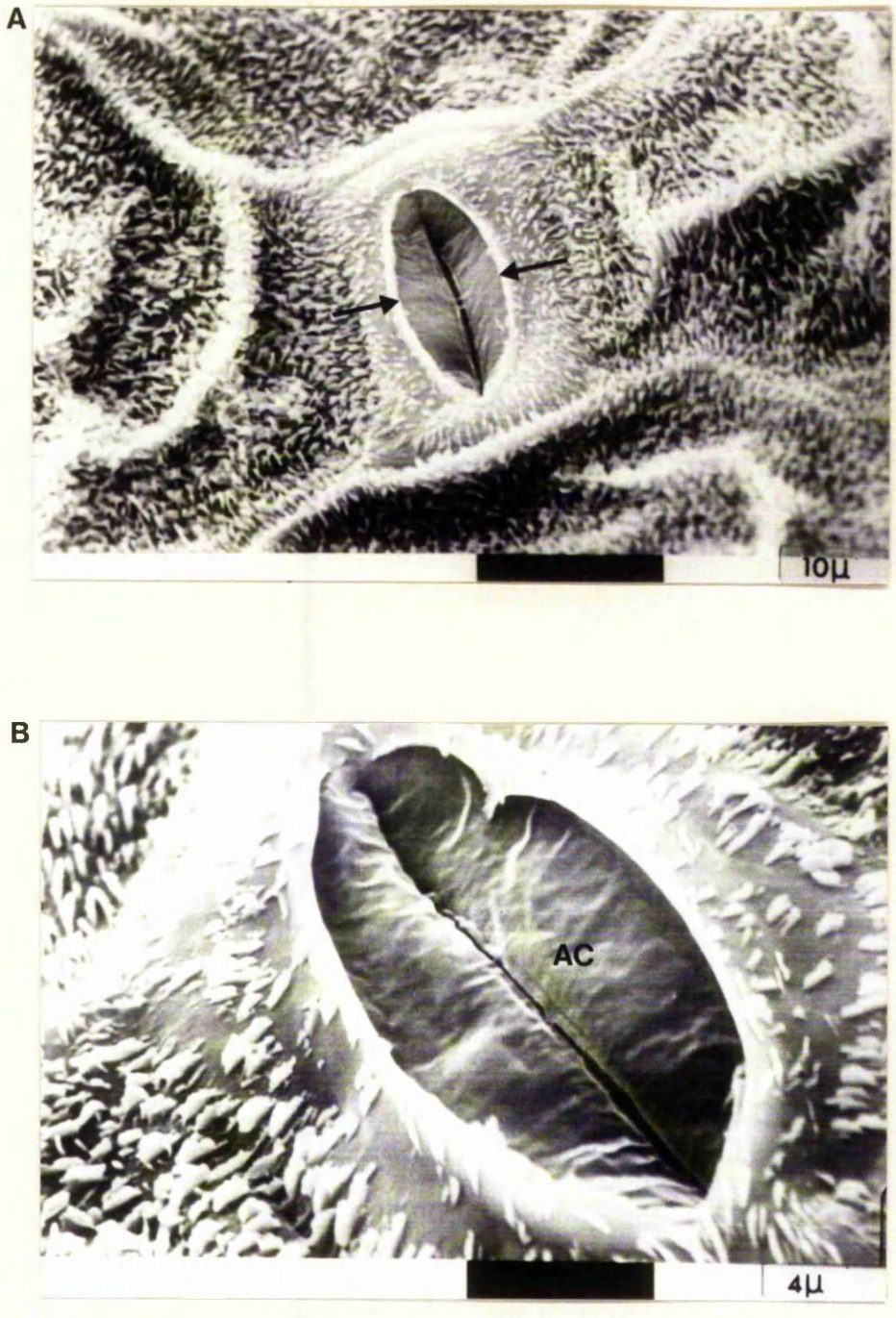
In all cases the time taken to make the stomatal measurements was kept to a minimum in order to reduce the interference of the microscope light. With experience this was reduced to a mean time of 15 minutes.

FIGURE 18. Stomatal morphology of C. album

a) Stoma on adaxial surface. Arrows indicate area for measurement of cuticular lip aperture.
Mag. 2.6 K

b) Stoma on adaxial surface showing large antechamber (AC) which is devoid of surface wax projections.
Mag. 6.4 K

FIGURE 18



5.3 RESULTS

5.3.1 Leaf surface morphology of C. album

Fig. 18 shows the scanning electron micrographs taken of the stomatal apparatus on the adaxial surface of C. album. The stomata are anomocytic, having no true subsidiary cells. The stomatal pore appears to be sunken and the ante-chamber is bordered by a well-defined, raised cuticular lip. The cuticle surrounding the guard cells is densely covered with wax projections, whilst the ante-chamber appears virtually wax-free.

5.3.2 The effect of Basagran on stomatal movement in C. album

5.3.2.1 Time course:- The effect of Basagran on stomatal movement was initially investigated over a 3-hour period. Epidermal peels were incubated at 21°C and at a light intensity of 1.85 Wm^2. Peels were periodically removed and 80 stomatal readings (40 per peel) were taken per treatment. The graph in Fig. 19 typical of several experiments performed, indicates a Basagran-induced response of stomatal opening. Maximum apertures were obtained in the presence of Basagran following an incubation period of 1 hour, apertures then being maintained at this level for the remaining 2 hours of the experiment. The opening process appears to be a rapid one, with increases in aperture being observed after as little as 5 minutes incubation. A similar pattern was observed in control treatments, although apertures measured were of a lesser magnitude.

A succeeding experiment was designed to investigate the effect of Basagran on stomatal movements in C. album

FIGURE 19. The effect of time on Basagran-induced stomatal opening in isolated epidermal peels of C. album

Epidermal peels were incubated at 21°C and a light intensity of 1.85 Wm^2 over a 3-hour period.

Each point represents the mean of 40 measurements taken from each of 2 epidermal peels.

■—■ - Basagran at field rate

○—○ - Hepes buffer control

Bars indicate standard deviation values

FIGURE 19.

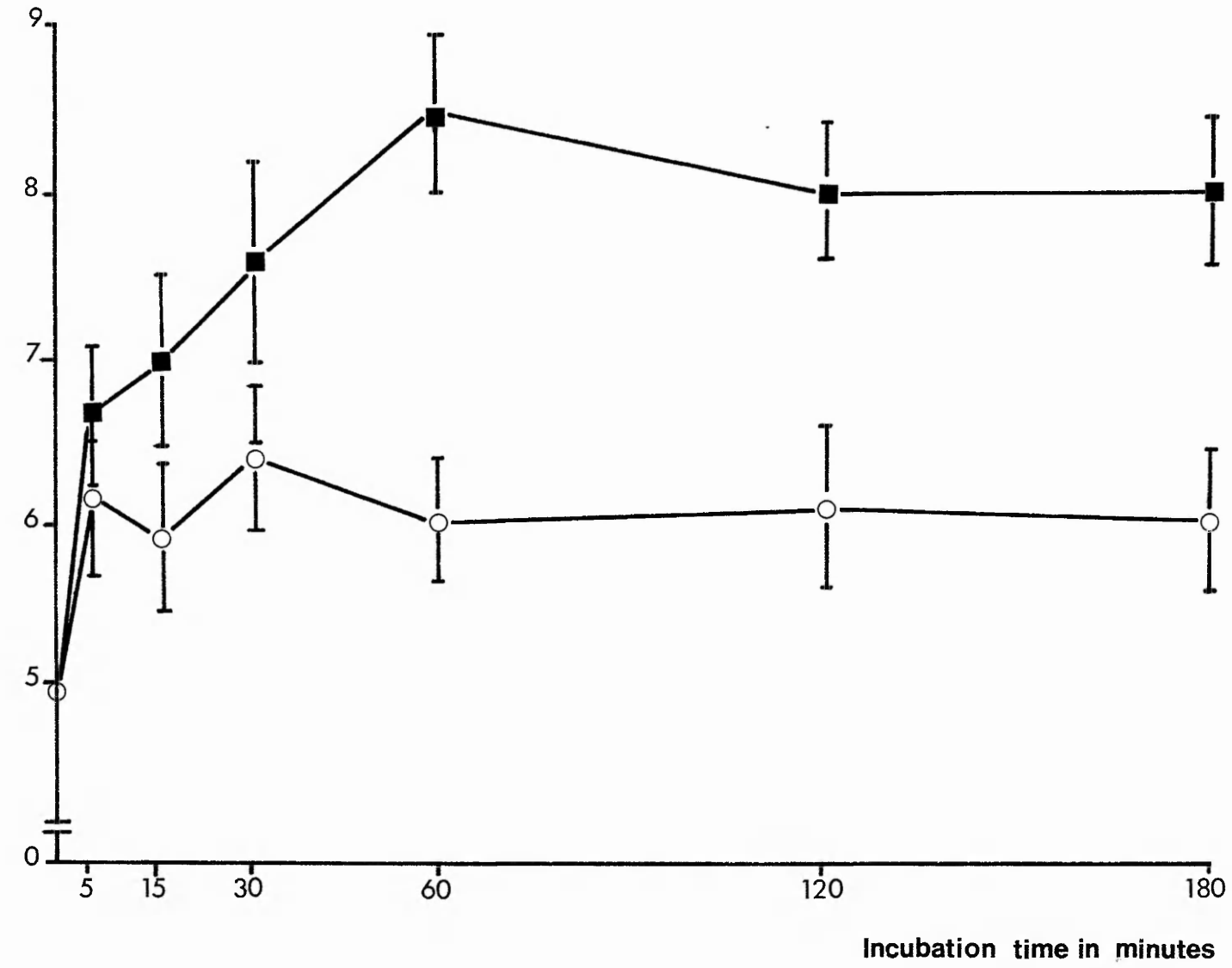

Stomatal aperture µm
9
8
7
6
5
0
5
15
30
60
120
180
Incubation time in minutes

FIGURE 20. The effect of prolonged incubation on Basagran-induced stomatal movements in C. album epidermis

Epidermal peels were incubated at 15°C and a light intensity of 1.85 Wm^2 for an 8-hour period.

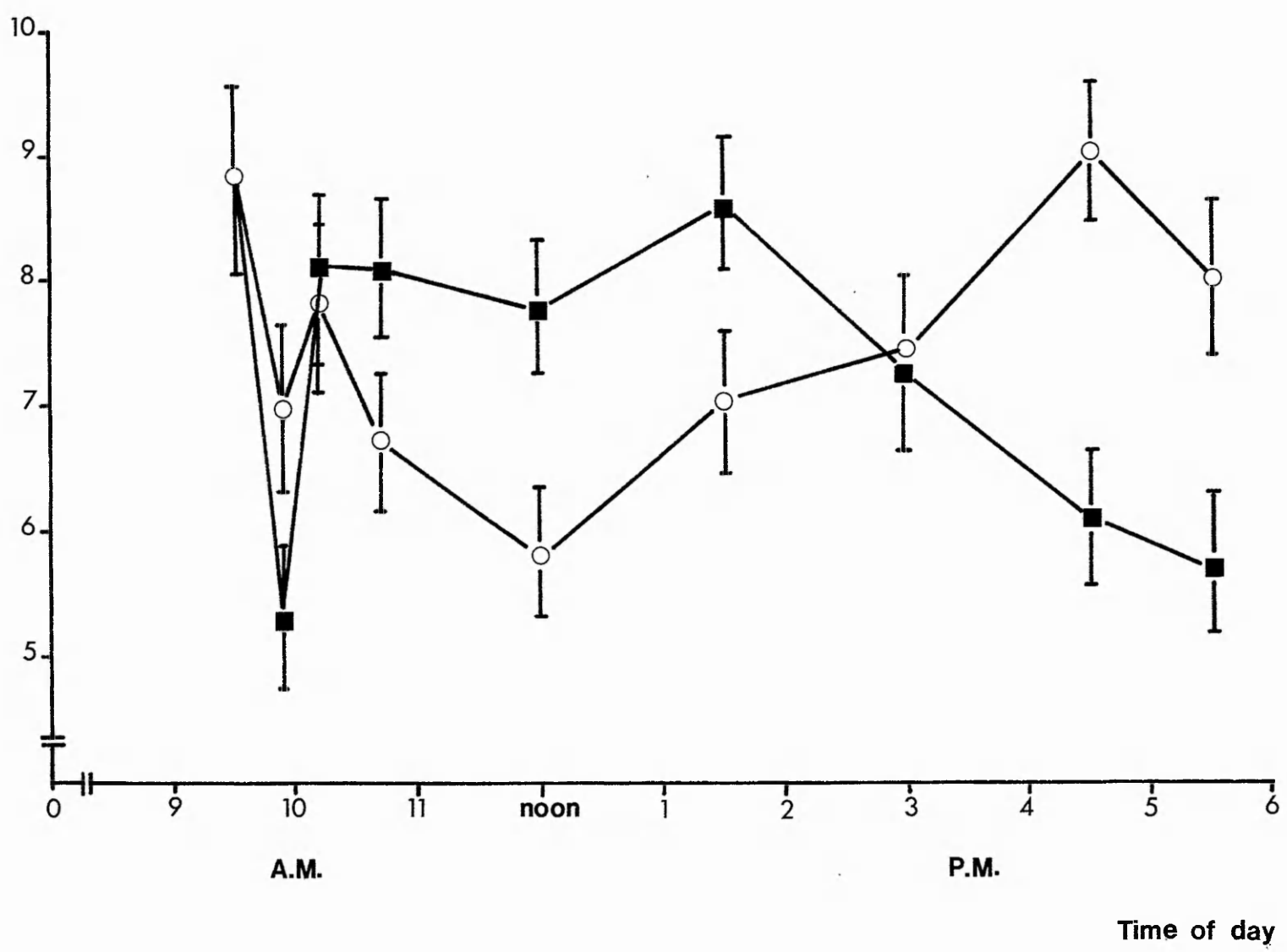
Each point represents the mean of 40 stomatal readings taken from each of 2 epidermal peels.

■—■ - Basagran at field rate

○—○ - Hepes buffer control

Bars indicate standard deviation values

FIGURE 20


Stomatal aperture µm
10
9
8
7
6
5
0
9
10
11
noon
1
2
3
4
5
6
A.M.
P.M.
Time of day

over an 8-hour period in order to establish the effects of long-term incubation.

Epidermal peels were incubated at 15°C and at a light intensity of 1.85 Wm^2. Results are expressed graphically in Fig. 20, each point representing the mean of 40 stomatal measurements taken from two epidermal peels.

Stomatal movements in control treatments followed a typical rhythmic pattern. Following an early morning opening, apertures diminished around mid-day, gradually increased as the afternoon progressed, and waned towards evening. Basagran-treated stomata, on the other hand, did not appear to display this rhythm. The results suggest that Basagran maintains stomatal opening initially, but that this effect diminishes with time.

5.3.2.2 Effect of temperature on Basagran-induced stomatal opening:- Stomatal apertures were measured over a temperature range of 9-29°C in a light incubator at 1.85 Wm^2, incubations being carried out over a period of 150 minutes. The results are expressed graphically (Fig. 21), each point representing the mean of 80 measurements taken on five separate occasions, a total of 400 readings for each value. In the presence of light, Basagran enhanced stomatal opening as a temperature-independent response. Under dark conditions Basagran had no effect on stomatal aperture when compared to a Hepes control.

An analysis of variance was carried out on the data (Appendix II, Table III), revealing the effects of Basagran and light to be highly significant ($p = 0.001$).

FIGURE 21. The effect of temperature on Basagran-induced stomatal opening in isolated epidermal peels of C. album

Epidermal peels were incubated in the dark and at a light intensity of 1.85 Wm^2 for a period of 150 minutes over a temperature range of 9-29°C. Each point represents the mean of 80 stomatal readings carried out on 5 separate occasions (i.e. 400 stomatal readings/point). An analysis of variance has been carried out on these data (Appendix II Table III).

■——■ - Basagran at field rate + light

■----■ - " " " " - light

○——○ - Hepes control + light

○----○ - " " - light

FIGURE 21

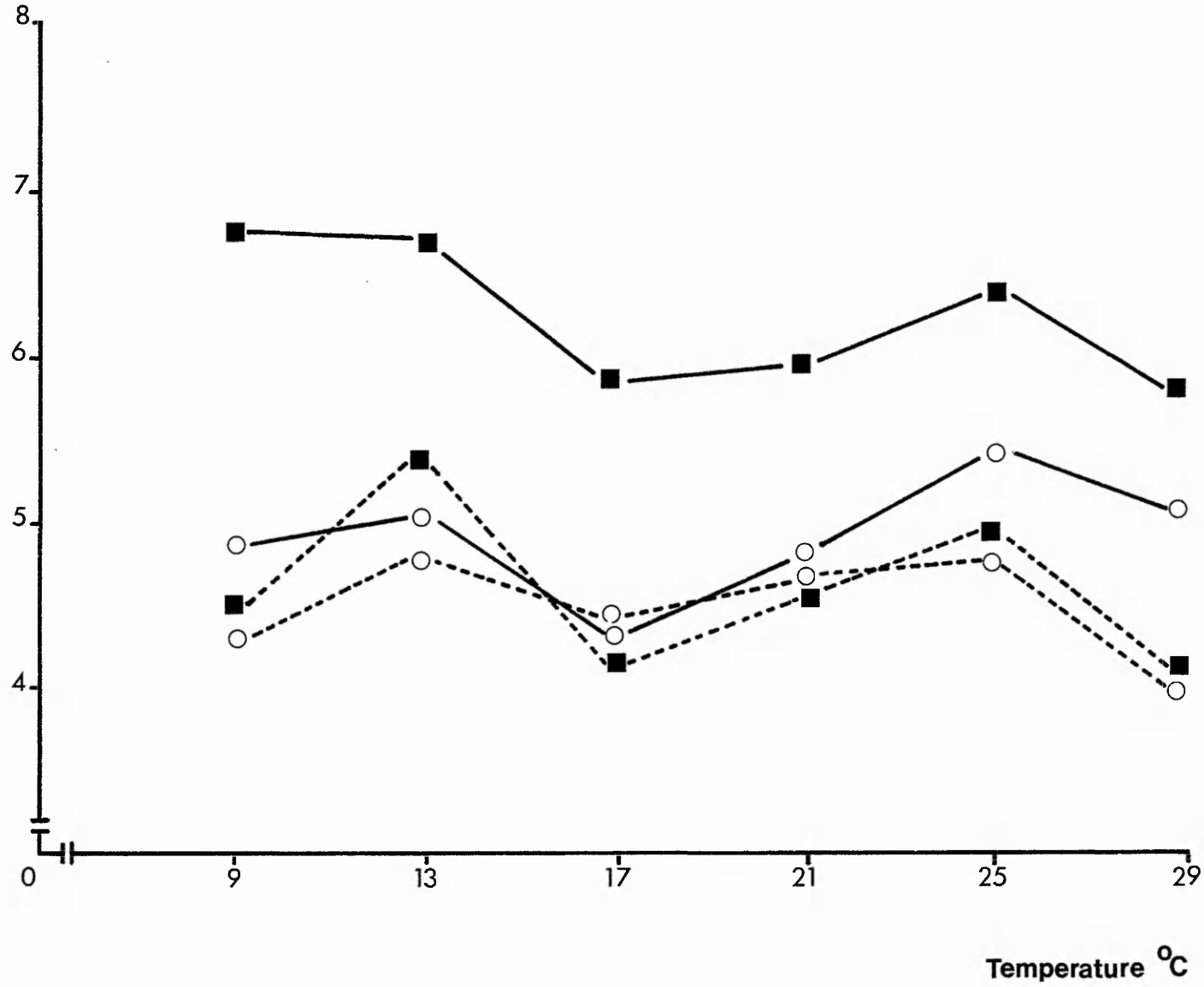


FIGURE 22. The effect of light intensity on Basagran-induced stomatal movements in C. album epidermal peels

Epidermal peels were incubated at a temperature of $21^{o}C$ for a period of 150 minutes over a light intensity range of 0-96 Wm^{2}.

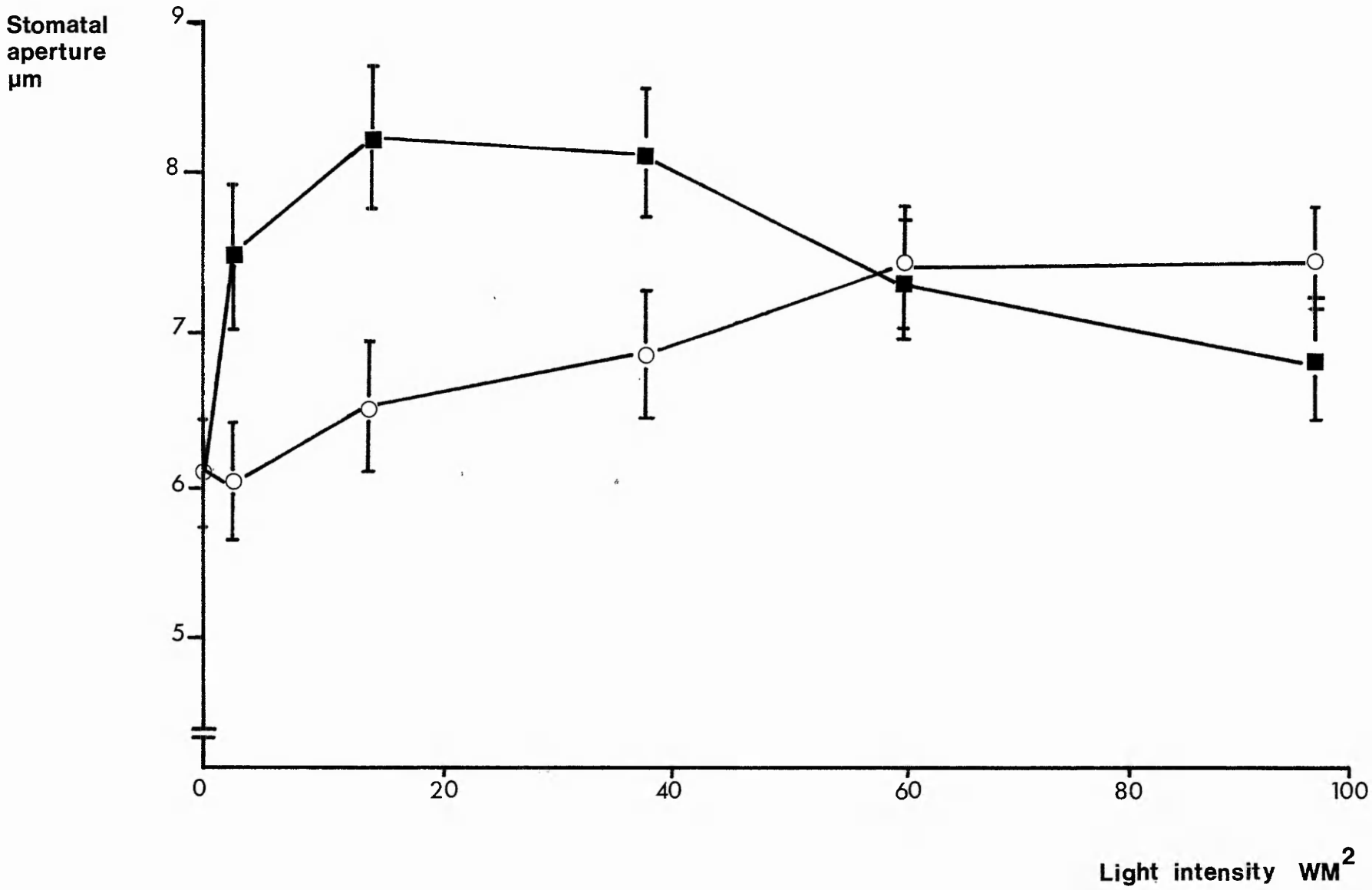
Each point represents the mean of 40 measurements taken from each of 2 epidermal peels.

■—■ - Basagran at field rate

○—○ - Hepes buffer control

Bars indicate standard deviation values.

FIGURE 22


Stomatal aperture µm
9
8
7
6
5
0
20
40
60
80
100
Light intensity WM2

The interaction between Basagran and light also proved highly significant ($p = 0.001$).

5.3.2.3 The effect of light intensity on stomatal opening in C. album:- The influence of Basagran on the stomatal apparatus of C. album was investigated over a range of light intensities from 1.85 - 96 Wm^2 at a constant temperature of 22°C. Incubations, over a 150 minute period, were carried out both in environmental cabinets and in a light incubator, both providing illumination by a series of Warm-white fluorescent tubes (Philips). The graph in Fig. 22 is typical of several investigations, each point representing the mean of 80 stomatal readings taken from two epidermal peels.

Basagran was observed to exert maximum effect at 12.95 Wm^2, declining as light intensity increased. At 96 Wm^2 the herbicide appeared to cause disruption of cellular membranes, resulting in plasmolysis in some areas of the tissue.

5.3.2.4 The effect of technical grade bentazone on stomatal movement in C. album:- The previous data were obtained using a formulation (Basagran) containing only 48% ($^w/_v$) bentazone. In view of this, a dose-response curve was determined with technical grade (98%) bentazone.

As bentazone has a very low water solubility, it was first converted to its sodium salt by dissolving in IN sodium hydroxide. A range of bentazone concentrations from 4×10^{-2} to 4×10^{-6}M was prepared in Hepes buffer and dose response curves were determined. Epidermal peels were

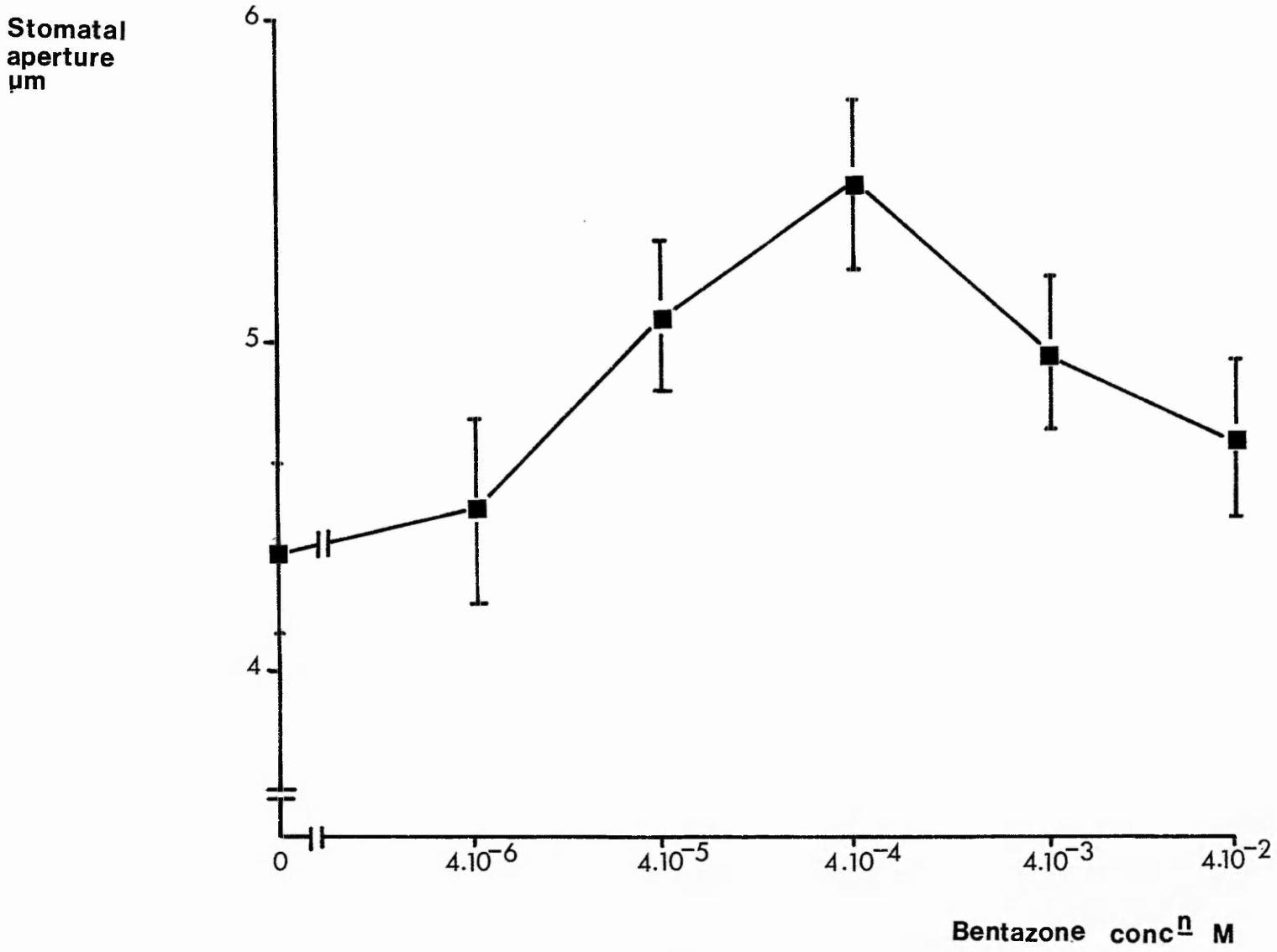
FIGURE 23. The effect of bentazone concentration on stomatal opening in isolated epidermal peels of C. album

Epidermal peels were incubated at 17°C with a light intensity of 1.85 Wm^2 over a period of 150 minutes with a range of bentazone concentrations from 4×10^{-2} to 4×10^{-6} M.

Each point represents the mean of 40 stomatal measurements taken from each of 2 epidermal peels.

Bars indicate standard deviation values.

FIGURE 23


Stomatal aperture µm
6
5
4
0
4.10⁻⁶
4.10⁻⁵
4.10⁻⁴
4.10⁻³
4.10⁻²
Bentazone concⁿ M

incubated in the solutions for 150 minutes at 17°C and a light intensity of 1.85 Wm^2.

A typical dose-response curve is presented in Fig. 23, each point representing the mean of 80 readings. This clearly demonstrates that the opening observed in the presence of the formulation, Basagran, is indeed principally due to the active ingredient, bentazone. Maximum apertures were obtained at a concentration of 4 x 10^{-4}M bentazone.

As bentazone was converted to its sodium salt, an additional control was set up to establish the effect of Na^+ on stomatal movements in C. album. It was found that, under the conditions of the experiment, Na^+ had no apparent influence on stomatal aperture when compared to a Hepes control.

5.3.2.5 The effect of Basagran "pulse" treatments on stomatal behaviour in C. album:- The principle aim of this experiment was to assess the length of time the herbicide was required to be in contact with epidermal peels in order to affect the pattern of stomatal movements in C. album. It was also hoped to determine the rate of recovery, if any, of treated peels from these different "pulse" treatments.

Epidermal peels were subjected to "pulse" treatments of Basagran for periods of 15, 30 and 60 minutes. Following Basagran treatment, peels were removed, washed and then returned to Hepes buffer for the remainder of the incubation period. Incubations were carried out at a temperature of 15°C and a light intensity of 1.85 Wm^2.

FIGURE 24. The effect of Basagran "pulse" treatments on stomatal movements in C. album epidermal peels

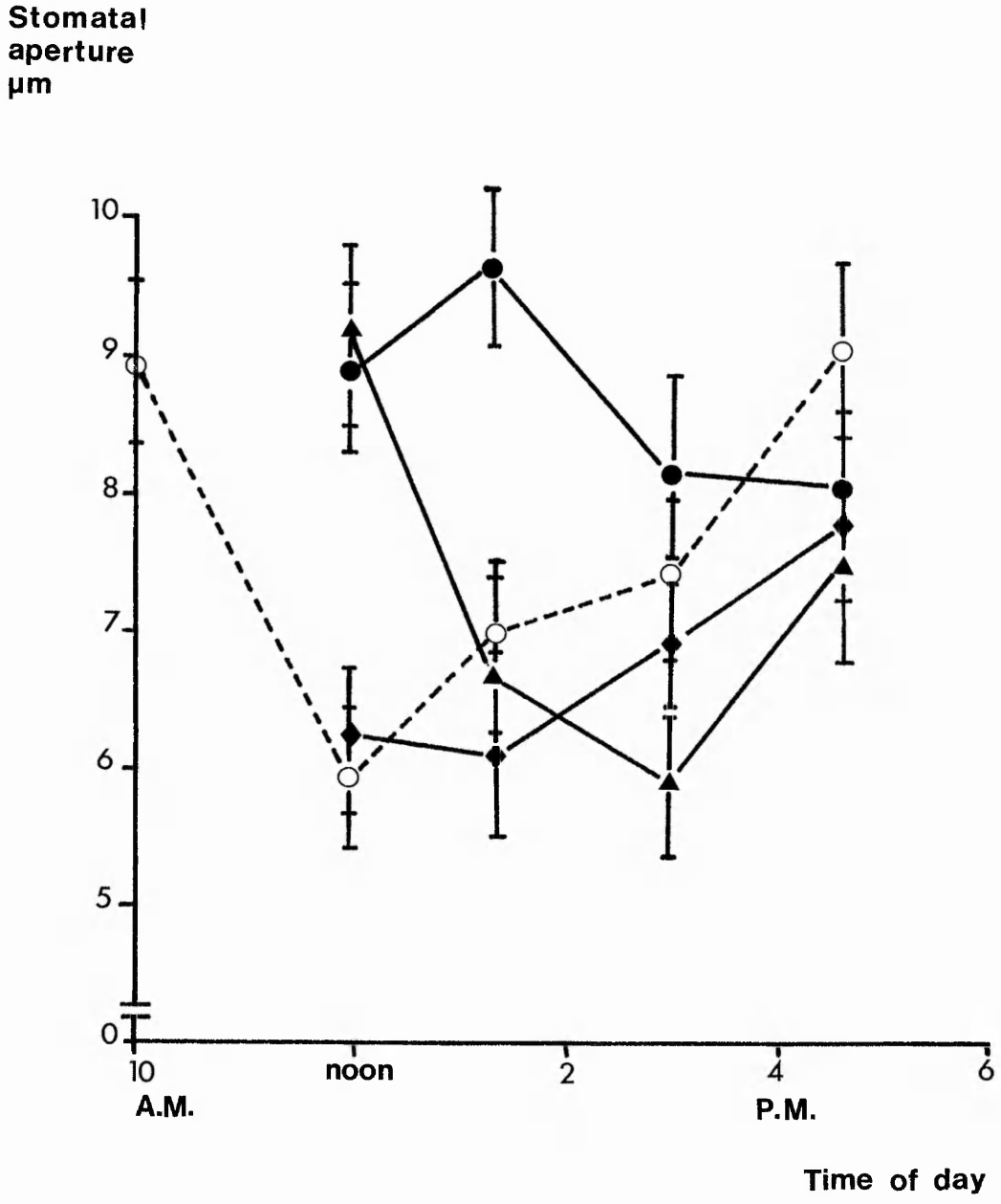
Epidermal peels received pulse treatments of Basagran for 15 (◆——◆), 30 (▲——▲) and 60 (●——●) minutes. Incubations were carried out at 15°C and a light intensity of 1.85 Wm^2 over an 8-hour period. Following treatment, the peels were returned to Hepes buffer.

Each point represents the mean of 20 stomatal readings from each of 2 epidermal peels.

O---O - Hepes control

Bars indicate standard deviation values.

FIGURE 24



The graph in Fig. 24 summarises the results obtained, each point being the mean of 40 stomatal measurements.

The control strips of C. album stomata displayed a typical rhythm of movement, with a notable reduction in aperture during the mid-day period. Epidermal peels receiving a pulse treatment of Basagran for 15 minutes showed little deviation from this pattern. Treatment with Basagran for 30 minutes, however, resulted in substantial increases in stomatal aperture around mid-day, although the effects of Basagran soon diminished in the early afternoon, as stomatal movements appeared to revert back to the normal rhythm of the control. This effect became more pronounced in epidermal peels subjected to a 60 minute Basagran treatment. Basagran appeared to maintain stomatal opening for a longer period of time, although again as the afternoon progressed, stomatal closure became evident.

5.4 CONCLUSIONS

From the results presented it can be concluded that:-

(1) Although the cuticle of *C. album* displays a dense covering of epicuticular wax, the stomatal antechamber appears virtually free of wax platelets.

(2) Basagran induces rapid stomatal opening in isolated epidermal peels of *C. album*.

(3) The Basagran-induced stomatal response is independent of temperature.

(4) Stomatal opening is only observed in the presence of light (optimum intensity 12.95 Wm^2). Higher light intensities of 96 Wm^2, however, result in cell damage in the presence of Basagran.

(5) The active ingredient, bentazone, is principally responsible for the induced opening, with maximum apertures observed at a bentazone concentration of 4×10^{-4} M.

(6) The response of *C. album* stomata to Basagran appears to be dependent on the dose of the active ingredient, bentazone.

5.5 DISCUSSION

The experiments reported demonstrates that Basagran induces rapid stomatal opening in *C. album* epidermal peels in a light-dependent response over a temperature range of 9-29°C. This response has not, however, been demonstrated in any of the other species investigated (*Pisum sativum* L., *Brassica napus* L., *Phaseolus vulgaris* L., *Capsella bursa-pastoris* L. and *Stellaria media* L.).

The opening process was a rapid one, with maximum apertures being observed following 1 hour incubation with the herbicide (Fig. 19). The observation that prolonged incubation with the herbicide resulted in stomatal closure (Fig. 20) cannot readily be explained. It could be argued, for example, that incubation of epidermal peels for a period of 8 hours would result in a loss of physiological function, the turgor

balance unable to be maintained with resultant stomatal closure. This does not explain, however, why control peels apparently continued to function over this period. Alternatively, Basagran could exert a direct effect on the epidermal cells at the plasmalemma, culminating in stomatal closure. It would be tempting to suggest that stomatal closure following prolonged incubation is as a result of the herbicide inhibiting photosynthesis. However, assuming the epidermal peels are free from contamination from the mesophyll (which, indeed, was the case in all experiments), the only cells containing chloroplasts are the guard cells themselves. This observation may be of considerable importance if guard cell chloroplasts provide the energy for stomatal opening in this species, a fact that currently remains unproven.

The stomatal response appeared to be independent of temperature (Fig. 21), which is rather surprising, since the general consensus of opinion (e.g. Stålfelt 1962; Willmer & Mansfield 1970) favours stomatal opening mediated by an active process requiring an energy source. It would seem likely, therefore, that higher temperatures would enhance stomatal opening. In the case of C. album, however, it may be that increases in temperature simply accelerate the opening process rather than cause actual increases in aperture.

The enhanced stomatal opening in the presence of Basagran was only observed in the presence of light (Figs. 21, 22). Indeed, only relatively low light intensities were required for a maximal response, and at higher light intensities of 96 Wm^2 (approximately equivalent to dull daylight), stomatal opening was not observed in the presence of Basagran. This latter response was accompanied by membrane disruption manifest in epidermal cell plasmolysis. This suggests an effect of the herbicide on cell membrane integrity, the effects of which are only apparent at

high light intensities. This could be related to the effects of prolonged incubation of Basagran with epidermal peels, where stomatal closure was observed. Cell damage, however, was not evident in this latter case and therefore suggests that the effect is light dependent, perhaps reflecting guard cell energy requirements for movement.

The stomatal opening observed following incubations of epidermal peels of C. album with technical grade bentazone (in 98% pure) confirm that the effects reported are principally due to the active ingredient and not to any other additive present in the formulation (Fig. 23). It is rather puzzling, however, that maximal opening was observed at a bentazone concentration of 4×10^{-4} M, and as yet no reasonable explanation can be offered for the reduced response observed at higher bentazone concentrations. These supra-optimal concentrations appear to be inhibitory to the opening movement. Although bentazone was made soluble by conversion to its sodium salt, the presence of Na^+ in the incubating medium was not found to influence stomatal movements (Mean stomatal apertures recorded for Na^+ at 2×10^{-2} M in Hepes buffer were 4.32 ± 0.36 μ compared with 5.18 ± 0.7 μ for Hepes control).

Although these investigations were largely conducted over relatively short periods of incubation, and, indeed, at the same time during the day, no account has been taken of the influence of endogenous rhythms on stomatal movements. It has been demonstrated in this laboratory (Smalley 1979) that the stomatal rhythm of C. album, measured on intact plants, follows a typical pattern of diurnal movement with a pronounced mid-day closure. The amplitude of this rhythm was found to be influenced by soil moisture conditions. Plants maintained under favourable moisture conditions displayed a wide response, whereas the amplitude of this response was somewhat reduced in water-stressed plants. Further investi-

gations revealed that this rhythm was still evident in isolated epidermal peels of C. album, albeit of a lower magnitude.

The influence of Basagran on the stomatal rhythm in detached epidermis was demonstrated in the final experiment on the effect of dosage on stomatal opening (Fig. 24). Short incubations of peels in Basagran (15 minutes) had little effect on the rhythm of movement, whereas 60 minute incubations resulted in extended periods of stomatal opening and even the effects of mid-day closure appeared to be totally opposed. These results infer that the efficiency of Basagran is dependent on exposure time, and although these results were obtained in totally artificial system, it could be argued that in situ the efficiency of Basagran would be dependent on the length of time the herbicide remained on the leaf surface in a penetrable form. Thus, any situation which might be expected to reduce herbicide availability, such as rainfall, high temperatures (droplet desiccation) or high light intensity (photo decomposition), would be likely to reduce the efficiency of the herbicide, a point further developed in the final discussion.

The exact nature by which Basagran affects stomatal movements in C. album is not known, nor is it apparent why C. album in particular is affected, since other susceptible species showed no response (e.g. C. bursa-pastoris, S. media). It could be argued, for example, that the observed response was due to epidermal cell damage leading to stomatal opening as a result of reduced pressure on the guard cells. Epidermal cell damage was only evident, however, at high light intensities and indeed it is difficult to envisage sufficient cell damage capable of affecting guard cell turgor after such a short period of incubation.

Such a rapid response of the guard cells to Basagran suggests a direct effect on the stomatal apparatus. It may be, for example, that

Basagran alters the permeability of the guard cell membranes, thus effecting a rapid accumulation of K^+ within the guard cells. From preliminary experiments it has been found that this opening process is dependent on the pH of the incubating medium. Incubations of Basagran with citrate buffer at pH 4.5, for example, failed to produce stomatal opening. This suggests that Basagran may facilitate the entry of K^+ into the guard cells at pH 6.5, but that this entry is restricted at pH 4.5. That the effect of Basagran is on K^+ movement was further illustrated in a subsequent experiment where stomatal opening was observed in peels incubated with citrate buffer at pH 4.5 + K^+, but that when Basagran was added to this incubating medium, stomatal opening was prevented.

This pH-dependence, although obviously requiring confirmation, suggests the involvement of a membrane-bound proton pump in C. album stomatal movement, and active transport of ions into and out of cells is often associated with high ATP-ase activity in the tissue (Leigh, Wyn Jones & Williamson 1973). Indeed, Raghavendra, Rao and Das (1976) have implicated the role of two iso-enzymes of ATP-ase which operate at different pH levels. They suggest from their results that the ATP-ase active at pH 7.5 is associated with stomatal opening and that the ATP-ase active at pH 5.5 is associated with stomatal closure in Commelina benghalensis. It is conceivable, therefore, that a similar system is present in C. album and that the iso-enzymes present are operative at pH levels of 6.5 and 4.5 which may be associated with stomatal opening and closure respectively in this species.

FINAL DISCUSSION

Experiments conducted under glass, in the field, and also under controlled environmental conditions have consistently shown that the response of C. album to Basagran is highly dependent on the environmental conditions before, during and following herbicide application. This statement, however, must be interpreted within the limitations of the experimental techniques adopted throughout this study. The response of C. album to Basagran, for example, is based on a visual assessment of herbicide damage over a relatively limited scale of 0-5, a factor previously discussed in Chapter 1. This is an arbitrary method of assessment but it must be emphasized that, particularly with controlled environmental work, severe limitations of space and, indeed, time existed throughout these experiments. Only two environmental cabinets were available for the study and since these could hold a maximum of 36 plants each, the scope for extensive herbicide assessments based on destructive methods of sampling (fresh/dry weight etc.) was severely restricted. For this reason it was felt that a continual visual assessment over a two-week period would, in this case, be of greater value. With reference to herbicide applications it is important to note that the herbicide was applied to plants in pots where the soil surface was unprotected from the herbicide. Since Basagran has been shown to be active in the soil (Mine & Matsunaka 1975), it could be argued that precautions should have been taken to eliminate this effect. However, it should also be pointed out that such protection is absent in the field and therefore results of herbicide damage where the soil surface has been protected could be misleading.

Throughout the controlled environmental experiments, plants were grown under normal glasshouse conditions prior to being placed under controlled conditions for an 8-day pre-treatment period. As discussed

previously (Chapter 4), the length of the pre-treatment period must be chosen to suit the requirements and, indeed, limitations of the experiments. In the present case the principle aim was to establish how changes in environment subsequently affect the susceptibility of C. album to Basagran and how environment influences the nature of the epicuticular wax in this species. In the field, however, it is highly unlikely that conditions will remain constant for any length of time. Nevertheless, it is evident from the results that such a short pre-treatment period resulted in substantial differences in susceptibility to the herbicide.

The most striking differences in response occurred as a result of a transition from low pre-treatment temperatures and light intensities to high post-treatment conditions. The apparently rapid response observed suggests that the effect is physiological in nature, i.e. as a direct result of the general metabolism of the plant, a premise previously discussed in Chapter 4. Since these results infer that the general metabolic rate of the plant will determine to a large extent its response, it follows that any factor which affects metabolism will correspondingly influence this response. It is well-known, for example, that actively growing plants, where metabolic turnover is high, are more susceptible to herbicides (Muzik 1976), and that this is particularly relevant in young plants, where meristem activity is high. The present study substantiates this view with the finding that C. album susceptibility is greatly dependent on plant size. The tolerance shown by more mature specimens may, then, be physiologically due to the lower rate of photosynthesis, and the generally lower levels of metabolic activity in older plants.

These physiological considerations, however, only partially explain the observed responses. The tolerance shown by plants maintained under conditions of high relative humidity, for example, cannot readily be

explained on a physiological basis, since plants grown under these conditions would be expected to be metabolically active and hence more susceptible to the herbicide. This finding is in complete contrast with previously published work (e.g. Nalewaja et al 1975, 1977; Babiker, Cook & Duncan 1976; Dannigkeit 1977). It is difficult, however, to make direct comparisons with the findings of other workers, since experimental techniques vary. Babiker et al (1976), for example, investigating the effect of relative humidity on amitrole activity in P. vulgaris, based their observations on plants under polythene bags. This is a totally artificial system, and involves additional factors such as changes in light intensity and, indeed, carbon dioxide concentration within the enclosed, saturated atmosphere. The work published by Nalewaja et al (1975, 1977) and Dannigkeit (1977) is of more direct relevance. In experiments involving the use of Basagran, it was generally found that high relative humidity promoted increased activity. Again, the methods of Nalewaja et al (1975, 1977) are subject to some degree of criticism. Plants were maintained under normal glasshouse conditions prior to being treated, and were only placed under controlled environmental conditions following herbicide application. From the experiments presented in this thesis it is clear that pre-treatment conditions play a significant role in governing the response of plants to herbicide treatments.

These differences in experimental technique may account for the contrasting results obtained, though it is also relevant to note that different plant species were used in the experiments. It may be, therefore, that C. album behaves differently to other plant species in relation to susceptibility under high relative humidity conditions, though the reason for this is not readily apparent.

The present investigations, together with field observations (King 1976), suggest that under prolonged periods of drought C. album shows

some degree of resistance to applications of Basagran. It is difficult to accept that this is solely as a result of lowered metabolic rate, since treated plants would still be expected to develop necrotic symptoms, albeit over a longer period of time. Observations in the field, however, revealed that such plants showed little visible signs of injury and suggest, instead, that resistance may be more directly related to the ability of the herbicide to penetrate the leaf surface and to reach its site of action.

In assessing the importance of foliar penetration, the majority of work carried out in this thesis supports the accepted view that the plant cuticle is the principal route by which solutes gain access into the leaf (Crafts & Foy 1962), and that the epicuticular wax is the principal barrier to foliar penetration (Leece 1976, 1978). However, contrary to the findings in other plant species (e.g. Baker 1974), the epicuticular wax in C. album did not change, either in composition, degree of deposition or, indeed, in ultrastructure as a result of the plants being maintained for 8 days under different conditions of temperature, light, relative humidity and soil moisture. It could be argued, however, that an 8-day pre-treatment period under constant conditions would result in little modification of surface wax projections, and that the nature of these wax deposits is largely a function of the glasshouse conditions under which the seedlings of C. album were raised. This is a point of contention, but studies by Baker (1974) and substantiated by others (Chapter 4) suggests that changes in the structure of wax may occur as little as 48 hr following changes in environmental conditions. The principle aim of this study was to establish how environmental changes can modify the surface wax, rather than to investigate the influence of environment on epicuticular wax development per se.

The difference in susceptibility observed as a result of changes in environment cannot, therefore, be correlated with changes in the nature of

the surface wax layer (Chapter 4). If the epicuticular wax is not the limiting factor, then some other component may be limiting the entry of Basagran into the leaf.

It is considered by many authors (e.g. Kamimura and Goodman 1964, Prasad and Blackman 1965) that the penetration of herbicides is effected by a combination of metabolic and non-metabolic processes, initial uptake being predominantly a diffusive process whereas subsequent absorption is metabolically controlled.

Although passage through the cuticle is considered to be a diffusive process, results presented by Norris and Bukovac (1969), using isolated cuticular membrane of pear leaf, have shown that increased penetration of NAA was associated with high temperature. The corresponding high Q_{10} values obtained are considered by these authors to be related to the lipoidal nature of the membrane. The high energy barrier associated with such membranes is considered to be an important rate-limiting step in penetration, although there does not, however, appear to be any direct evidence to support this view. The validity of such experiments is difficult to assess, since the process of isolation of cuticles by either chemical or enzymic methods, followed by wax extraction will undoubtedly lead to changes in the structure and hence penetration properties of the delicate cuticular membrane. Results based on such investigations cannot therefore be directly extrapolated to the situation one might expect in the intact plant. However, on a comparative basis, this type of work may give some idea of the differences in permeability between different plant species.

Passage across the epidermal cell plasmalemma is considered to be a metabolic process, and Sargent & Blackman (1969) have discussed the possibility of this membrane as the principal barrier to uptake and consider that differences in penetration may be as a result of the level

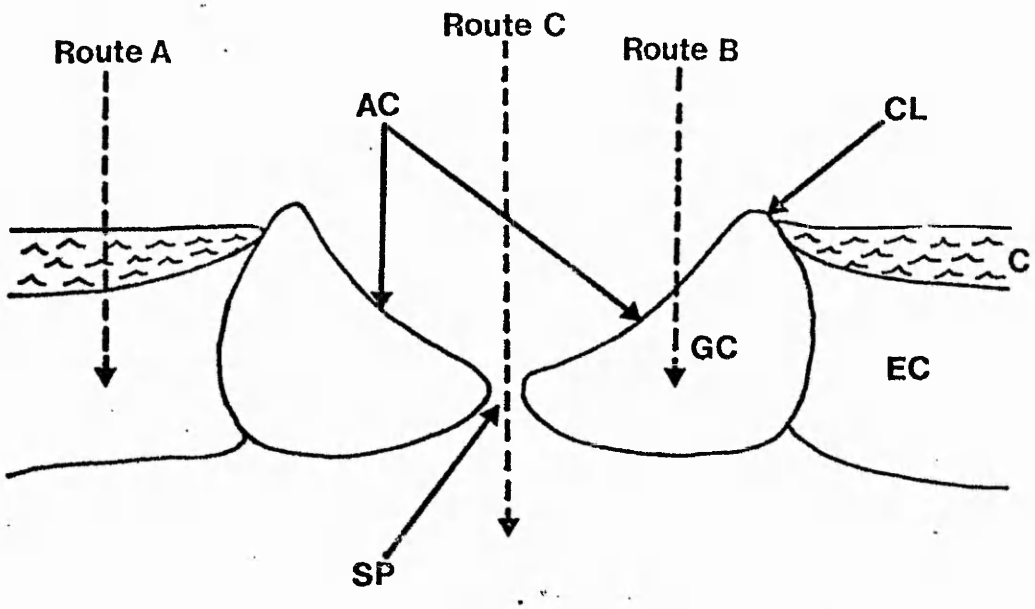
of ATP available to effect transfer, which in turn is influenced by factors such as light and temperature. The environmental effects described, therefore, could conceivably be due to differences in the rate of penetration governed by this metabolic component.

To reach its site of action, however, bentazone must also penetrate yet another membrane system, namely that associated with the chloroplast, so introducing a further rate-limiting step towards penetration. Thus, the amount of exogenously-applied herbicide would be expected to be considerably greater than the amount that actually reaches the site of action. Indeed, it has been reported from previous studies in this laboratory (McFarlane, 1978) that in pea (Pisum sativum var Feltham First), the amount of Basagran required to inhibit the Hill reaction by 33.5% was only 1.9% of the amount actually applied to the leaf surface. It should be emphasized, however, that pea is relatively tolerant to Basagran and therefore, it would be expected that in more susceptible plants, such as C. album, the amount of Basagran reaching its site of action would be of a greater magnitude or that the target biochemical process is more sensitive.

Although the performance of Basagran in the control of C. album may well be related to its uptake by membranes within the leaf, the exact route by which the herbicide initially gains entry should also be considered.

The schematic diagram presented below (Fig. 25) displays the possible routes by which the herbicide may gain access into the leaf:-

Fig. 25. Possible routes for the penetration of Basagran into C. album leaves



Route A – via cuticle

" B – via antechamber

" C – via stomatal pore

C - Cuticle
EC - Epidermal cell
GC - Guard cell
CL - Cuticular lip
AC - Antechamber
SP - Stomatal pore

The present investigation has shown that the epicuticular wax in C. album is principally composed of long-chain aldehydes which form a dense covering on both abaxial and adaxial leaf surfaces. Although the epicuticular wax of C. album has been investigated by other workers (e.g. Allebone et al 1970, 1972; Baker & Bukovac 1971; Hamilton 1976), detailed accounts of methods of plant cultivation, development and leaf sampling are very scant and prevent accurate comparisons to be made. Allebone et al (1970, 1972), for example, harvested plants of C. album from fields in The Wirral, Cheshire. No mention was made of plant age at harvest, nor was any reference made to the range of leaves analysed. Similarly, a more recent paper by Hamilton (1976), studying the penetration of 2,4-D, gave

little mention of the growth of plants or harvest details. In the present investigations care was taken in the selection of leaf material for subsequent wax analysis. Leaves from nodes 5-8 were sampled, which avoided very mature and very young specimens. Despite the lack of experimental detail, Hamilton's paper on 2,4-D penetration yielded some very interesting results. Following the application of 2,4-D to C. album leaves (again, no mention was made of the method of application or details of plant age), after 1 hour 93% of the herbicide still remained within the surface waxes in contrast to Stellaria media, where more than half of the applied 2,4-D had penetrated after 1 hour. One week following application there still remained 28% of the herbicide on C. album leaf surfaces. Hamilton explains this by reference to the nature of the surface wax of C. album, which is rich in non-polar components. In addition, he measured contact angles of 140^{o} on the leaf surface which he considers a significant factor. These findings are in general agreement with observations reported in this thesis. This strong correlation makes the earlier findings of Challen (1962) rather dubious. He studied a range of plants and classified the leaf surfaces as either unwettable, partially wettable or completely wettable. C. album was classified as completely wettable according to his experimental techniques adopted, which involved dipping leaves in aqueous suspensions of lycopodium spores and an investigation of spore dispersal. Again samples were harvested from the field and details of plant age would have been of value. The results of Baker and Bukovac (1971) cast further doubt over Challen's results, since these workers also found that C. album wax contained significant quantities of aldehydes. From penetration studies using filter paper impregnated with these waxes, they concluded that hydrocarbons and aldehydes strongly impede the penetration of water and polar molecules.

From the present investigations and from previously published work (Baker & Bukovac 1971; Hamilton 1976) it is concluded, therefore, that the surface wax of C. album presents a considerable barrier to foliar penetration and that other, more preferential sites of entry may be of greater significance in this species.

An additional and perhaps alternative route for the penetration of foliar-applied herbicides is the stomatal apparatus, and many workers (e.g. Schonherr and Bukovac 1972) believe that, under certain conditions, stomata may play a principal role. Indeed, the question of stomatal penetration has been greatly debated, with much ambivalence in the literature. This is largely as a result of the variations in the experimental methods used by various workers. Currier & Dybing (1959) and Dybing & Currier (1961), for example have argued the case for stomatal penetration based on experiments with fluorescent dyes as tracers. While it may be said that fluorescent dyes may not behave in a similar manner to herbicides, these workers have indicated, using radioactive maleic hydrazide, that these chemicals produce similar results. Their investigations are based on a five minute immersion period of cut leaves in the dye solution, followed by rinsing. In leaves with open stomata, rapid infiltration of the substomatal chambers followed when a surfactant was added. Penetration when stomata were closed, on the other hand, was very much reduced. It is of interest to note that from their experiments on a range of plant material, the open stomata of C. album were infiltrated even in the absence of a surfactant, though penetration was substantially improved by its presence.

Some of their experimental techniques are open to criticism. The immersion of cut leaves into solutions for 5 minutes, for example, would result in substantial pressure being exerted at the leaf surface, which

would be much greater than that under normal field conditions. In a subsequent paper by Currier et al (1964), this was overcome by spraying plants directly to run-off and allowing them to drip, inverted for five minutes prior to rinsing. Penetration was again found to be largely stomatal, as assessed by examination of dye in the substomatal chambers. The use of dye again correlated well with applications of dalapon. It is interesting to note that in C. album there was little difference in stomatal penetration between upper and lower leaf surfaces, and that sufficient herbicide had penetrated following five minutes treatment to induce an injury response to dalapon.

Skoss (1955) favours stomatal involvement for a number of reasons:- 1) a number of workers indicated that internal injury is localized in the immediate vicinity of stomata, both with polar and non-polar herbicide sprays, 2) in vitro, the cuticle is impermeable to the passage of certain organic salts in solution and, 3) from results of field applications of herbicidal sprays at various times of the day, he has concluded that greatest herbicide effectiveness coincided with periods of widest stomatal opening.

While it is generally conceded that aqueous solutions cannot penetrate the stomatal pore directly, due to their high surface tension (Schonherr & Bukovac 1972), there is considerable evidence to suggest that the guard cells themselves are preferential sites of entry (e.g. Sargent & Blackman 1962). However, it should be mentioned that these authors treated bean (P. vulgaris) leaf discs for 8-hour periods. It is the opinion of workers such as Currier et al (1964) that stomatal penetration is a rapid process, occurring as little as five minutes following treatment, whereas cuticular penetration occurs over a longer period of time. It may be in this case, therefore, that these workers are dealing principally with cuticular penetration and not strictly stomatal penetration per se. In addition these experiments were conducted using bean discs - a totally artificial

system for investigating herbicide penetration. Franke (1969), however, also supports the idea of differential penetration into the guard cells. Using a Spinacia extract containing ^{14}C- incorporated sucrose and amino acids, he has shown by microradioautography that after application to leaf surfaces of Spinacia and Viola, radioactive materials were associated with guard cells but not the stomatal pore. These sites are rich in ectodesmata and Franke concludes that these are the main sites of entry. Yamada et al (1966) have similarly studied the possibility of specific binding sites within membranes in relation to the passage of ions and molecules and discovered that these binding sites were concentrated in areas adjacent to stomata, above the periclinal cell walls and in the continuum of the membrane within the substomatal chamber.

The results presented in this study, drawn from various areas of research provide further evidence to suggest that stomatal penetration may play a significant role in the control of C. album.

The experiments described in Chapter 5, for example, demonstrate a rapid Basagran-induced stomatal opening in isolated epidermal peels of C. album (Fig. 19). The rapid response of the guard cells supports the view that entry may take place directly through these structures. It must be stressed, however, that this series of experiments was conducted in the laboratory under in vitro conditions, and extrapolations to whole plants are hazardous. More recently, however, the rapid stomatal response in C. album has been demonstrated under field conditions (Smalley 1979) in mature plants, where the herbicide was applied directly to the plant surface. Such a rapid response in situ certainly implies that the cuticle is not traversed directly, since cuticular penetration is thought to be rather a slow process (Crafts & Foy 1962).

The rapid entry through the stomatal apparatus correlates well with observation that the stomatal antechamber in these species is apparently

devoid of surface wax projections (Fig. 18) and so penetration via the antechamber may avoid this formidable wax barrier. The observation that Basagran induces stomatal opening would suggest that, following initial penetration, the herbicide may facilitate further entry by making more of this wax-free area available for penetration, i.e. by increasing the distance between the epicuticular lips. It is not yet known whether the antechamber is covered with a smooth form of wax undetectable by standard SEM procedures. It would certainly seem likely that some form of wax covering is present to overcome water loss by transpiration. The nature of this wax layer may be elucidated by means of transmission electron microscopy through a transverse section of the stomatal pore. The practical probabilities of obtaining such sections across the pore are, however, very small, though an unsuccessful attempt was made during this study.

Further evidence implicating the role of stomata in foliar penetration in C. album is provided by Smalley's work in this laboratory on stomatal frequency and her study of rhythmic movements in this species. Both abaxial and adaxial surfacers are well-endowed with stomata (a ratio of 2:2:1 respectively) and stomatal frequency was found to be inversely proportional to leaf age. Indeed, this relationship correlates well with the finding that young leaves of C. album are more susceptible to Basagran than older leaves, where the stomatal frequency or density is much reduced.

It is proposed, therefore, that the differences in susceptibility observed under different environmental conditions is correlated with the degree of stomatal opening under such conditions. Hence, the poor control observed under prolonged periods of hot, dry weather may be due to the reduced area available for the penetration of the herbicide following stomatal closure, perhaps as a result of increased ABA production. Similarly the observation that the stomata of C. album display a typical

diurnal rhythm of movement with a pronounced mid-day closure (Smalley 1979) would also explain why herbicide applications made during this period of stomatal closure have little effect. Further evidence to support this view is provided by recent unpublished work by Dunleavy (personal communication) in this laboratory. Continuing the present investigation, he has found from porometry that leaf diffusive resistance in C. album varies according to an endogenous circadian rhythm, with increased stomatal resistance at night and mid-day. Furthermore, this rhythm is affected by Basagran application.

It would appear, however, that the tolerance shown by plants maintained under high relative humidity is not correlated with stomatal opening, since under such conditions, the stomata would be expected to be open. It may be that since the water tension within the plant would be low, the much reduced transpiration pull expected under these conditions may consequently limit the entry of the herbicide. Water relations within plants are, however, extremely complex and considerable research is required in this area before such statements can be reliably presented.

It would be tempting to suggest, from the schematic diagram presented, that the herbicide may enter directly through the stomatal pore. However, as pointed out by Schonherr and Bukovac (1972), pore dimensions would be unlikely to permit the entry of a solution, such as Basagran, whose surface tension is in the order of 60-70 dynes cm^{-1}. Before such movement could occur, the surface tension of the solution would need to be reduced to approximately 32 dynes cm^{-1}, as is the case when the oil adjuvant, Actipron, is added to the formulation. The role of Actipron in overcoming poor control under adverse conditions may, therefore, be directly as a result of mass movement through the stomatal pore. In the case of C. album, however, the pore is so small (only 1-2 μ) that this seems unlikely to be the principal route. It is more likely that enhanced

herbicide performance is as a result of improved coverage resulting from the lowered surface tension, thus extending the area of contact between herbicide and leaf surface. Thus, although the influence of Basagran was found to be dependent on plant size, the presence of Actipron eliminated this size-effect by the greater efficiency of surface wetting and subsequent penetration of the herbicide.

As yet no mention has been made of the importance of trichomes as portals of entry. Leaf hairs have been reported by numerous workers as being possible routes for herbicide penetration. However, the trichomes present on the surface of C. album are very delicate, and as a result, soon disappear from the leaves of field populations. It is therefore considered unlikely that these make any substantial contribution to herbicide penetration in this species.

From the outset of this work, the principle aim was to assess the role of epicuticular wax of C. album leaves in relation to herbicide uptake. This has been achieved to a great extent. It has been concluded from these investigations that the epicuticular wax of C. album provides a considerable barrier to the penetration of foliar applied herbicides by virtue of its high aldehyde content. This finding was further substantiated by means of contact angle determinations on both adaxial and abaxial leaf surfaces. Though the surface of C. album is densely covered with surface waxes of a strongly hydrophobic nature, it is evident from SEM studies that this does not form an homogenous covering and that these projections appear to be absent on the stomatal antechamber. It can thus be stated that the analytical methods adopted throughout this study, i.e. TLC, GLC, SEM and contact angle determinations, have contributed significantly to our understanding of epicuticular wax composition and ultrastructure in this species.

By means of controlled environmental studies much has been learned on the influence of environment on susceptibility of C. album to Basagran. It has been concluded that these effects are physiological in nature, rather than being due directly to changes in the rate of penetration through the surface wax. However, it must be stressed that in these investigations, the surface wax barrier alone was considered, and as mentioned previously, various other barriers exist, the relative importance of which still provide considerable scope for research.

The major development to emerge from these studies has been the investigation into the role of stomata in foliar penetration. This was not originally considered to be among the principle aims of the study, but as investigations developed, it became evident that these structures may be of particular relevance with respect to foliar penetration in C. album. The work conducted in this area has gone far to implicate the role of stomata in foliar penetration, though the evidence is largely indirect, and obviously calls for further extensive research. The use of field porometers, for example, can provide direct information on stomatal resistance in whole plants in situ, thus providing valuable information on the state of plants during herbicide applications. Furthermore, the use of an infra-red gas analyser can provide a more quantitative assessment of herbicide damage on treated plants by recording changes in the carbon dioxide uptake by plants in situ.

Although Basagran has been shown to induce stomatal opening in C. album, the mechanism by which this is effected is still unknown, and therefore further work should be conducted in this area by means of detailed metabolic studies.

Finally, it is proposed that, with the use of radio-active labelled herbicide, the precise route for penetration in C. album could readily be determined. Furthermore, such studies could also be extended to investigate

the possible role of internal membranes in governing uptake and thus enable an accurate determination to be made of the amount of herbicide which actually reaches its site of action within the chloroplast.

It is hoped that the experience gained from the present study, together with the proposed work outlined, will lead to a clearer understanding of the factors governing C. album control by Basagran in french bean crops.

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APPENDIX 1

Table I. The effect of Basagran and additives on the susceptibility of C. album

Treatment	Mean score/Plot (0-10)					
	Days after spraying					
	5	s.d.	12	s.d.	19	s.d.
Control	0	-	0	-	0	-
Basagran 3l/ha	5.3	2.0	8.6	0.57	9.3	1.15
" " + 2-methoxyethanol 2l/ha	6.0	1.73	9.0	0	9.6	0.57
" " " 1l/ha	6.3	1.52	8.3	1.52	9.0	0.99
" " " 0.5l/ha	5.0	0.99	8.6	0.57	10.0	0
" " + $(NH_4)_2SO_4$ 2%	5.0	1.73	8.6	0.57	10.0	0
" " " 1%	5.0	0.99	9.0	0	9.6	0.57
" " " 0.5%	4.6	1.0	8.6	0.57	9.6	0.57
Basagran 0.3l/ha	1.3	0.57	4.0	0.99	3.3	0.57
" " + 2-methoxyethanol 2l/ha	2.3	1.52	4.6	0.57	3.3	0.57
" " " 1l/ha	1.0	0	3.3	1.15	2.3	1.15
" " " 0.5l/ha	1.6	0.57	3.0	2.0	2.3	1.52
" " + $(NH_4)_2SO_4$ 2%	1.6	0.57	2.6	0.57	2.0	1.0
" " " 1%	1.3	0.57	2.6	2.08	2.6	2.08
" " " 0.5%	1.3	0.57	2.6	1.32	1.0	0

Application Details

Spray equipment : Shandon Sprayer

Date of application : 9/6/76 (14.00-16.00 hr)

Weather : very warm and dry

Site : Trent Polytechnic grounds

Expt[1] design : plots, 0.5m x 0.5m arranged in 3 randomised blocks.

Table II. The effect of Basagran concentration on the susceptibility of C. album

Treatment	Mean Score/Plot (0.10)					
	Days after spraying					
	4	s.d.	7	s.d.	11	s.d.
Control	0	-	0	-	0	-
Basagran 3l/ha	0.3	0.28	4.0	1.73	7.3	1.52
" " + Actipron 1l/ha	1.6	0.57	7.0	0	8.3	0.57
Basagran 2.25l/ha	0	0	3.3	2.08	7.3	0.57
" " + Actipron 1l/ha	1.3	0.57	7.3	0.57	8.6	0.57
Basagran 1.5l/ha	0.3	0.57	3.3	0.57	6.3	0.57
" " + Actipron 1l/ha	1.3	0.57	6.6	1.15	8.0	1.0
Basagran 0.75l/ha	0	0	2.0	1.73	3.6	2.51
" " + Actipron 1l/ha	1.0	0	5.3	0.57	6.6	0.57

Application Details

Spray equipment : Shandon sprayer

Date of application : 17/6/76 (14.00-16.00 hr)

Weather : Warm, slight wind

Site : Trent Polytechnic grounds

Expt[1] design : Plots, 0.5m x 0.5m arranged into 3 randomised blocks.

Table III. The effect of Basagran and Actipron on the susceptibility of C. album

Treatment	Mean Score/Treatment (0-5)	
	Days after spraying	
	5	11
Control	0	0
Basagran 3l/ha	1.66	4.11
" " + Actipron 0.5l/ha	2.19	4.53
" " " 1.0 "	1.80	4.47
" " " 1.5 "	2.52	4.32
" " " 2.0 "	2.31	4.47
" " " 2.5 "	1.85	3.99
Basagran 2l/ha	1.17	3.94
" " + Actipron 0.5l/ha	1.44	4.25
" " " 1.0 "	1.37	4.23
" " " 1.5 "	1.50	3.68
" " " 2.0 "	2.58	4.03
" " " 2.5 "	1.30	3.66

Application Details

Spray equipment : Binks-Bullows compressor sprayer at a pressure of 10 p.s.i.

Date of application : 24/6/77 (11.00-15.00 hr)

Weather : Warm, sunny, slight wind

Site : L & K Fertilisers, Saxilby, Lincs.

Expt[1] design : Plots 1m x 2m arranged into 3 randomised blocks

Assessment : 50 plants scored/plot along a transect

Plant size : most of plants approx. 25cm in height

Table IV. The effect of various oils and additives on the susceptibility of C. album to Basagran

Treatment	Day 4		Day 6	
	mean score per plant	s.d.	mean score per plant	s.d.
Control	0	-	0	-
Basagran	2.85	1.35	3.71	1.60
Actipron 1l/ha	0	-	0	-
" " + Basagran	4.6	0.40	5.0	-
Ammonium Sulphate 1% ($^{W}/_{v}$)	0	-	0	-
" " + Basagran	4.8	0.40	5.0	-
2-methoxyethanol 1l/ha	0	-	0	-
" + Basagran	5.0	0	5.0	-

Replication: 8 plants/treatment.

Table V. The effect of Actipron, ammonium sulphate and 2-methoxy-ethanol on the susceptibility of C. album to Basagran

Treatment	Mean Score/Plot (0-10)							
	Days after spraying							
	5	s.d.	7	s.d.	12	s.d.	19	s.d.
Control	0	-	0	-	0	-	0	-
Basagran 3l/ha	0.38	0.25	1.5	0.57	5.25	0.50	3.75	0.50
" " + Actipron 1l/ha	1.12	0.63	5.5	2.38	8.25	1.50	7.0	2.30
" " + $(NH_4)_2SO_4$ 1%	0.62	0.75	2.75	0.50	7.0	1.41	5.0	1.63
" " + 2-methoxyethanol 1l/ha	0.50	0	4.25	2.62	6.75	0.95	4.75	0.95

Application Details

Spray equipment : Shandon sprayer

Date of application : 16/6/76 (11.00 hr)

Weather : Rain early morning, cool with slight wind

Site : Trent Polytechnic grounds

Expt[1] design : Plots, 0.5m x 0.5m arranged into 4 randomised blocks.

Table VI. The effect of plant height on the susceptibility of C. album to Basagran and Actipron

Treatment	Mean Score/Plant (0-5)							
	Days after Spraying							
	1	s.d.	5	s.d.	8	s.d.	16	s.d.
5-10cm Basagran 3l/ha	0.8	0.8	5.0	-	5.0	-	5.0	-
" " +								
Actipron 1l/ha	3.6	1.9	4.8	0.44	5.0	-	5.0	-
10-15cm Basagran 3l/ha	0.2	0.44	2.8	1.01	2.8	1.61	5.0	-
" " +								
Actipron 1l/ha	3.8	0.44	5.0	-	5.0	-	5.0	-
15-20cm Basagran 3l/ha	0.2	0.44	1.8	0.83	1.8	0.83	3.8	0.83
" " +								
Actipron 1l/ha	2.6	0.54	3.8	0.44	3.8	0.44	5.0	-
20-25cm Basagran 3l/ha	0	-	1.0	0.7	1.0	0.7	3.8	0.78
" " +								
Actipron 1l/ha	2.0	-	4.0	-	4.0	-	5.0	-
25-30cm Basagran 3l/ha	0.4	0.54	0.8	0.44	1.0	0.7	4.0	0.7
" " +								
Actipron 1l/ha	2.4	0.54	4.0	-	4.0	-	5.0	-
30-40cm Basagran 3l/ha	0.2	0.44	0.4	0.34	2.0	0.7	4.0	-
" " +								
Actipron 1l/ha	2.8	0.44	4.0	-	4.0	-	4.4	0.54

Replication: 10 plants/treatment

Table VII. The effect of Basagran and Actipron on C. album, three days following application

Plant Height	Basagran 3l/ha	Basagran 3l/ha + Actipron 2l/ha
5 cm	4.20	4.75
5-10 cm	3.50	4.50
10 cm	3.15	3.90
MEAN	3.62	4.38

Application Details

Spray equipment : Shandon Sprayer

Date of application : 8/6/76

Weather : Hot and dry

Site : Codnor, Derbyshire

Expt[1] design : Exptl area (5.6 x 5.6m) marked out as randomised block design with 64 small plots (0.7 x 0.7m)

Table VIII. The effect of application time on the response of C. album to Basagran

Application Time	Temperature (°C)	Mean Score (0-5)	s.d.
08.00 hr	18	3.4	0.93
10.30 hr	22	3.7	0.95
13.00 hr	23	2.3	0.92
15.30 hr	23	3.1	0.84
18.00 hr	24	4.13	0.81

Application Details

Spray equipment : Knapsack sprayer

Date of application : 17/7/79

Weather : cloudy with sunny intervals, very dry

Site : Codnor, Derbyshire

Expt[1] design : plots 3m x 3m, unreplicated.

Assessment : 30 plants scored/plot along a transect

Table IX. The effect of temperature on the susceptibility of C. album to Basagran and Actipron : 12°C vs 28°C

Treatment	Mean Score/Plant (0-5)					
	Days after Spraying					
	2	s.d.	5	s.d.	8	s.d.
28°C-28°C Basagran	0	-	0	-	1.00	1.00
" " + Actipron	0	-	0	-	1.80	1.20
28°C-12°C Basagran	0	-	0	-	0.30	0.46
" " + Actipron	0	-	0.58	0.50	1.43	0.93
12°C-12°C Basagran	0	-	0.12	0.34	0.50	0.51
" " + Actipron	0.56	0.62	1.87	0.65	2.18	0.91
12°C-28°C Basagran	0.57	0.51	1.18	0.65	3.28	1.61
" " + Actipron	2.46	0.74	3.00	0.59	4.13	1.03

Table X. The effect of temperature on the susceptibility of C. album to Basagran and Actipron : 17°C vs 28°C

Treatment	Mean Score/Plant (0-5)					
	Days after Spraying					
	3	s.d.	8	s.d.	11	s.d.
28°C-28°C Basagran	0	-	0.25	0.44	2.0	1.03
" " + Actipron	0.81	0.94	3.37	1.36	4.5	1.09
28°C-17°C Basagran	0	-	0.06	0.25	0.38	0.50
" " + Actipron	0	-	1.41	1.17	2.72	1.63
17°C-17°C Basagran	0.18	0.39	0.20	0.41	1.23	0.43
" " + Actipron	1.29	0.91	3.27	1.77	3.88	1.45
17°C-28°C Basagran	0.43	0.81	2.28	1.48	4.27	1.27
" " + Actipron	1.50	0.70	3.72	1.63	4.84	0.55

Table XI. The effect of temperature on the susceptibility of C. album to Basagran and Actipron : 22°C vs 28°C

Treatment	Mean Score/Plant (0-5)					
	Days after Spraying					
	1	s.d.	3	s.d.	5	s.d.
28°C-28°C Basagran	0	-	1.86	0.83	3.87	0.95
" " + Actipron	0.63	0.64	4 35	0.74	4.92	0.26
28°C-22°C Basagran	0	-	0.64	0.49	1.28	0.61
" " + Actipron	0.83	0.77	3.53	0.66	4.76	0.43
22°C-22°C Basagran	0	-	1.00	0.60	1.76	0.83
" " + Actipron	0.94	1.76	4.90	0.34	4.94	0.24
22°C-28°C Basagran	0	-	1.66	0.72	3.86	0.99
" " + Actipron	0.67	1.80	4.46	0.63	5.0	-

Table XII. The effect of light intensity on the susceptibility of C. album to Basagran and Actipron : $12Wm^2$ vs $96Wm^2$

Treatment	Mean Score/Plant (0-5)					
	Days after Spraying					
	2	s.d.	5	s.d.	7	s.d.
$96Wm^2$-$96Wm^2$ Basagran	0.06	0.24	0.89	0.90	2.18	1.04
" " + Actipron	2.30	1.43	3.86	1.50	4.53	0.77
$96Wm^2$-$12Wm^2$ Basagran	0	-	0.15	0.37	0.61	1.04
" " + Actipron	1.72	1.00	3.91	1.37	4.75	0.62
$12Wm^2$-$12Wm^2$ Basagran	0.38	0.50	1.15	0.37	2.30	0.67
" " + Actipron	2.23	0.72	4.92	0.26	5.0	-
$12Wm^2$-$96Wm^2$ Basagran	0.33	0.47	2.47	0.94	4.47	0.87
" " + Actipron	2.77	0.66	5.0	-	5.0	-

Table XIII. The effect of light intensity on the susceptibility of C. album to Basagran and Actipron : $37Wm^2$ vs $96Wm^2$

Treatment	Mean Score/Plant (0-5)					
	Days after Spraying					
	1	s.d.	3	s.d.	6	s.d.
$96Wm^2$-$96Wm^2$ Basagran	0	-	0	-	1.43	0.72
" " + Actipron	1.0	0.68	4.23	1.03	5.0	-
$96Wm^2$-$37Wm^2$ Basagran	0	-	0	-	1.35	0.92
" " + Actipron	1.14	0.79	4.25	0.96	5.0	-
$37Wm^2$-$37Wm^2$ Basagran	0	-	0.51	0.77	2.42	0.85
" " + Actipron	1.23	0.72	4.35	0.92	5.0	-
$37Wm^2$-$96Wm^2$ Basagran	0	-	0.19	0.40	2.33	0.76
" " + Actipron	1.35	0.48	4.90	0.30	5.0	-

Table XIV The effect of relative humidity on the susceptibility of C. album to Basagran and Actipron

Treatment	Mean Score/Plant (0-5)			
	Days after Spraying			
	3	s.d.	8	s.d.
80%-80% Basagran	0.70	0.53	2.28	0.46
" " + Actipron	1.30	0.60	3.60	0.34
80%-40% Basagran	0.66	0.48	1.70	0.67
" " + Actipron	1.80	1.01	4.20	0.77
40%-40% Basagran	1.00	0.70	3.94	0.74
" " + Actipron	2.60	0.94	4.95	0.21
40%-80% Basagran	0.70	0.71	3.31	1.10
" " + Actipron	1.80	0.88	4.60	0.69

APPENDIX II

ANALYSIS OF VARIANCE DATA

Table I. The effect of Basagran and Actipron on the susceptibility of C. album. Assessment 5 days following application

Source	Sums of Squares	Degrees of freedom	Means Square	Variance ratio	Significance at 1% level
Basagran	3.33	1	3.33	9.65	YES
Actipron	2.16	5	0.432	1.25	-
B/A interaction	0.41	5	0.082	0.237	-
Error	8.28	24	0.345		
TOTAL	14.18	35			

Table II. The effect of Basagran and Actipron on C. album. Assessment 11 days following application

Source	Sums of Squares	Degrees of freedom	Means Square	Variance ratio	Significance at 1% level
Basagran	1.01	1	1.01	7.842	YES
Actipron	1.62	5	0.324	2.516	-
B/A interaction	0.246	5	0.049	0.383	-
Error	3.09	24	0.128		
TOTAL	5.96	35			

Table LII. The effect of temperature on the response of C. album stomata to bentazone

Source	Sums of Squares	Degrees of freedom	Means Square	Variance Ratio	Significance at 1% level
Replicates	19,230.115	4	4,807.52	0.99	-
Temperature	127,469.94	5	25,493.98	5.27	YES
Light	319,197.67	1	319,197.67	65.99	YES
Bentazone	128,773.00	1	128,773.00	26.62	YES
Temp/Light interaction	27,731.08	5	5,546.21	1.146	-
Temp/ Bentazone interaction	21,415.15	5	4,283.03	0.885	-
Light/ Bentazone interaction	106,029.08	1	106,029.08	21.92	
Temp/Light/ Bentazone interaction	19,358.07	5	3,871.61	0.800	-
Residual	444,989.88	92	4,836.84	-	-
TOTAL	1 214,193.99	119	4,807.52	0.99	-

APPENDIX III PUBLICATIONS

The work presented in Chapters 5 and 6 has been published in the following journals:-

DAVIES L. G., COBB A. H. and F. ELIZABETH TAYLOR (1979) The susceptibility of C. album to bentazone under different environmental conditions. Proceedings of the European Weed Research Symposium. Mainz, W. Germany. 97-104.

(A copy of this paper is enclosed)

F. ELIZABETH TAYLOR, COBB, A. H. and DAVIES, L. G. (1979) The effect of bentazone on stomatal behaviour in Chenopodium album L. New Phytologist.

(Proofs not yet available)

Proc. EWRS Symp. The Influence of Different Factors on the Development and Control of Weeds, 1979

THE SUSCEPTIBILITY OF CHENOPODIUM ALBUM TO BENTAZONE UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Summary

The susceptibility of C. album to bentazone is dependent on temperature, light and relative humidity, both before and after application of the herbicide. Transition from a low to a high regime of temperature and light after application significantly increases susceptibility and vice versa. These effects are explained by reference to the mode of action of the herbicide. C. album appears to be more susceptible to bentazone at low humidity. Differences in susceptibility due to environmental changes are not correlated to the production and deposition of epicuticular wax on the leaf surface. Stomatal penetration is considered to play a significant role in the entry of bentazone into the leaf in this species.

INTRODUCTION

Bentazone is used for selectively controlling broad-leaved weeds in french bean crops, Phaseolus vulgaris L. (May, 1974; King and Handley, 1976). Application during warm, dry periods in the summer of 1975 and 1976 however, resulted in reduced efficiency, particularly in the control of Chenopodium album L., the predominant weed in this crop.

This paper describes the effects of bentazone on C. album under different environmental and physiological conditions.

METHODS AND MATERIALS

Seedlings of C. album were raised in John Innes compost No. 2 in a greenhouse at a temperature of 18 $\pm$ 3°C, with supplementary lighting provided by a series of Warm-white fluorescent tubes for a 16 hour photo-period.

Uniform plants, at least twelve per treatment, with four to six expanded leaves were transferred into two environmental cabinets which were set at a low and high regime of the parameter under investigation, for seven days prior to the application of the herbicide. Fisons environmental cabinets (models 600G3/TTL and 600G3/THTL) were used throughout except for the experiment on relative humidity which was performed in environmental cabinets at the National Vegetable Research Station, Wellesbourne.

Plants were removed from the cabinets and sprayed with bentazone (formulation BAS 35107 H; Basagran, 48% w/v a.i.) at the recommended field rate of 3 l/ 280 l water/ ha, using a Shandon chromatography sprayer. Following application the plants were returned to the same cabinet or transferred to different conditions in the other cabinet, thus allowing pre- and post-treatment conditions to be investigated.

Herbicide damage was assessed on an individual plant basis on a scale of 0 - 5 (0 representing no damage and 5 plant death).

Epicuticular wax was extracted from the leaf surfaces by immersion in chloroform for 30 seconds. Analysis of wax was carried out on a Perkin-Elmer F11 gas chromatograph. Scanning electron microscopy of the leaf surfaces was performed using a Cambridge Stereoscan 600 electron microscope at a H.T. voltage of 25 kV.

Leaf epidermal strips were peeled from abaxial surfaces and floated on 50 mM Hepes buffer, pH 6.5, for various times and treatments, including technical grade bentazone (98%). Incubation of strips was performed in the Fisons environmental cabinets. Stomatal apertures and frequency were measured by direct microscopic observations under oil immersion.

RESULTS

1. Environmental conditions versus susceptibility and wax production.

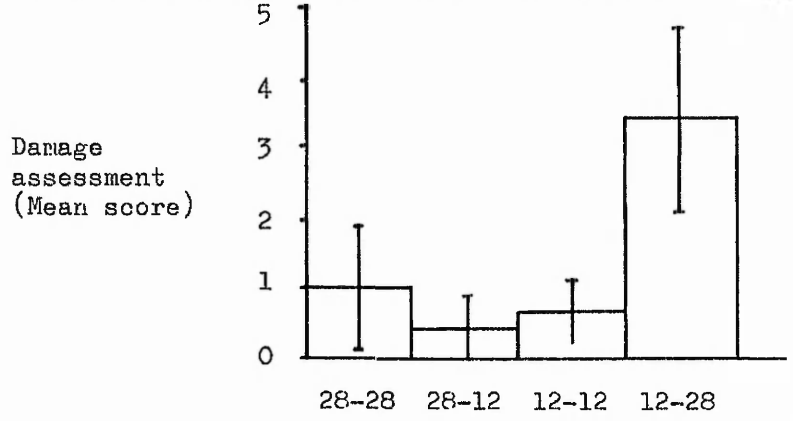
Temperature

Most damage was evident in plants given a low temperature pre-treatment prior to bentazone application, followed by transfer to a higher temperature. This response is illustrated in Figure 1a. Generally, the greater the temperature gap between pre- and post-treatment conditions, the greater the observed damage.

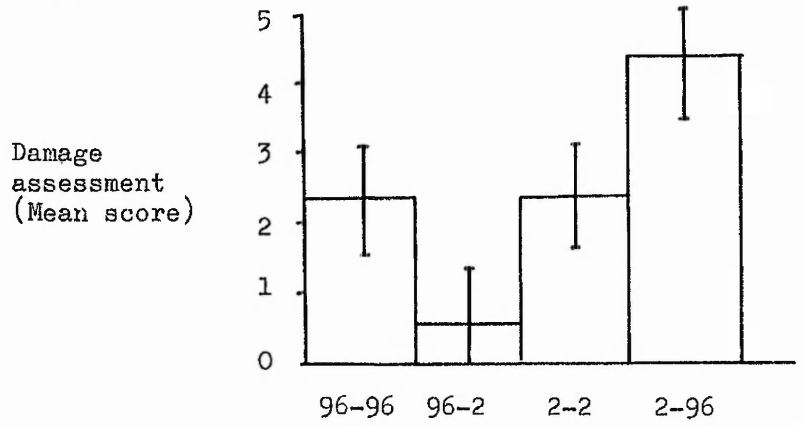
There was no significant difference in the composition and deposition of leaf epicuticular wax in plants grown at 12, 17, 22 and 28°C for seven days. The yield of epicuticular wax was approximately 0.22 mg/cm^2.

Figure 1. The response of C. album to bentazone under different environmental conditions, eight days after application. (Bars indicate standard deviation)

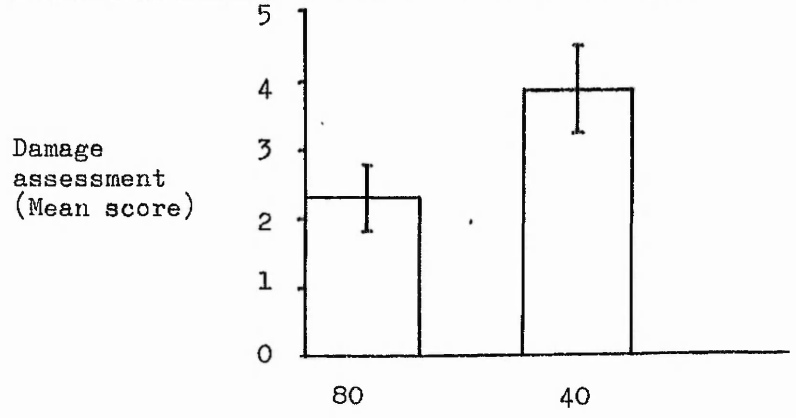
(a) Temperature (light intensity at 96Wm^2; R.H. at 70 ± 10%)



(b) Light (temperature at 22°C; R.H. at 70 ± 10%



(c) Relative humidity (temperature at 21°C; light at 80Wm^2)



Light

Constant low ($2Wm^2$) and high ($96Wm^2$) light regimes resulted in no difference in susceptibility of C. album to bentazone (Figure 1b). However, plants transferred from the low to the high light regime following application showed more damage than plants kept in a constant light environment.

There was no significant difference in the composition and deposition of leaf epicuticular wax in plants grown at the two light regimes for seven days.

Relative Humidity

Intact plants of C. album are more susceptible to bentazone at lower (40 ± 10%) than at higher (80 ± 10%) relative humidity (Figure 1c). This effect has also been observed in detached leaves of this species.

There was no significant difference in the composition and deposition of leaf epicuticular wax in plants grown at the two humidity regimes for seven days.

Soil Moisture

We have not observed marked variation in susceptibility of C. album to bentazone over a wide range of soil moisture regimes. However, some plants maintained at near wilting point showed less damage, although further experimental data is needed to confirm this effect.

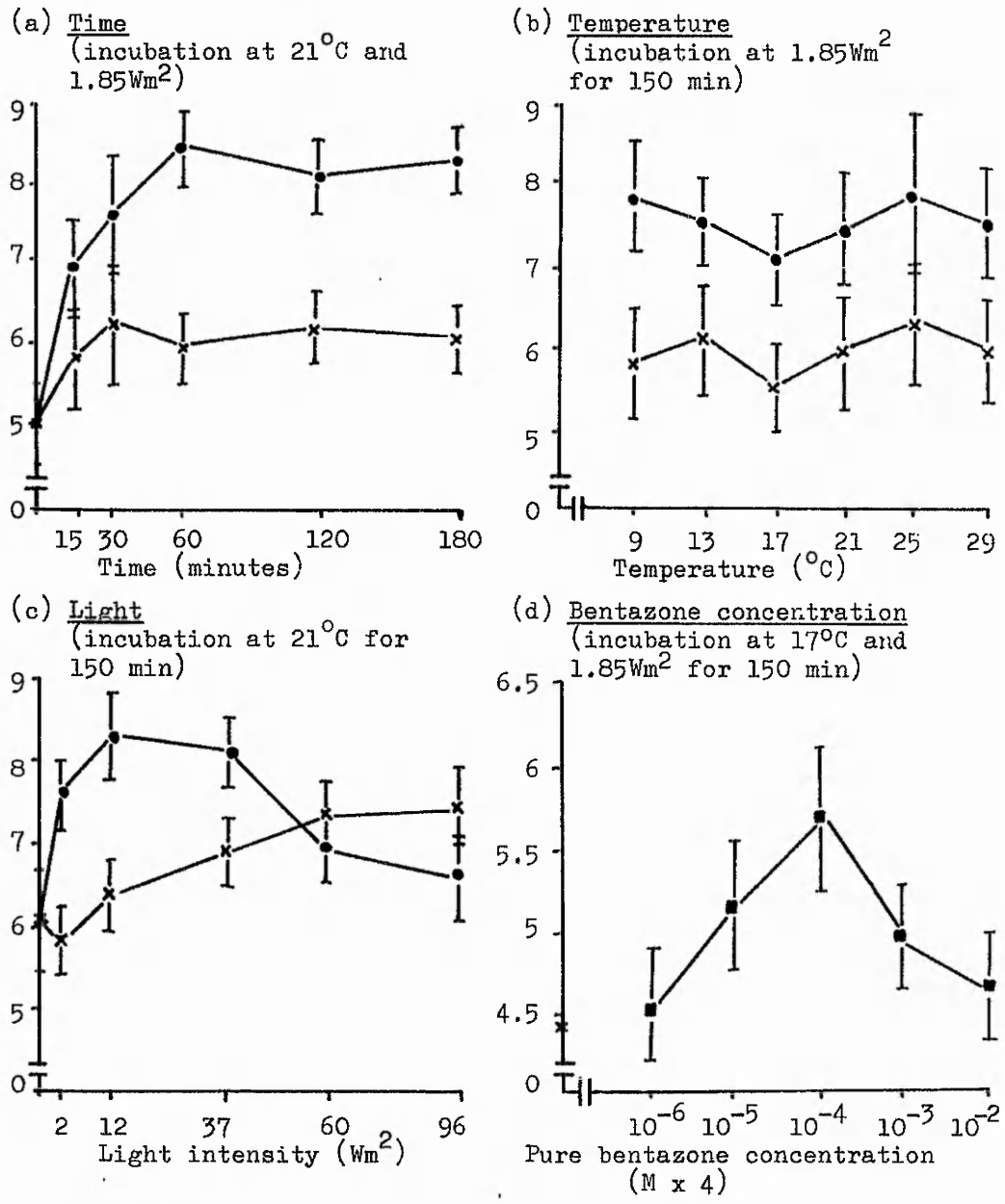
2. Environmental conditions versus stomatal behaviour

As there is no relationship between susceptibility to bentazone and epicuticular wax production, stomatal characteristics were studied in an attempt to account for these observations.

The leaf surface of C. album is densely covered with wax projections and displays a distinctive stomatal apparatus. The stomata are anomocytic, having no true subsidiary cells, and the sunken pore is surrounded by wax-free antechamber with a raised cuticular lip (Taylor, et al., 1979 in the press). There is a significant number of stomata on both upper and lower leaf surfaces. Stomatal frequency is inversely related to leaf size ($Y = 199x + 6.85$ for upper leaf surface; $Y = 229x + 74.9$ for lower leaf surface).

The data in Figure 2a-d show a bentazone-induced opening of the stomatal apparatus with respect to time, concentration, temperature and light. The opening of the stomatal apparatus in epidermal strips, over a temperature range of 9 - 29°C, is a rapid light dependent response with an optimum at $4 \times 10^{-4}M$.

Figure 2. The effect of time, temperature, light and concentration on bentazone-induced stomatal opening in epidermal strips of C. album. (Bars indicate standard deviations)



Vertical axis: Mean stomatal aperture in µm (80 readings)
●: bentazone concentration at field rate
✖: Hepes buffer, control.

DISCUSSION

The efficiency of bentazone in the control of C. album depends on the environmental conditions both before and after application of the herbicide. Furthermore, a sudden change in environment results in altered susceptibility to bentazone, (Figure 1a and 1b). These effects may be explained by reference to the mode of action of bentazone, an inhibitor of photosynthetic electron transport (Mine and Matsunaka, 1975). Thus, plants may die through lack of photosynthate and/or membrane damage as a result of excess free radical production (Halliwell, 1978). Hence, transition from a low to a high regime may increase weed damage by enhanced metabolic activity (Figure 1a), and higher light intensity would increase damage as a result of increased free radical production (Figure 1b). Therefore leaves with greater photosynthetic demand are more susceptible to bentazone than leaves where the demand is relatively constant, a situation observed in the field with actively growing plants of C. album and P. vulgaris (Taylor, 1979). Conversely, decelerated metabolic and photosynthetic rate, as a result of transition from a high to a low regime of temperature and light, decreases susceptibility, also observed with mature plants in field conditions.

The decreased susceptibility of C. album to bentazone during the prolonged warm, dry periods in the summer of 1975 and 1976 may have been due to a lowered soil moisture content limiting the metabolic rate. However, the finding that C. album at low humidity is more susceptible to bentazone than at high humidity (Figure 1c) is difficult to interpret. This finding differs from previous reports (eg. Nalewaja, et al., 1975) although knowledge of transpiration rate, leaf water potential and stomatal resistance under these conditions are needed to explain this observation.

A lack of correlation between susceptibility and epicuticular wax production suggests that the response of C. album is physiological in nature rather than induced by a physical change in the leaf surface. Consideration must therefore be given as to how the environmental changes affect the penetration of bentazone into the leaf.

The movement of herbicides through the leaf cuticle is passive whereas entry into the epidermal cells is thought to be an active process (Kirkwood, 1972), hence increased temperature may be expected to enhance bentazone uptake. An additional route of entry is the stomatal apparatus. In this species the leaf surface is well endowed with epicuticular wax, which may impede cuticular penetration and a significant number of stomata are present on the upper leaf surface, with wax free antechambers (Taylor, et al., 1979). Furthermore, the relationship between stomatal frequency and leaf size correlates well with the finding that young leaves are more susceptible to the herbicide than older leaves. This inference, that stomatal involvement in bentazone penetration is of significance in this species, is further supported by the data presented in Figure 2a-d, discussed in

detail in Taylor, et al., 1979. We envisage that environmental conditions favouring stomatal opening expose more wax-free antechamber to the herbicide, which may then be the principal route of bentazone entry in this species. Hence, the decreased susceptibility of C. album to bentazone under periods of prolonged drought may therefore have resulted from reduced stomatal penetration. The enhanced herbicidal action of bentazone when amended with an oil adjuvant, such as Actipron (King and Handley, 1976), may be due to greater penetration of bentazone through the stomata by improved coverage of the leaf surface.

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We wish to thank BASF Ltd for the gifts of Basagran and technical grade bentazone, the National Vegetable Research Station for the use of their environmental cabinets and Miss Jean Smalley for the data on stomatal frequencies.

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Résumé

La susceptibilité de C. album à bentazone dépend de température, lumière et humidité relative autant avant qu'après l'application de l'herbicide. La transition d'un régime bas à un régime de température et lumière élevé après cet application augmente la susceptibilité et vice versa. On peut expliquer ces effets en signalant le mode d'action du herbicide. Il pavait que C. album est plus sensible à bentazone pendant une humidité légère. Les différences de susceptibilité causées par des modifications déterminées par le milieu ne sont pas en corrélation avec la production d'une couche de cire épicuticulaire sur la surface de la feuille.

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THE EFFECT OF BENTAZONE ON STOMATAL BEHAVIOUR IN *CHENOPODIUM ALBUM* L.

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Summary

The herbicide bentazone induces stomatal opening in epidermal strips of *C. album* in a light-dependent response, over a temperature range of 9 to 29 °C. The relevance of these findings is discussed in relation to stomatal structure and the foliar penetration of the herbicide.

Introduction

The site of herbicide penetration into leaves has been greatly debated in the literature. The cuticle is generally considered to be the principal site of entry, although under certain conditions the stomatal apparatus may play a significant role (Crafts and Foy, 1962; Sargent and Blackman, 1962; Franke, 1964; Currier, Pickering and Foy, 1964). Stomatal movement is known to be affected by chemical stimuli, such as abscisic acid (Jones and Mansfield, 1970), kinetin (Meidner, 1967) and farnesol (Ogunkanmi, Wellburn and Mansfield, 1974); and more recently the work of Pemadasa and Jeyaseelan (1976) suggests that herbicides may also influence stomatal movements in susceptible plants.

In the early 1970s the herbicide bentazone emerged as one of the most successful means of selectively controlling broad-leaved weeds in french bean crops, *Phaseolus vulgaris* L. (May, 1974; King and Handley, 1976). Applications during hot, dry periods in the summers of 1975 and 1976, however, resulted in reduced efficiency, particularly in the control of *Chenopodium album* L., the predominant weed in this crop.

It has been suggested that environmental conditions may alter epicuticular wax production of leaf surfaces (Whitecross and Armstrong, 1972; Baker, 1974), although investigations in this laboratory indicate that factors other than wax production may be of greater significance in the susceptibility of *C. album* to bentazone (Davies, Cobb and Taylor, 1979). Hence, the purpose of this paper is to describe the effects of bentazone on the stomatal apparatus of *C. album* under different environmental conditions, and to establish the potential role of stomata in bentazone penetration.

Materials and Methods

In all experiments fully expanded leaves were selected from 8 to 10-week-old *C. album* plants grown in John Innes No. 2 compost. Greenhouse conditions were maintained at 18 ± 3 °C, supplementary lighting being provided by a series of warm-white fluorescent tubes for a 16 h photo-period. These provided a light intensity of approximately 25 W m^2 at the leaf surface.

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Epidermal strips were peeled from the abaxial surface and floated immediately, cuticle upwards, on 50 mM Hepes (Sigma) buffer, pH 6·5, and stored in the dark until required for use. The strips, approximately 1 cm^2 and free of mesophyll contamination, were easily obtained, and epidermal and guard cells appeared to be microscopically intact. Incubations were performed in environmental cabinets (Fisons, models 600G3/TTL and 600G3/THTL) and in a temperature-controlled light incubator (Astell).

The bentazone [3,isopropyl-2,1,3-benzothiadiazinone-(4)-2,2-dioxide] formulation used throughout was Basagran (BAS 35107 H), kindly provided by BASF Limited, containing 48% (w/v) of the active ingredient, bentazone. This formulation was diluted in Hepes buffer to a concentration of 10^{-2}, which was approximately equivalent to the recommended field rate of 3 litres to 280 litres of water ha^{-1}. A pure preparation of bentazone (98% technical grade) was also used in some experiments. In all cases the treatment solutions were prepared in petri dishes to a volume of 20 cm^3. Parameters examined were incubation time (0 to 3 h), temperature (9 to 29 °C), light intensity (0 to 96 W m^2) and bentazone concentration.

As the stomatal pore of *C. album* was very small (1 to 2 μm, compared with pore widths of 12 μm for *Commelina communis* L., Willmer and Mansfield, 1969), the maximum distance between the cuticular lips surrounding the pore was taken as the criterion of measurement (see Plate 1), since this also reflected changes in guard cell turgor. Apertures were measured by direct microscopic observation under an oil immersion lens using a calibrated eyepiece micrometer. In these experiments the stomatal pore remained between 0 and 2 μm, whereas the distance between the cuticular lips varied between 4 and 5 μm (dark) to 9 μm. Mature plants in the field showed opening up to 15 μm.

For the scanning electron microscopy, leaf sections of *C. album* were mounted on stubs using double-sided adhesive tape, and coated with gold using a Polaron Mark II E 5000 sputter coater. The pressure in the coating unit was reduced to 0·01 to 0·02 Torr over a period of 2 min. Gold was then evaporated from the target, forming a cloud which provided an even coating of the specimens. Scanning electron micrographs were taken of stomata from the abaxial surfaces using a Cambridge Stereoscan 600 electron microscope at an H.T. voltage of 25 kV.

RESULTS

Leaf surface topography of C. album

Plate 1 shows scanning electron micrographs of the stomatal apparatus on the abaxial surface of *C. album*. The stomata are anomocytic, having no true subsidiary cells. The stomatal pore appears to be sunken and the antechamber is bordered by a well-defined, raised cuticular lip. The cuticle surrounding the guard cells is densely covered with wax projections, whilst the stomatal antechamber appears virtually wax-free.

Time course

The effect of bentazone (Basagran formulation) on stomatal movement was investigated over a 3-h period (Fig. 1). This graph, typical of several experiments performed, shows a bentazone-induced stomatal opening. Maximum apertures were obtained in the presence of bentazone after an incubation period of 1 h, thereafter

remaining virtually constant. The opening process appears to be a rapid one, with increases in aperture being observed after 5 min incubation. A similar pattern was observed in control treatments, although smaller apertures were measured.

The effect of temperature on bentazone-induced stomatal opening

Stomatal apertures were measured over a temperature range of 9 to 29 °C in a light incubator at 1·85 W m² (Fig. 2). In the presence of light, bentazone enhanced stomatal opening as a temperature independent response. In darkness Basagran had no effect on stomatal aperture when compared to a Hepes control.

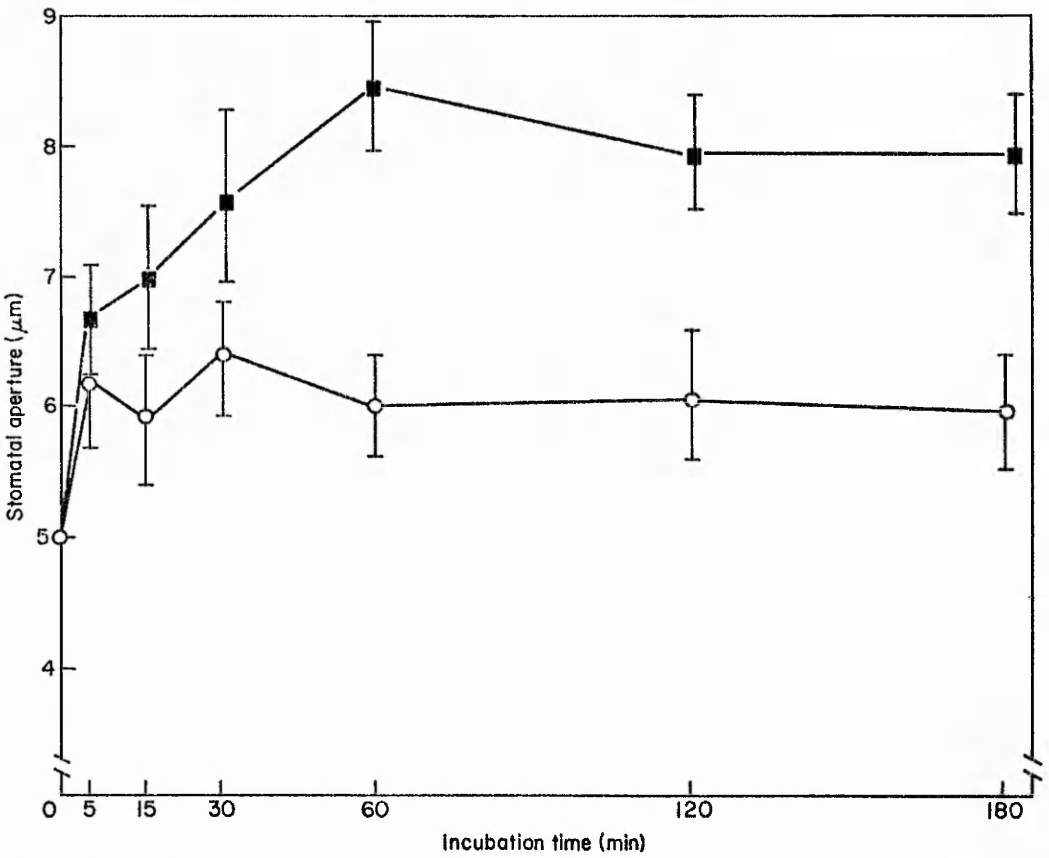

Fig. 1. The effect of bentazone on stomatal movement. Epidermal strips were incubated at 21 °C with a light intensity of 1·85 W m² for up to 3 h. Strips were periodically removed for measurement. Each point represents the mean of 40 measurements taken from two epidermal peels. ■, 10^{-3} bentazone (in Hepes); ○, Hepes control. Bars indicate standard deviation values.

An analysis of variance was carried out on the data from each of five experiments performed, revealing the effects of bentazone and light to be highly significant ($P < 0{\cdot}001$). The interaction between bentazone and light also proved highly significant ($P < 0{\cdot}001$).

The effect of light intensity on stomatal opening

The influence of bentazone on the stomatal apparatus was determined over a range of light intensities from 1·85 to 96 W m² at a constant temperature of 22°C (Fig. 3). Incubations were carried out both in environmental cabinets and in an

incubator, both providing illumination by a series of warm-white fluorescent tubes (Philips).

Bentazone was observed to exert maximum effect at 12·95 W m², this effect declining as light intensity increased. At 96 W m² the herbicide appeared to cause disruption of cellular membranes, resulting in plasmolysis in some areas of the tissue.

The effect of pure bentazone on stomatal movement

The previous data were obtained using a formulation containing only 48% (w/v) bentazone. In view of this, a dose response curve was determined with technical grade (98%) bentazone (Fig. 4).

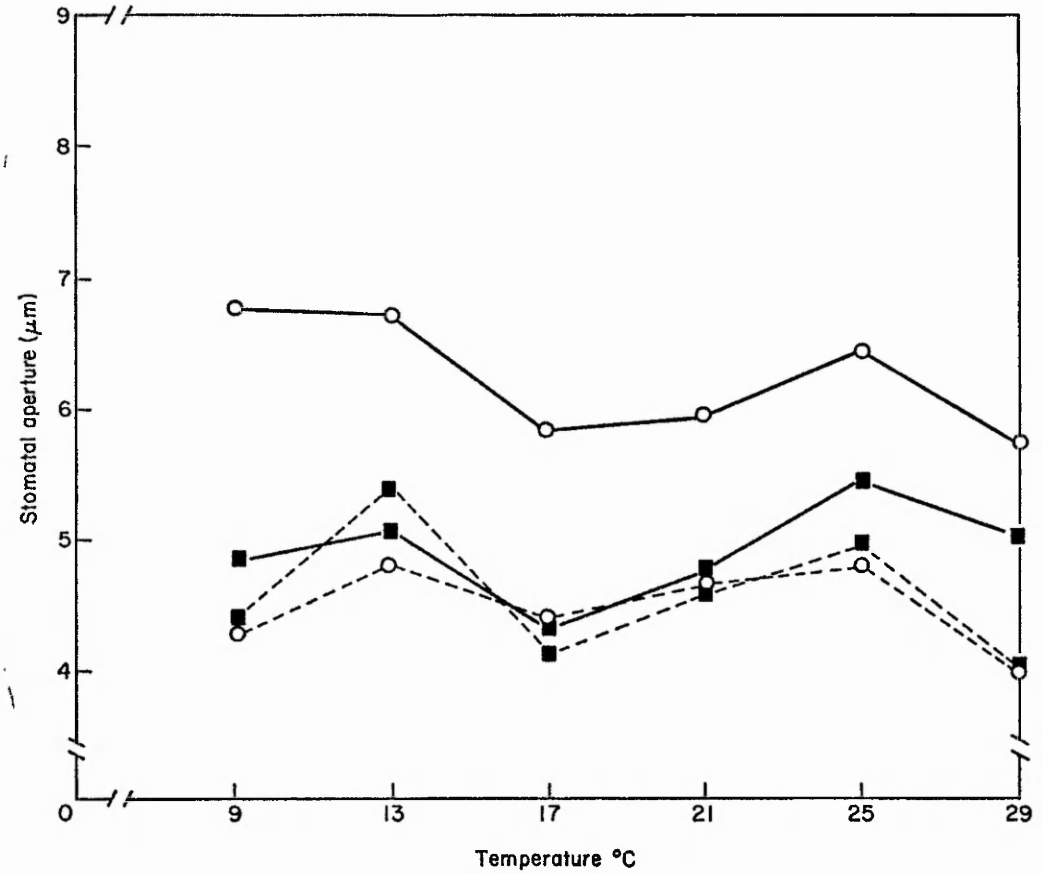

Fig. 2. The effect of temperature on bentazone-induced stomatal opening. Epidermal strips were incubated at a range of temperatures for 150 min in either light (1·85 W m²) or total darkness, prior to stomatal measurement. Each point represents the mean of 80 readings taken on five separate occasions. O—O, 10^{-2} bentazone (in Hepes) + light; ■—■, Hepes control + light; O- -O 10^{-2} bentazone (in Hepes) + dark; ■- -■, Hepes control + dark.

As bentazone had a very low water solubility, it was first converted to its sodium salt by dissolving in 1 N NaOH. A range of bentazone concentrations from 4×10^{-2} to 4×10^{-6} M was prepared in Hepes buffer, and dose response curves were determined.

The results in Figure 4 clearly demonstrate that the opening observed in the presence of the formulation Basagran is principally due to the active ingredient, bentazone, maximum apertures being obtained at a concentration of 4×10^{-4} M bentazone.

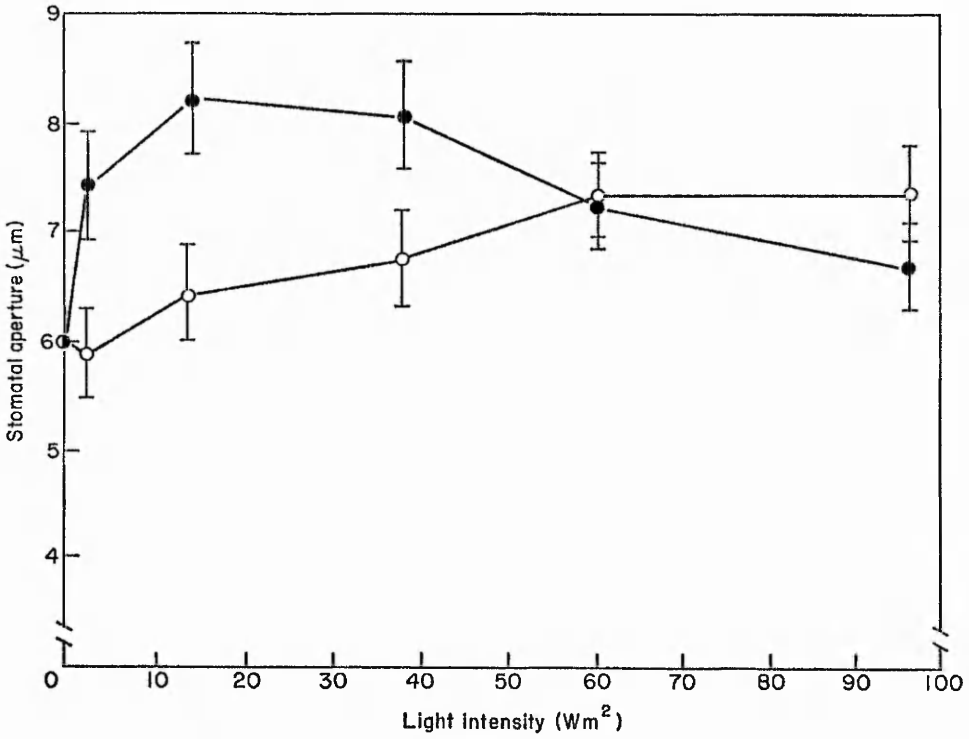

Fig. 3. The effect of light intensity on stomatal aperture. Epidermal strips were incubated for 150 min in a range of light intensities at a constant temperature of 22 °C. Each point represents the mean of 40 measurements taken from two epidermal peels on three separate occasions. ●, 10^{-2} bentazone (in Hepes); ○, Hepes control. Bars indicate standard deviation values.

Discussion

The experiments reported above show that bentazone opens the stomatal apparatus of *C. album* epidermal peels in a light-dependent response over a temperature range of 9 to 29 °C. This response was not observed in other species tested (*Pisum sativum* L. and *Brassica napus* L.). It is rather puzzling that this effect is independent of temperature (Fig. 2), since the general consensus of opinion (e.g. Stålfelt, 1962; Willmer and Mansfield, 1970) is that stomatal opening is mediated by an active process, requiring an energy source. One would assume, therefore, that higher temperatures would result in a greater degree of opening. However, in the case of *C. album*, increases in temperature may simply accelerate the opening process rather than cause increased stomatal apertures. The enhanced stomatal opening obtained with bentazone was only observed in the presence of light (Figs 2 and 3). Only low light intensities were required for this process, since at the higher light intensities of 96 W m² (approximately equivalent to normal daylight), stomatal opening was not observed. This latter response was accompanied by cellular disruption manifest in epidermal cell plasmolysis, and suggests photodestruction due to the presence of the herbicide at its primary site of action. Indeed, cell plasmolysis has been reported as one of the initial toxic effects of bentazone by Potter and Wergin (1975). In studying the role of light in bentazone toxicity to cocklebur (*Xanthium pennsylvanicum*), these authors found that light was necessary for necrosis to develop, and at high light intensities toxicity symptoms developed more rapidly. The development of necrosis was preceded in all cases by the cessation of photosynthesis.

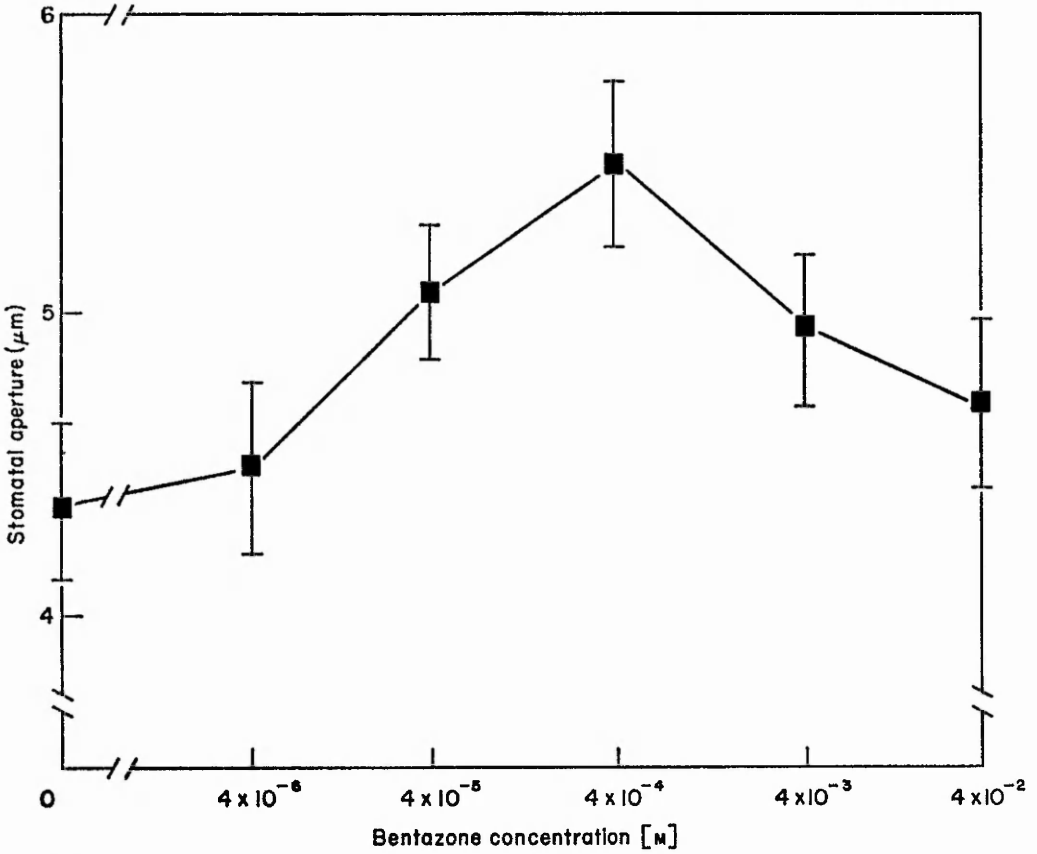

Fig. 4. The effect of pure bentazone on stomatal movement. Epidermal strips were incubated for 150 min at 17 °C and a light intensity of 1·85 W m^2. Each point represents the mean of 80 measurements from two epidermal peels. Note, buffer control had a value of 4·36 μm. Bars indicate standard deviation values.

The chloroplast is the primary site of action of bentazone, and it has been suggested (e.g. Mine and Matsunaka, 1975; Böger, Beese and Miller, 1977) that non-cyclic electron transport is specifically inhibited. Thus, at high light intensities, a block in the electron transport chain would result in a build up of excitation energy unable to be harnessed or efficiently dissipated, and cellular destruction may rapidly ensue as a result of lipid peroxidation (Halliwell, 1978). However, to exert this effect, the herbicide must first penetrate the cuticle and/or the stomatal apparatus of the leaves.

In studying the routes of herbicide penetration, many authors (e.g. Crafts and Foy, 1962) consider that stomatal penetration is a rapid process, whereas cuticular penetration is thought to occur at a much slower rate. In these experiments bentazone has been shown to induce the rapid opening of the stomatal apparatus in isolated epidermal peels of *C. album* (Fig. 1), supporting the view that the entry of bentazone through the stomata may be of great importance in this species.

Evidence to substantiate this comes from various sources. Firstly, we have observed that applications of bentazone are less effective in the control of *C. album* at mid-day than at early morning and evening. These findings cannot be explained solely in terms of differences in epicuticular wax present at the time of spraying, since wax structure is unlikely to change appreciably within an 8 to 12 h period. The observation that stomata of many species exhibit 'mid-day closure' (Meidner and Heath, 1959) may

be of interest in this respect, since poor control observed following application at mid-day could be due to reduced penetration through closed stomata.

Secondly, the scanning electron micrograph of the stomatal apparatus of *C. album* (Plate 1) clearly shows that the antechamber surrounding the stomatal pore is virtually devoid of wax projections. Such an area may offer less resistance to the penetration of the spray droplets. Other workers have reported diminished wax deposits in the vicinity of stomata (Juniper, 1960; Wortman, 1965) and suggest that these areas may be preferential sites of entry. The walls of the guard cells are also believed to be well endowed with ectodesmata (Franke, 1964) which are also considered to be portals of entry.

Thirdly, the varying susceptibility of *C. album* to bentazone in response to different environmental conditions (light, temperature and humidity) is not correlated to the degree of wax deposition or indeed wax orientation on the leaf surface (Davies, Cobb and Taylor, 1979).

Thus, we envisage that conditions favouring stomatal opening cause the guard cells to swell, pushing the cuticular lips further apart, and so exposing the wax-free antechamber to the herbicide. This may then be the principal route by which bentazone gains entry into the mature *C. album* leaf to reach its site of action. Hence, assuming the observations recorded above are consistent with the situation in the field, an assumption currently unproved, the poor control of *C. album* observed in the 1975 and 1976 seasons may have been a result of reduced stomatal apertures induced by the dry conditions prevalent at that time.

An additional, and perhaps alternative, interpretation of the rapid stomatal response in Figure 1 is that bentazone may cause epidermal cell damage, and a rapid loss of turgor. This could cause a reduction in pressure on the guard cells resulting in the increased stomatal apertures observed. However, damaged epidermal cells were only observed at high light intensities.

The full significance of these findings, and the exact route of entry of bentazone into the leaf, will only be determined with further studies.

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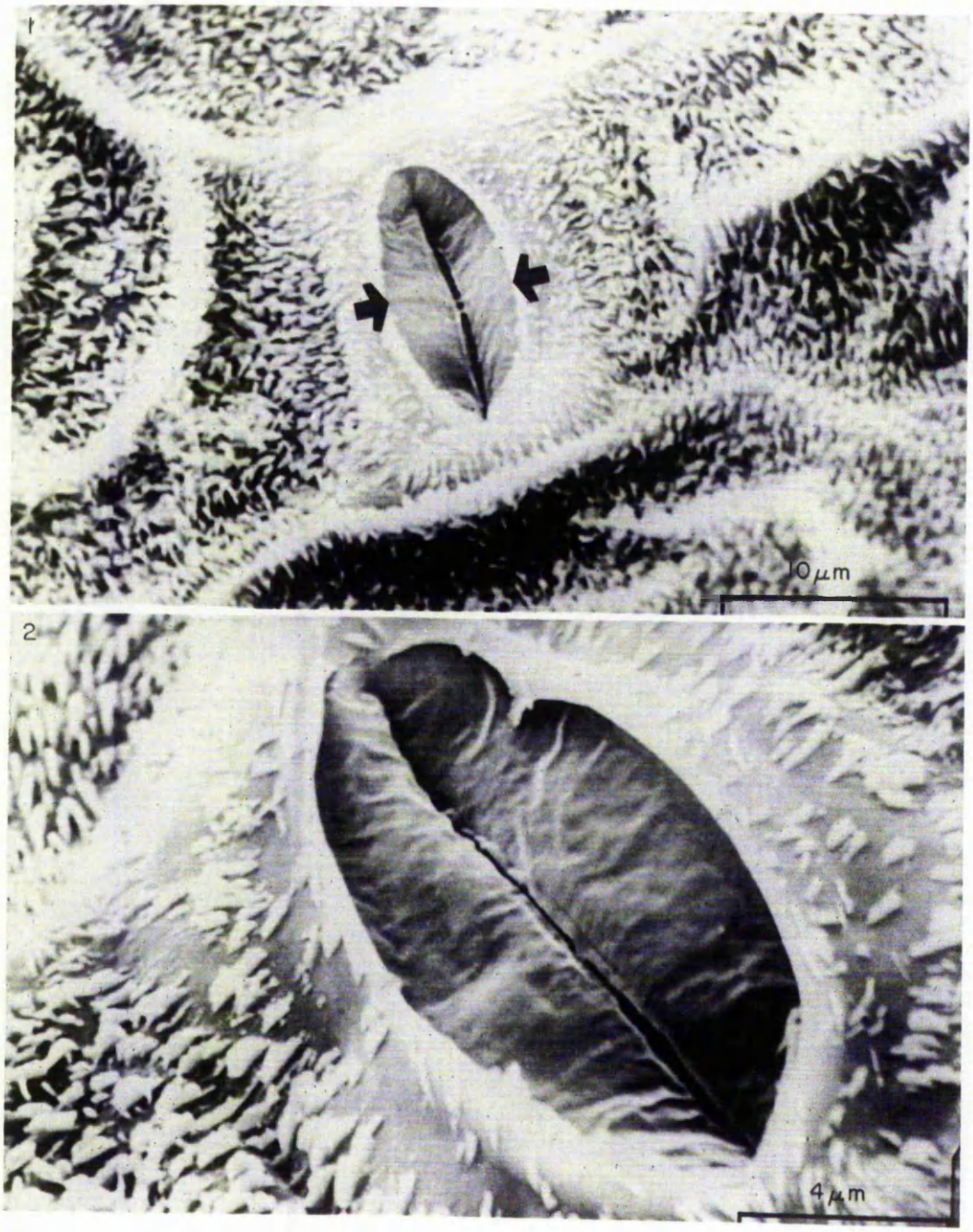
EXPLANATION OF PLATE

PLATE 1

Scanning electron micrographs of abaxial surface of leaves of *C. album*. Arrows refer to the distances measured between the cuticular lips. Note the relatively wax-free antechamber. Magnification (1) $\times$ 2,000, (2) $\times$ 5,000.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