UPTAKE OF PICLORAM BY EUCALYPT LEAF DISCS

by

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 ORIGINALITY OF THESIS

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Except where acknowledged this thesis is my own original work.

R. Sands

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SUMMARY

Factors affecting the uptake of picloram (4-amino-3,5,6-trichloropicolinic acid) by *Eucalyptus viminalis* and *Eucalyptus polyanthemos* leaf discs were examined.

Surfactants generally increased uptake, and uptake correlated well with the contact angle made on the leaf surface by the applied solution. Uptake correlated better with contact angle than with the surface tension of the applied solution. Leaf surface characteristics were important in uptake-surfactant relationships, and it was concluded that surfactants influenced uptake largely by altering the wettability of the leaf surface by solutions.

The effects of the leaf surface on uptake and wettability were examined after mechanical removal and chloroform extraction of the surface wax. Wax removal increased the wettability of *E. polyanthemos* leaf surfaces, but not as well as the surfactants: uptake was increased after mechanical removal, but not after chloroform extraction. The surfactants themselves dissolved some surface wax which may have played a part in their effects on uptake.
The stomata differed between the species in some characteristics, and possible effects of these differences on wettability and penetration via stomata are discussed.

Uptake was greater in light than dark, and increased with increasing light intensity. Uptake increased with temperature, and more so in light than dark. Far red light stimulated uptake by *E. viminalis* leaf discs but not by *E. polyanthemos*, and this stimulation was not phytochrome activated. The metabolic inhibitors DCMU and F-CCP inhibited the light-stimulated uptake by *E. polyanthemos* but not by *E. viminalis* leaf discs. Uptake in atmospheres of nitrogen, oxygen, and air indicated oxidative metabolism was not required.

Comparison of uptakes in light and dark by upper and lower surfaces of *E. viminalis* juvenile leaf discs correlated well with stomatal numbers and degree of opening. Determination of stomatal apertures under conditions leading to differences in uptake led to the conclusion that, in these experiments, stomata could be major portals of entry into eucalypt leaf discs.

Some basic assumptions about the role of stomata in uptake are questioned, and the literature in this field is critically reviewed.
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CHAPTER 1

FOLIAR UPTAKE OF HERBICIDES - A LITERATURE REVIEW

1.1. Introduction

Weeds are undesirable for a variety of reasons (Klingman 1961), and their control is of considerable economic importance. Various control methods are used (King 1966), but the use of chemicals has assumed a major role, particularly since the discovery of the auxin type herbicide, 2,4-D (Zimmerman and Hitchcock 1942).

Herbicides may be applied to the soil, or to the aerial parts of plants as contact herbicides (killing tissue on contact) or as systemic herbicides (absorbed by the plant and translocated to sites of action). A considerable part of commercial usage involves the spraying of foliage with systemic herbicides which have to be absorbed to exert their toxic effect. It is this absorption or uptake which is reviewed in detail, and is the subject of examination in the research described later.

Foliar uptake is the process by which externally applied materials are absorbed into the leaf. Translocation within or from the leaf may occur simultaneously with uptake and it is difficult, and
perhaps unwise, to say where one begins and the other ends. This review will concentrate on penetration of the immediate surface layers, and the factors that may affect this. For successful foliar uptake, a solution containing the material of interest must be retained by, and adequately wet, the leaf surface. Foliar uptake is reviewed under these aspects of wettability, retention, and penetration. For a more complete understanding of the process, uptake of substances other than herbicides may be referred to where necessary. Common names of herbicides are used and their full chemical description is given in the Appendix.

1.2. Foliar Uptake

(1) Wettability: The contact angle assumed by a droplet on a leaf surface is regarded as an index of wettability, spreadability, and adhesion. Ebeling (1939) considered static contact angle measurements indicated the spreading and penetrating properties of a liquid after deposition on a leaf surface. Fogg used advancing contact angle as an index of wettability (Fogg 1947) and adhesion (Fogg 1948a), while Evans and Martin (1935) used receding contact angle for the same purpose. Contact angle measurements (static, advancing, and receding) have been used widely as an index of wettability (Challen 1960, Foy and Smith 1965, Silva Fernandes 1965a, Troughton and Hall 1967, Holloway 1969, Rentschler 1971).

Elliott and Riddiford (1964) reviewed the physical aspects of contact angle formation in terms of
Interfacial tensions and work of adhesion, and gave a mathematical representation of the factors involved. A simplification of this shows that an equilibrium contact angle value depends on the surface free energies at the interfaces (surface tensions), and the degree of roughness and porosity of the solid surface.

From contact angle theory, reduction of the surface tension of a liquid should increase its wettability. This is so in practice (Ebeling 1939, Brunskill 1956), and improved herbicidal performances have been associated with such reduced surface tensions. The effect of surface tension will be discussed further when dealing with surfactants. Surface roughness will affect and often inhibit wettability (Fogg 1947, 1948a, Challen 1960, Gray 1967), and surface porosity also affects wettability (Cassie and Baxter 1944, Dettre and Johnson 1967).

Contact angle (and thus wettability) may vary between leaves of different species, between different leaves of the same species (Ebeling 1939, Fogg 1947), and between opposite sides of the same leaf (Ebeling 1939, Troughton and Hall 1967). It may also vary with age of leaf (Linskens 1950, 1952a, b) and external influences such as cigarette and diesel smoke which substantially reduce contact angles on leaves (Rentschler 1971). Contact angle may show a characteristic diurnal variation, and variation with the turgidity of the leaf (Fogg 1947). This is explained in terms of changes in surface roughness produced by changes in the water content of the tissues.
Challen (1960) suggested that the chemical nature of the leaf surface and submicroscopic roughness were major factors affecting the wetting of the upper surface of *Agropyron repens* Beauv., and Juniper (1959a) considered leaf surface ultrastructure to largely determine leaf wettability. Juniper and Bradley (1958) found that difficult-to-wet surfaces possess a great variety of structures on their surfaces, while wettable surfaces are generally smooth or covered with coarse ridges or undulations arising under the actual surface. They considered the easily wettable species likely to be the most susceptible to herbicides.

Many plant species exhibit a waxy bloom over their cuticle (Mueller et al. 1954, Juniper 1959a), and differences in wettability have been attributed to this. Wortman (1965) showed the poor wettability of some leaves to be due to the presence of submicroscopic wax structures, and found the removal of such wax by brushing to greatly increase wettability. Linskens (1950) showed wettability to be negatively correlated with the amount of surface wax, and Silva Fernandes (1965a) found wettability to be influenced by the amount, chemical composition, and particularly physical configuration of surface wax. Martin (1966) considered the physical form of surface wax to be the major factor governing affinity for water. Holloway (1969) acknowledged the importance of superficial waxes in determining wettability in many species, but cautioned that they played little part in some species.
Bukovac et al. (1971) found that removal of the epicuticular wax on isolated cuticles of tomato fruit decreased their wettability by water. Brunskill (1956) discussed the phenomenon of droplet bounce on some difficult to wet surfaces. He attributed droplet bounce on pea leaves to the fine waxy granular deposit on the leaf surface. Any factor of environment or growth affecting leaf wax deposition may consequently affect wettability.

Juniper and Bradley (1958) found that peas grown under low light intensities had less leaf surface wax and greater leaf wettability. Slow growth of leaves can lead to thicker wax projections on the surface and a possible increased resistance to herbicides (Juniper 1960). Mechanical abrasion of surface wax (Juniper 1960, Hall and Jones 1961) and the discarding of surface wax by older leaves (Rentschler 1971) may increase leaf wettability. Rainfall (Hammerton 1968) and wind (Hall and Jones 1961) can damage surface wax and increase wettability, and wax deposition may be modified by temperature (Banks and Whitecross 1971, Armstrong 1969).

Soil applications of TCA and dalapon may increase the susceptibility of pea plants to later applications of foliar-applied herbicides (Dewey et al. 1956, Pfieffer et al. 1957), due possibly to the soil-applied herbicides increasing the wettability of the leaf surfaces (Dewey et al. 1956). TCA in the soil reduces the amount (Pfieffer et al. 1957, Juniper 1959b) and changes the form (Juniper 1959b) of wax on pea leaves, and this has been associated with large increases in
wettability (Juniper 1959b). Dalapon has similar effects (Juniper and Bradley 1958). Increased wettability of cabbage leaves from EPTC inhibition of surface wax deposition may increase susceptibility to DNPB (Gentner 1966).

(2) Retention: Foliar retention is a measure of the amount of spray liquid retained on the leaf surface after application. Retention may vary with leaf area and arrangement with respect to overlapping (Holly 1964), angle of tilt of leaf (Ennis et al. 1952, Bikerman 1958, Furmidge 1962a), or angle of incidence of spray droplets impinging on the leaf surface (Brunskill 1956, Blackman et al. 1958). Surface characteristics of foliage such as surface roughness and protruding veins (Furmidge 1962b), and leaf pubescence (Ennis et al. 1952, Holly 1964), also affect retention. Therefore, differences in morphology may markedly affect retention between species and between different stages of development of one species. Blackman et al. (1958) considered retention to be maximum when the ratio of leaf area to shoot weight was high, the surfaces were completely wetted, and the leaf laminae lay in a horizontal plane. Bischof et al. (1970) found retention decreased with increasing age of the plant, and Furmidge (1962b) noted leaf movement by wind could reduce retention.

Most foliar applications of herbicide are carried out by spraying, and size (Fogg 1947, Brunskill 1956, Riepma 1960), and velocity of droplets (Brunskill 1956,
Furmidge 1962a, b) will affect retention. Retention depends on the volume of spray applied (Blackman et al. 1958, Riepma 1960, Bischof et al. 1970). Furmidge (1962a) considered volatility and viscosity of the spray liquid to be factors involved in retention, but Brunskill (1956) found viscous changes had little effect on the retention of spray droplets on pea leaves. Spray liquid density (Furmidge 1962a) and the use of surfactants (Stanley 1958, Furmidge 1962b) can also affect retention.

Foliar retention may be related to contact angle measurements (Bikerman 1958, Furmidge 1962a, b) and the surface tension of the spray liquid (Brunskill 1956, Furmidge 1962a, b), and is therefore related to wetting. However, there are differences of opinion or emphasis in the literature concerning the nature of this relationship. Stanley (1958) considered that significant increases in retention could be expected under field conditions when wetting agents were added to a spray formulation. Fogg (1947) found water, which has a higher contact angle (less wetting) on wheat than on charlock, was retained more on charlock, and Brunskill (1956) found the retention of spray droplets on pea leaves increased with decreasing surface tension. On the other hand, Furmidge (1962b) noted that the use of surface active agents generally reduced retention, and considered that wetters should be used in concentrations just sufficient to give adequate cover of the target surface. Reduced retention due to too great wettability is most likely with high volume rates of application (Furmidge 1959a, Holly 1964).
It appears that the use of wetters must be considered in terms of volume rates of application. With high volume rates, the object is to drench the leaves, often to run-off conditions, in order to retain as much liquid as possible and place the necessary amount of herbicide on the leaf. Under these circumstances, uncontrolled addition of wetters may reduce the desired high retention, especially on surfaces which are easily wetted naturally. With low volume rates of application, the amount of spray liquid is at a premium, the leaves are not drenched, and increased wettability might increase foliar retention. It is possible also, that under certain circumstances it would be advantageous to have improved wetting and spreading at the expense of retention. Furmidge (1962b) discussed retention when leaves were sprayed to run-off conditions. Retention is a maximum at the point of run-off (or incipient run-off) (Blackman et al. 1958) after which it generally is less, particularly on smooth leaf surfaces (Furmidge 1962b).

Differences in retention between species can be important in determining selective action of herbicides (Blackman et al. 1958), and Holly (1964) concluded that the diversity of behaviour of spray droplets on plant surfaces could contribute a great deal towards the selective behaviour of herbicides.

Increased herbicide susceptibility following mechanical damage may be due in part to increased
retention (Dewey et al. 1956, Dorschner and Buchholtz 1956), and such changes in retention may themselves be due to changes in wettability (Juniper 1960).

Wettability and retention, though very much interrelated, are not the same thing, and confusion reigns in the literature when they are used interchangeably. It is suggested that these two phenomena be envisaged as distinct entities when viewing them in their interrelated aspects.

(3) The Nature of the Barrier: In examining the penetration process, it is important to have some understanding of the physical and chemical nature of the barrier that has to be negotiated. Brogniart (1830, 1834) described a non cellular membrane covering the outer walls of epidermal cells of leaves which he named cuticle. For a foliar-applied penetrant to contact living cytoplasm, it must pass through the cuticle into the cell walls and ultimately traverse the plasmalemma.

(i) The cuticle: The boundary between the cuticle and the epidermal cell wall is not distinct. A layer of pectin has frequently been reported above the cell wall (Orgell 1957, Scott et al. 1958, Bolliger 1959, Frey-Wyssling and Mühlethaler 1965, Norris and Bukovac 1968), as a continuation of the matrix of the cell wall material. Isolated cellulose molecules as in the cell wall beneath, and cutin and cuticular waxes as in the cuticle above, extend into this pectinaceous transition
zone (Frey-Wyssling 1953, Frey-Wyssling and Mühlethaler 1965, Martin 1966). Cuticular waxes have been recorded in the cell wall (Schieferstein and Loomis 1959).

Towards the surface, this boundary zone grades into a membrane of cutin which may contain occluded wax lamellae (Martin 1966, Norris and Bukovac 1968). Though most workers accept this as a continuous, uniform membrane (Scott et al. 1958, Sitte 1965, Martin 1966, Norris and Bukovac 1968), others have claimed that cutin platelets may be separated by pectin (Roberts et al. 1948, Orgell 1955) forming a continuous pectin pathway from leaf surface to cell wall (Roberts et al. 1948).


The cuticle is considered to be a polymolecular reticular framework of hydroxy acid, fatty acid, and di-carboxylic acid units, called cutin (Martin 1966), embedded in a lipophilic wax matrix (Kolattukudy 1970). Cutin has lipophilic properties due to -CH₂ and -CH₃ groups (Frey-Wyssling 1953). The lipophilic nature of cuticle is responsible for its generally accepted major role of reducing water loss. However, cutin has hydrophilic properties due to free hydroxyl and carboxyl groups (Frey-Wyssling 1953), and may become hydrated and swell (Frey-Wyssling 1953, Crafts and Foy 1962, Martin 1966, Franke 1967). This hemihydrophilic nature of cutin might facilitate the passage of both polar and
apolar molecules. The surface wax attracts the lipophilic parts of cutin (Frey-Wyssling 1953), and polymerisation of cutin which requires oxygen is more complete towards the surface (Franke 1967). This contributes to the gradient of apolarity or low polarity at the surface to high polarity within the epidermal cell wall (Crafts and Foy 1962). The high negative charge of cutin gives the cuticle a potential for selective cation permeability (Franke 1967).

The lipophilic cuticular waxes may contain long chain paraffins, alcohols, ketones, fatty acids, triterpenoid compounds, esters of alcohols and acids, aldehydes, and acetals (Martin 1966, Eglington and Hamilton 1967).

Cuticle is a very variable component. It can change its nature with time throughout the life of a plant (Skoss 1955, Crafts and Foy 1962, Sargent 1965). Within the same leaf it may vary in thickness over anticlinal cell walls (Crafts and Foy 1962), over veins (Crafts and Foy 1962, Martin 1966, Norris and Bukovac 1968), and between upper and lower leaf surfaces (Norris and Bukovac 1968). Cuticle may vary between species in the chemical nature (Crafts and Foy 1962, Baker and Bukovac 1971) and the proportion (Richmond and Martin 1959, Baker and Bukovac 1971) of its constituents. Factors of environment such as exposure to sunlight, wind, rain, and insects (Crafts and Foy 1962), shading and water stress (Skoss 1955), temperature (Skoss 1955, Hull 1958), and nutrient supply (Richmond and Martin 1959) can add to cuticular variability.
There is some controversy as to whether pores are present in the cuticular membrane. Scott et al. (1958), Juniper and Bradley (1958), Schieferstein and Loomis (1959), Bayer et al. (1968), and Hallam (1970b) found no evidence of pores in the cuticular surface; whereas Hall and Donaldson (1962) and Hall (1967) reported pores in the cuticle for wax extrusion, and Hülsbruch (1966a, b) described a system of very fine tubes extending from the cell wall into the cuticle. Orgell (1957) found that isolated cuticle of several species contained minute cracks and perforations.

Ectodesmata are very fine thread-like structures in the outer walls of epidermal cells (Franke 1960a, b, 1962). Franke (1964c) suggested they are interfibrillar spaces containing protoplasmic excretion products which render ectodesmata visible upon staining with mercuric chloride (Franke 1961a, 1962, 1964b, Schönherr and Bukovac 1970). They extend from the cuticle which they do not perforate: some appear to terminate in the cell wall, and others pass through the lumina (Franke 1964c). Schönherr and Bukovac (1970) claimed ectodesmata were not specific cell wall structures but that their distribution was determined by the cuticle. Ectodesmata may be concentrated in guard cells, conical hairs, anticlinal walls, and epidermal cells adjacent to leaf veins (Franke 1961b), and they have been observed in the walls lining stomatal chambers (Lambertz 1954, Franke 1964d). Tukey et al. (1962) claimed that ectodesmata
appeared to increase in number at night and during leaf development, and that they were at a maximum in bright green leaves and decreased as the leaves yellowed.

(ii) The cell wall: The outer epidermal cell wall is composed of a framework of microfibrils of cellulose (a $\beta 1/4$ glucose polymer) embedded in a matrix of non-cellulosic polysaccharides. The cellulose framework which consists of lateral aggregations of longitudinal micellar strings, gives rise to intermicrofibrillar spaces up to 100 Å diameter, and intramicrofibrillar spaces about 10 Å diameter (Frey-Wyssling and Mühlethaler 1965). The amorphous matrix of the non-cellulosic polysaccharides is composed of hemicelluloses and pectin (Scott et al. 1958, Mühlethaler 1967, Lamport 1970). This matrix material fills many of the interstices, and any unoccupied spaces may be filled with imbibed water (Franke 1967).

The cell wall is composed of a primary and a secondary wall. The primary wall contains more matrix relative to framework material, and the microfibrils lack the organization of the more closely textured secondary wall (Frey-Wyssling and Mühlethaler 1965). The matrix material may also differ in composition (Lamport 1970).

Pectin consists of the acidic and neutral non-cellulosic polysaccharides; hemicellulose is the alkali soluble portion, though there may be some degree of overlapping of these substances (Lamport 1970). The
acidic polygalacturonides which are a major component of pectin (Beavan and Jones 1947), carry free carboxyl groups which allow the formation of salts and give pectin its cation exchange properties (Crafts and Foy 1962). These carboxyl groups, and also the free hydroxyl groups of cellulose and the hemicelluloses, make the cell walls highly hydrophilic and antagonistic to the passage of apolar solutes (Franke 1967).

(iii) Stomata: Stomata are the most dynamic feature of a leaf surface with the stomatal aperture varying from closed to fully open depending on turgor changes in the accompanying guard cells. Stomata and their physiology have been recently reviewed by Meidner and Mansfield (1968) and Zelitch (1969). They can vary in shape, size, density, distribution, and physiological response between species and within a species, be similar in number on both surfaces, or be significantly less or absent on the upper surface (Weintraub et al. 1954, Goodman and Goldberg 1960, Goodman and Addy 1963, Darlington and Cirulis 1963, Kamimura and Goodman 1964a, Sargent and Blackman 1965). Stomata may be considered as invaginations of the cuticular membrane (Yamada et al. 1966), as the cuticular layer is continuous over the walls of the stomatal chamber (Scott 1950, Goodman and Addy 1963, Yamada et al. 1966). However, this 'inner' cuticle is thinner (Goodman and Addy 1963) and more hydrated (Crafts and Foy 1962) than the 'outer' cuticle. Scott and Lewis...
(1953) found no plasmodesmata in walls adjacent to intercellular spaces, but reference has been given to the presence of ectodesmata in the chamber wall.

(iv) The plasmalemma: Permeability of the plasmalemma and other membranes is central to plant physiology, and yet there is no general agreement concerning their structure or the pathways and mechanism of their penetration. Davson and Danielli (1952) described the plasmalemma as a bimolecular lipid leaflet stabilized by hydrophobic interactions ionically bound with protein on both surfaces. A variety of other structures have subsequently been proposed (see Branton 1969, Cook 1971) in an attempt to explain some of the uptake phenomena observed, but Branton (1969) in his review considered the classical Davson and Danielli model best explained the unspecialized properties of the membrane. Permeability of membranes has recently been reviewed by Stein (1967), Leggett (1968), and Stadelmann (1969). Park (1960) cited nine possible mechanisms by which biological membranes may be penetrated.

(4) Penetration: Cuticular penetration is demonstrated in the penetration of astomatous leaf surfaces (Biswas et al. 1962, Sharma and Vanden Born 1970), and astomatous cuticular membranes (Darlington and Cirulis 1963). Also, in penetration via stomata, the inner lining of cuticle must be penetrated. Cracks and perforations (Orgell 1957, Martin 1966, Franke 1967), trichomes (Hull 1963), and extrusion pores, if they do exist, may account for some penetration in
isolated circumstances, but the cuticle in general remains an overall barrier to penetration.

The cuticle is penetrated more readily by lipophilic than by hydrophilic molecules (Mitchell et al. 1960, Crafts and Foy 1962, Darlington and Cirulis 1963, Sargent 1965). Wax-covered leaf surfaces are more easily wetted by lipophilic solutions (Crafts and Foy 1962), and waxes may assist in their entry (Martin 1966). Penetration is effected by solubilization in the lipophilic wax and cutin constituents (Crafts and Foy 1962, Franke 1967), the extent being determined by the solubility, partition, and molecular size of the penetrant (Franke 1967).

The lipophilic leaf waxes and partly lipophilic cutin chemically oppose penetration by hydrophilic molecules. Silva Fernandes (1965b) obtained no penetration of isolated astomatous leaf cuticles by copper and mercury from aqueous solutions of their salts when membranes contained 0.1 mg/cm² of cutin or more. Wax in particular is an important barrier to hydrophilic penetration (Skoss 1955, Crafts and Foy 1962, Thrower et al. 1965, Silva Fernandes 1965b, Martin 1966, Sharma and Vanden Born 1970, Bukovac et al. 1971). Baker and Bukovac (1971) found sorption of 2,4-D by cuticular membranes to be inversely proportional to the amount of cuticular wax, and Sharma and Vanden Born (1970) noted penetration of adaxial surfaces of poplar leaves by picloram to be increased up to four times after removal of surface wax. Crafts and Foy (1962) considered
surface wax exerted its greatest influence by inhibition of wettability, whereas cuticular waxes were more important as a chemical barrier to hydrophilic entry. Reference, however, has been made to the hemihydrophilic nature of the cuticle. Water penetrates through the cuticle to the atmosphere in cuticular and peristomatal transpiration (Seybold 1961-1962, Maercker 1965a, b), and from the atmosphere into the leaf under conditions of high humidity (Breazeale et al. 1950). Cuticle has been likened to a sponge which, in a dry atmosphere, has its cutin matrix wax pockets close together forming a relatively impermeable barrier to water; but which in a humid atmosphere becomes hydrated and swells, and the wax pockets are separated to a maximum (Martin 1966). This hydrated cuticle may facilitate passage of aqueous solutions (Crafts and Foy 1962, Franke 1967).

Penetration of the cuticle by polar substances is considered to be by simple diffusion (Darlington and Cirulis 1963, Martin 1966). However, Yamada et al. (1964a) found penetration of isolated cuticles by radioactive cations and anions to be much greater from the outer to inner surfaces than vice versa, and suggested the greater ion binding capacity on the inside of the cuticular membrane could facilitate foliar absorption. Conversely, Goodman and Addy (1963) noted greater penetration by labelled pesticides and model compounds from the inner to outer surface of isolated cuticle.
Crafts (1961) postulated the presence of specific polar and apolar penetration pathways, though Foy (1964) considered that adequate experimental support for their existence was lacking. Roberts et al. (1948) suggested a pectin pathway from the surface of apple leaves to the vein extensions allowed specific passage of hydrophilic molecules. Sargent et al. concluded that their data on the penetration of 2,4-D (Sargent and Blackman 1969) and dalapon (Sargent et al. 1969a) into Phaseolus leaf discs was consistent with polar and apolar pathways. From data obtained using surfactants, Jansen (1965b) suggested herbicides moved into plants by distinct hydrophilic and lipophilic pathways.

There is disagreement about the significance of stomata in foliar penetration. Since stomata are lined with cuticle (which would still need to be penetrated), 'penetration via stomata' is perhaps preferable terminology to 'stomatal penetration'. Solutions entering stomatal cavities would have the advantage of a larger surface area for absorption (Franke 1967), and a thinner and more hydrated cuticle for penetration. Solutions within stomatal cavities would evaporate less and could act as reservoirs.

Although vapours (Fogg 1948b), and oils (Turrell 1947, Van Overbeek and Blondeau 1954) may enter stomata, many agree that aqueous solutions with surface tensions close to that of water do not (see Sargent 1965, Franke 1967). In the presence of surfactants, however, fluorescent tracers in aqueous solutions can enter open
stomata (Dybing and Currier 1959, 1961, Currier et al. 1964), the amount depending on the degree of opening (Currier et al. 1964). Skoss (1955) considered stomata to be the major portals of entry irrespective of the nature of the sprayed material. Dybing and Currier (1959, 1961) found that stomatal entry, when it occurred, was very rapid, but cuticular penetration was very slow.

Schuster and Karr (1969) found night application of herbicide to be more effective in controlling plains prickly pear, a species in which stomata are open at night. It has often been shown that uptake through the lower surface of a leaf is greater than through the upper when the species has no, or fewer stomata on the upper surface (Cook and Boynton 1952, Crafts and Yamaguchi 1958, Dybing and Currier 1959, Prasad et al. 1962, Biswas et al. 1962, Sargent and Blackman 1962, 1965, Goodman and Addy 1963, Kamimura and Goodman 1964a, Davis et al. 1968a, Sharma and Vanden Born 1970). Goodman and Addy (1963) and Kamimura and Goodman (1964a) suggested that both the increased contribution of stomata and the thinner cuticle on the under surface caused this effect.

Franke (1969) suggested that ectodesmata were favoured penetration pathways for aqueous solutions. Special sites (guard cells, conical hairs, anticlinal walls, and epidermal cells adjacent to leaf veins) known to be rich in ectodesmata may correspond to known sites of ion binding (Yamada et al. 1966, Franke 1969),
and permeability to water (Maercker 1965a, b, Franke 1961a, b), solutions, and dyes (Franke 1961a, b). Ectodesmata in stomatal cavity walls may assist penetration via stomata (Franke 1964a). Sargent and Blackman (1962) considered the difference in uptake of 2,4-D between upper and lower surfaces of Phaseolus leaf discs was caused by increased penetration through guard cells rather than via stomatal pores, and Jyung et al. (1965a) found uptake of rubidium $^{86}$ by Phaseolus leaves to be highly correlated with stomatal frequency, but not with stomatal opening.

Movement in the cell wall has been demonstrated with fluorescent dyes (Dybing and Currier 1959) and by microautoradiography (Pickering 1965). The hydrophilic cell walls are porous and freely permeable to polar substances including large molecules (Currier and Dybing 1959). Movement through the cell walls is by diffusion (Fogg 1948b, Currier and Dybing 1959, Franke 1967, Bayer et al. 1968), though Currier and Dybing (1959) reviewed evidence suggesting that movement within cell walls may be under some degree of protoplasmic control. Franke (1967) admitted that cell wall structures were well suited to free diffusion, but considered penetration occurred through separated pathways such as ectodesmata.

Less is known about the movement of lipophilic substances through cell walls. Lipophilic lined channels in the epidermal cell wall for extrusion of wax
and cutin precursors may exist (Bolliger 1959, Hülsbruch 1966a, b) and these may provide passage for lipophilic molecules (Franke 1967).

Membrane penetration can be passive or active (Jennings 1963). Active foliar uptake requires respiratory or photosynthetic energy (van Lookeren Campagne 1957, Kylin 1960), and high energy phosphate compounds may be involved (Jennings 1963, MacRobbie 1965). Absorption may be correlated with protein synthesis (Jyung et al. 1965b) and proteinaceous carriers could be important (Jyung and Wittwer 1964). Further aspects of active uptake are discussed later in the section on metabolism.

Penetration by some polar molecules, though passive in nature, occurs at a much greater rate than would be expected by simple or activated diffusion through a homogeneous lipoid membrane (Jennings 1963). This is termed facilitated diffusion and depends on the breaking of H-bonds between the penetrant and water, and the forming of H-bonds between the penetrant and a membrane molecule. Stein (1967) reviewed possible mechanisms for facilitated diffusion.

2,4-D can reduce the integrity of cell membranes (Hallam 1970b, Bachelard and Ayling 1971) and it is possible that herbicide damage to membranes could affect its own penetration behaviour.
1.3. **Factors affecting foliar uptake**

(1) **The Plant:** Åberg (1964) reviewed effects of stage of plant development, vigour and nutrient status, previous history, state of health, varietal differences, and population trends and acquisition of immunity of plants on their susceptibility to herbicides. Some of these effects may be due to effects on penetration as variations in leaf wettability, leaf morphology, and the nature of leaf cuticle within, between, and at different stages of development of plant species can modify penetration behaviour.

Penetration is often better in younger leaves (Fang et al. 1951, Bukovac 1965, Way 1969), and Schiefterstein and Loomis (1959) found that permeability of isolated cuticles of *Hedera helix* L. to 2,4-D can decrease up to fifty times with age. Conversely, Agbakoba and Goodin (1969) noted that foliar uptake of picloram and 2,4,5-T was greater in adult than seedling field bindweed. Bischof et al. (1970) showed that penetration of phenmedipham was dependent on the stage of growth of plants. Kamimura and Goodman (1964a) noted differences in uptake of leucine at different positions on apple leaves (apex versus base, and veinal versus interveinal regions) as well as with position of the leaf on the shoot. Bukovac (1965) found marked differences in uptake of 3-chlorophenoxy-α-propionic acid between different areas of peach leaf cuticle, but could not associate this localization with visible morphological characteristics.
Herrett and Linck (1961) considered amitrole was more toxic to Canada thistle than field bindweed because of more efficient penetration in the thistle. Gallup and Gustafson (1952) suggested the slower absorption of 2,4-dichloro-5-iodo-131 phenoxyacetic acid by monocotyledons contributed to their resistance, and Davis et al. (1968a) found the foliar uptake of 2,4,5-T by tree species to be related to their 2,4,5-T susceptibility.

(2) The Herbicide: Various herbicides differ in their effectiveness, but this need not necessarily reflect difference in uptake but in intrinsic toxicities. Veldstra (1953) reviewed structure-activity relationships of auxin-type herbicides. Penetration properties of a substance may depend on particle size, chemical composition and structure, pH, steric form, polarity, and ionic properties (Crafts and Foy 1962 and references cited). pH will be discussed in detail later. Mitchell et al. (1960) emphasized the importance of size of the penetrating particle whether it be an ion, molecule, or molecular aggregate, and added that large crystalline deposits would limit absorption.

Reference has been made already to lipophilic molecules having an advantage in penetration over more hydrophilic ones. Sargent et al. (1969b) examined the effect of progressive chlorination of phenoxyacetic acid and benzoic acid on their uptake into Phaseolus leaf discs, and the rate at which they moved from one aqueous phase to another through a lipid (octanol) in a
model system. They found chlorination increased the rate of phenoxyacetic acid but decreased the rate of benzoic acid penetration in both the model system and leaf discs. Bukovac et al. (1971) reported a similar effect on rate of transfer through the isolated cuticle of tomato fruits. Okuda and Yamada (1962) considered lipid solubility an important controlling factor in the foliar uptake of phosphoric acid, and Norris and Freed (1966) found that, with 2,4-D and 2,4,5-T and their triethanolamine salts and 2-ethylhexyl esters, absorption increased with decreasing polarity. Hauser (1955) also demonstrated different penetration rates by different formulations of 2,4-D.

Penetration rates may depend on herbicide concentrations: Hauser (1955) noted 2,4-D uptake increased with increasing concentration, and Davis et al. (1968a) found foliar uptake of 2,4,5-T and picloram by oaks to be approximately proportional to their concentration outside the leaf.

Some combinations of herbicides improve toxicity (Colby and Warren 1963, Colby et al. 1965, Bovey et al. 1968, Agbakoba and Goodin 1970). Putnam and Ries (1967) found paraquat to have a synergistic phytotoxic effect when added with simazine or diuron, but considered that this was not due to increased uptake. Davis et al. (1968b) showed paraquat increased the uptake of picloram by yaupon; and 2,4,5-T increased picloram uptake, and picloram reduced 2,4,5-T uptake, in mesquite.
Oils, emulsifiers, and cosolvents may modify foliar penetration characteristics. Oils can penetrate stomata (Turrell 1947, Van Overbeek and Blondeau 1954) and move through intercellular spaces (Minshall and Nelson 1949). Triplett (1968) found foliar penetration by atrazine increased with increasing proportions of oil in the mixture. Cosolvents can increase the solubility of herbicides in water and maintain a high degree of dispersion of herbicide molecules (Mitchell et al. 1960). As water evaporates from an aqueous herbicide solution on a leaf surface, a non-volatile carrier may progressively become the major carrier in which most of the herbicide is finally dissolved (Mitchell and Linder 1957). Dimethyl sulfoxide (DMSO) has recently been publicised as a chemical having a whole range of remarkable properties (Davidson 1964, Anonymous 1966). It may stimulate foliar uptake of nutrient ions (Leonard 1967, Bajaj et al. 1970) and herbicides (Kiel 1965, Burns et al. 1969). However, it is a powerful solvent affecting membrane permeability and metabolism, and may have to be used with caution in biological systems (Bajaj et al. 1970).

Other adjuvants may enhance foliar penetration. Surfactants will be discussed in detail later. Currier and Dybing (1959) reviewed the use of carbowax, glycerol, and molasses as humectants (retardents of evaporation of spray solutions), and propylene glycol as both a humectant and cosolvent. Plant growth substances may affect herbicide penetration (Sargent and Blackman 1962, Sargent 1968). Brady (1970) found the addition of
ammonium nitrate and phosphoric acid increased the absorption of the iso-octylester of 2,4,5-T by leaves of various tree species. Szabo and Buchholtz (1958, 1961) showed that penetration of a collodion membrane and the peeled and intact epidermis by 2,4-D was influenced by ionic additives, while Goodman and Goldberg (1960) demonstrated that streptomycin competed with metallic ions for absorption sites in homogenised leaf tissue. Yamada et al. (1965b) found urea enhanced the penetration of rubidium and chloride ions through astomatous cuticular membranes of tomato fruit cuticle.

(3) pH: Orgell (1957) considered that acids and bases were best sorbed at acid and base pH values respectively. Better absorption of 3,5-dinitro-o-cresol (Simon et al. 1952), 2,4-D (Hansen and Buchholtz 1952, Weintraub et al. 1954, Hauser 1955, Orgell and Weintraub 1957, Wedding and Erickson 1957, Sargent and Blackman 1962), 2,4,5-T (Baur et al. 1971), dalapon (Sargent et al. 1969a), and picloram (Sargent and Blackman 1970a, Baur et al. 1971) has been registered at lower pH values. At least part of the reason for this may be that the presence of the undissociated more lipophilic molecule is favoured by lower pH, and this penetrates more easily than the anion (Crafts 1957, Baur et al. 1971).

Simon and Beevers (1952) acknowledged that the effect of pH on the degree of dissociation of weak acids played a major role in biological activity, but considered that the ions were also effective. Sargent
et al. (1969a) found significant entry of dalapon into Phaseolus leaf discs at a pH corresponding to nearly complete dissociation, and Blackman et al. (1953) considered both the molecule and anion of growth regulating weak acids to be biologically active. Weintraub et al. (1954) reported the typical relationship of 2,4-D uptake with pH, but found, in the presence of certain cations, that absorption at neutral and alkaline pH values could approach that of acid. Similarly Szabo and Buchholtz (1961) showed anion and cation additives could modify the pH effect. Nobel (1970) found a logarithmic correlation between the pK of some organic acids and their light stimulated uptake, in the presence of potassium, into pea leaf fragments.

Baur et al. (1971) obtained improved uptake of 2,4,5-T and picloram at lower pH values, but considered 2,4,5-T to be more dependent on the degree of dissociation than picloram. Sargent and Blackman (1970a) reported decreased penetration of picloram into Phaseolus leaf discs as pH was increased from 4.2 to 7.2, but this decrease was not as much as might be expected if penetration were confined to unionized molecules. They suggested that permeability changes in the cuticle or epidermal cell membranes might explain this effect. This is supported by their observation (Sargent and Blackman 1970b) that penetration, in the light, of young bean leaves by chloride ions is increased as pH falls: any explanation in terms of undissociated molecule
penetration cannot apply here. Hansen and Buchholtz (1952) also suggested increased membrane permeability as an alternative explanation of the pH effect.

Care is needed in interpreting pH effects in the plant body on the basis of the pH of the externally applied solution. Crafts (1957) described the changes in pH that the free acid of 2,4-D (pH about 3.3) would encounter as it moved through the leaf surface layers (pH about 5.5) and ultimately into the phloem (pH about 7.0). A more rational interpretation of observed pH effects may require examination of undissociated molecule and ion concentrations in the micro-environment within the cell (Simon and Beevers 1952). Also, it may be difficult to determine whether a pH effect is due to pH per se, or to the presence of buffer or other chemical species as interacting molecules or ions in their own right.

(4) Surfactants: Surfactants (surface active agents) have a wide application in industry and technology (Young and Coons 1945). Their use in biological systems other than for herbicidal enhancement was reviewed by Parr and Norman (1965). Surfactants have balancing hydrophobic and hydrophilic groups in the one molecule, and can reduce surface tension of solutions and interfacial tensions at liquid and solid interfaces (Parr and Norman 1965). Black (1964) recognized several classes of surfactant: anionic (surface active species an anion), cationic (surface active species a cation), nonionic (uncharged),
amphoteric (contain both acidic and basic functional groups), and polymeric (macromolecules exhibiting protective colloidal properties).

The addition of surfactants is known to enhance herbicidal effectiveness (Hitchcock and Zimmerman 1948, Staniforth and Loomis 1949, McWhorter 1963a, b, Foy and Smith 1965). The uptake of herbicides is enhanced by surfactants (Bryan et al. 1950, Weintraub et al. 1954, Barrier and Loomis 1957, Dybing and Currier 1959, Sargent and Blackman 1962, Prasad et al. 1962, Foy 1962, 1963). Weintraub et al. (1954) found, when using the most effective type and concentration of surfactant they tested, that only seven per cent as much 2,4-D was needed to give the same herbicidal response as was achieved without surfactant. However, in some circumstances surfactants have little effect (Zukel et al. 1956, Bukovac 1965).

The lowering of surface and interfacial tensions by surfactants and the consequent increase in wettability as discussed previously is considered the cause of at least part of the increased uptake of herbicide. Mueller et al. (1954) used polystyrene macromolecules in sprays with or without surfactants on waxy leaf surfaces. When surfactants were used, the polystyrene molecules came into contact with the cuticle between the wax groups; in the absence of surfactants, the polystyrene molecules did not penetrate beyond the wax layer. Staniforth and Loomis (1949) regarded decreased surface tension as the likely reason for surfactant effectiveness, but added
it was not a simple relationship. Similarly, Freed and Montgomery (1958) and Hughes and Freed (1961) considered the reduction of surface tension important, but no more so than the molecular interaction between surfactant and herbicide. Jansen (1964) supported this but extended it to species, claiming a strong herbicide-surfactant-species interaction. Surfactant structure-activity relationships were examined by McWhorter (1963b), Jansen (1965a), and Smith et al. (1966).

The surfactant concentration (or range of concentrations) in aqueous systems above which surfactant molecules are not independent but add to a micellar aggregate is known as the critical micelle concentration (or range). Surface tension usually is reduced up to this range but beyond this it remains relatively constant (Parr and Norman 1965). Maximum enhancement of herbicidal effects has been recorded at surfactant concentrations well above this range (Staniforth and Loomis 1949, Jansen et al. 1961, Foy and Smith 1965) suggesting that something more than a reduction of surface tension is involved in surfactant effects. Jansen et al. (1961) considered observed surfactant effects correlated best with the colloidal state of the surfactant system.

There is a tendency in the literature to assume that because surfactant effects cannot be explained fully in terms of surface tension, they are equally inexplicable in terms of wettability and associated surface energy relationships. However, the surface
tension of a solution alone does not necessarily reflect the wettability of that solution on a surface, as the nature of the surface, and interactions between it and the solution, are also involved. Theoretically, herbicide-surfactant-species interactions may all be involved in determining surface energy relationships. However, Foy and Smith (1965) found surfactant enhancement of herbicide effects did not correlate with surface tension, contact angle, observed wettability, or initial surfactant toxicity. They suggested interactions more specific than those involved in wetting were a part of total surfactant action.

Jansen (1964) and Smith et al. (1966) suggested surfactants may modify cuticle absorption pathways, but Darlington and Barry (1965) considered surfactants could not alter the permeability of hydrated leaf cuticle. Furmidge (1959b) claimed surfactants could dissolve and remove large areas of surface wax, and Wortman (1965) found surfactants could increase wettability by altering surface wax structures. Van Overbeek and Blondeau (1954) suggested increased herbicide penetration could result from a solubilizing effect of surfactants on the plasmalemma. Surfactants may also alter turbidity (McWhorter 1963b, Jansen 1965b), increase the solubility of a herbicide in water (Temple and Hilton 1963), stabilize emulsions or suspensions, increase tenacity of deposits by acting as stickers (Furmidge 1959a), and act in a minor role as a humectant (Currier and Dybing 1959). Surfactants may markedly enhance penetration by
assisting stomatal entry of aqueous solutions as discussed previously. Foy and Smith (1963) and Smith and Foy (1966) found surfactants themselves do not penetrate surfaces readily.

The addition of surfactants may reduce variation in toxicity of different formulations of a herbicide (Hughes and Freed 1961), and variation in herbicidal effectiveness induced by climatic changes (Hauser 1955). Some surfactants may be phytotoxic (Furmidge 1959b, c) or stimulatory to growth (Stowe 1960, Jansen et al. 1961).

5 Light: Prior illumination of leaves may affect uptake (Sargent and Blackman 1962) and effectiveness (Blackman and Robertson-Cuninghame 1955a, Minshall 1957, Jordan et al. 1960) of herbicides applied subsequently. Weaver and Nylund (1963) found MCPA caused greater damage to peas in which carbohydrate levels were high as a result of exposure to light, and Moorby (1964) considered reduced uptake of Caesium¹³⁷ in the dark by pea leaves to be associated with changes in carbohydrate levels. Cook and Boynton (1952) found light pretreatment (to modify carbohydrate levels) had no effect on the absorption of urea by apple leaves. Effects of light intensity on leaf cuticle and surface wax, discussed previously, will modify penetration behaviour: other morphological changes in leaves induced by light (Blackman 1956, Schwabe 1963) may contribute.

Uptake of 2,4-D (Sargent and Blackman 1965), 2,4-dichloro-5-iodophenoxyacetate (Blackman and Robertson-Cuninghame 1955b), 2,4,5-T (Brady 1969),
dalapon (Sargent et al. 1969a), picloram (Sargent and Blackman 1970a), ions (Gustafson 1956, Sargent and Blackman 1970b), antibiotics, and amino acids (Kamimura and Goodman 1964b) may increase with light intensity. Conversely, no effect (Rice 1948, Herrett and Linck 1961) or an inhibitory effect (Davis et al. 1968a) of increasing light intensity on uptake has also been recorded. Penetration in light is usually greater than in dark (Sargent and Blackman 1962, 1965, 1970a, 1970b, Sargent et al. 1969a), though Rice (1948) recorded greater, and Weintraub et al. (1954) equal, absorption in the dark.

Light stimulated uptake has been explained in terms of increased stomatal aperture (Currier and Dybing 1959) and dependence, or part dependence, on photosynthesis and associated metabolism. This relationship with metabolism will be discussed later.

(6) Temperature: Previous reference was given to variations in cuticle and surface wax with temperature, and this, together with temperature-induced morphological changes in leaves (Milthorpe 1956, Njoku 1957, Schwabe 1963) may affect subsequent foliar penetration.

Increased temperature may enhance the herbicidal effectiveness of 2,4-D (Marth and Davis 1945, Kelly 1949, Bryan et al. 1950, Blackman and Robertson-Cuninghame 1955a, Jordan et al. 1960) and dinitrophenols (Meggit et al. 1956), and can increase foliar penetration by 2,4-D (Barrier and Loomis 1957, Pallas 1960, Sargent and Blackman 1962, Muzic and Mauldin 1964),
ammonium 2,4-dichlorophenoxyacetate (Rice 1948), 2,4,5-T (Morton 1966), dalapon (Prasad and Blackman 1965a, Prasad et al. 1967, Sargent et al. 1969a), picloram (Sargent and Blackman 1970a, Sharma and Vanden Born 1970), phenylmercuric acetate (Silva Fernandes 1965b), streptomycin (Goodman and Goldberg 1960), cations (Gustafson 1956), and anions (Sargent and Blackman 1970b). The herbicidal effectiveness of potassium cyanate (Antognini 1951), and the foliar penetration of urea (Cook and Boynton 1957) have been increased with decreasing temperature, while Zukel et al. (1956) and Smith et al. (1959) showed little effect of temperature on the foliar absorption of maleic hydrazide.

The increase in penetration with temperature is frequently greater in the light than in the dark (Sargent and Blackman 1962, 1970a, b, Sargent et al. 1969a). Higher $Q_{10}$ values have been observed in the light than in dark: this is further discussed in the section on metabolism. Stomatal apertures may increase with temperature in the light (Stalfelt 1962, Prasad et al. 1967, Raschke 1970) which could increase penetration via stomata. Van Overbeek (1956) reviewed evidence suggesting temperature could modify penetration by causing viscosity changes in the plasmalemma.

Supraoptimal temperatures may lessen penetration due to rapid evaporation of spray solutions, stomatal closure, and wilting (Hammerton 1967). Mauldin and Muzic (1964) and Mauldin et al. (1966) found that the
addition of thiamin, which restores normal growth to coast fiddleneck at suboptimal temperatures, increases its susceptibility to 2,4-D at this temperature.

(7) Relative Humidity: Increased relative humidity can increase the toxicity of foliar applied herbicides (Antognini 1951, Jeffrey and Nalewaja 1968). Foliar penetration by 2,4-D (Pallas 1960, Clor et al. 1963, Burns et al. 1969), amitrole (Herrett and Linck 1961, Clor et al. 1963), maleic hydrazide (Zukel et al. 1956, Crafts et al. 1958, Smith et al. 1959), diquat and parquat (Brian 1966), and dalapon (Prasad et al. 1962) can also increase with increased relative humidity, but Morton (1966) found little effect of relative humidity on 2,4,5-T absorption, as did Went and Carter (1948) with sucrose absorption.

Rapid evaporation of the herbicide solution on the leaf surface can reduce uptake (Rice 1948), and since low relative humidity favours evaporation (Foy and Sukartaatmadja 1963) this may explain in part the reduced penetration found at low relative humidities (Antognini 1951, Prasad et al. 1962, 1967). Holly (1956) found glycerol to be beneficial as a humectant only at low humidity.

At high humidities, stomata are more widely open (Wilson 1948, Prasad et al. 1962, 1967), and may be open at low light intensities (Foy 1962). Therefore increased penetration via stomata may contribute to humidity effects (Prasad et al. 1962). Pallas (1960) found increased uptake at high humidities to be correlated
with the degree of stomatal opening, but Brian (1966) considered there was no stomatal component in humidity effects. Prasad et al. (1967) found high relative humidity promoted uptake by astomatous as well as stomatous surfaces. Previous reference was made to the swelling and hydration of cuticle under high humidity, thereby making enhanced cuticular penetration by polar substances explicable.

Leaves under moisture stress may absorb less 2,4-D (Hauser 1955), picloram (Davis et al. 1968c), and cobalt (Gustafson 1956), though Merkle and Davis (1967) and Davis et al. (1968c) found little effect of moisture stress on uptake of 2,4,5-T, as did Basler et al. (1961) on 2,4-D, and Merkle and Davis (1967) on picloram. High relative humidity may enhance foliar uptake by reducing moisture stress, though Brian (1966) found high humidity combined with low soil moisture gave better uptake of diquat and paraquat than did low humidity combined with high soil moisture.

(8) Metabolism: There are several lines of evidence suggesting that foliar uptake is, in part, under some form of metabolic control, either directly or indirectly. Light-stimulated absorption of chloride ions (van Lookeren Campagne 1957), streptomycin, and leucine (Kamimura and Goodman 1964b) had action spectra similar to that of photosynthesis, though Davis et al. (1968a) considered light quality had no significant effect on the foliar uptake of picloram and 2,4,5-T by ten woody species. Brady (1969) noted that foliar uptake of
2,4,5-T by oak species was a maximum at the light intensity giving most efficient photosynthesis. Walker (1961-1962) found light could reduce the membrane resistance of Chara cells, and again the action spectrum was very similar to that of photosynthesis. In the dark, foliar uptake of ions may be associated with utilization of respiratory energy (van Lookeren Campagne 1957, Kylin 1960).

Foliar absorption of streptomycin, leucine (Kamimura and Goodman 1964b), and cobalt\textsuperscript{60} (Gustafson 1956) was increased by the exogenous addition of sugars in the dark. Jhung et al. (1965c) found light, succinate, and bicarbonate promoted ion absorption in cells enzymatically isolated from green tobacco leaves.

High Q\textsubscript{10} values for temperature effects on the foliar absorption of 2,4-D (Sargent and Blackman 1962, 1965), dalapon (Sargent et al. 1969a), and ions (Jhung and Wittwer 1964, Goodman and Goldberg 1960) in the light have been recorded. Though a low Q\textsubscript{10} could indicate a simple diffusion process, a high Q\textsubscript{10} does not necessarily imply active uptake, as it may be completely compatible with facilitated passive mechanisms as well. Uptake of 2,4-D (Sargent and Blackman 1962, 1965), dalapon (Sargent et al. 1969a), picloram (Sargent and Blackman 1970a), chloride ions (Sargent and Blackman 1970b), and sucrose (Vickery and Mercer 1964) is markedly reduced at low temperatures.

A variety of chemical inhibitors of metabolism may reduce foliar absorption (van Lookeren Campagne 1957, Kamimura and Goodman 1964b, Vickery and Mercer 1964,
Jyung and Wittwer 1964, Jyung et al. 1965c, Prasad and Blackman 1965b). Ultra violet light (Sargent and Blackman 1965) and oxygen withdrawal (van Lookeren Campagne 1957) may also reduce absorption, and aeration may stimulate it (Jyung and Wittwer 1964). Jyung et al. (1965c) found that DNP inhibited rubidium uptake by tobacco leaf cells but the addition of ATP reversed this effect. Sargent and Blackman (1969) examined the effect of a range of inhibitors chosen to affect respiration, phosphorylation, photosynthesis, membrane permeability, protein synthesis, and the binding capacity of membrane systems on the uptake of 2,4-D by Phaseolus leaf discs, and found most active inhibition was in the light by inhibitors which blocked ATP production. They suggested that ATP generated in the light might influence permeability of the leaf surface to 2,4-D (Sargent and Blackman 1969), dalapon (Sargent et al. 1969a), and picloram (Sargent and Blackman 1970a). Oxidative phosphorylation might be a significant factor in any observed respiration-dependent uptake in the dark (Kylin 1960, Jennings 1963).

Jyung and Wittwer (1964) recorded the accumulation of rubidium against a concentration gradient, a prerequisite for active uptake. Sargent and Blackman (1965, 1970a, b) and Sargent et al. (1969a) observed an accelerated phase in the time course of penetration in the light, which suggests something more complex than simple diffusion through surface layers of constant permeability.
1.4. The Purpose of the Work

Ever since man has been involved in land management there has been a need to control unwanted vegetation. Mechanical removal is often expensive, and efficient biological control, though ecologically attractive, is not common. The expeditious use of chemicals in weed control has been and remains of vital economic importance. Unfortunately there are often inconsistencies in herbicidal results at different times and in different places. This emphasizes the need for the more efficient use of herbicides. One approach is to develop and test new chemicals, but this may not detract from but add to the problem. Chemicals currently available are more than adequate, and the need is to understand how these work and how to use them most efficiently.

Foliar uptake is a necessary and often limiting factor in the successful foliar application of a herbicide, and an understanding of it may have significant economic repercussions. Reference has been made to the considerable activity in this field, but also to the lack of agreement and gaps in existing knowledge. This research attempts to examine some aspects of foliar uptake to better understand it as a process and to evaluate how various factors affect it. The effects of time, surfactants, light intensity, light quality, temperature, and metabolic inhibitors on the uptake of picloram by eucalypt leaf discs were examined. Results prompted examination of the nature of the leaf surfaces
(particularly surface wax) and their relationships to wettability, and to the role of stomata in the penetration process.

CHAPTER 2

EFFECT OF PERIOD OF TREATMENT ON UPTAKE

2.1. Introduction

Much of the background, rationale, materials and methods described in this chapter refer to the work as a whole. As such, they will be discussed fully here, and referred to or understood subsequently.

The species chosen for this study were Eucalyptus viminalis Labill. and Eucalyptus polyanthemos Schau. Eucalypts were chosen because of their predominance in Australian forests and woodlands; because of difficulties in forest, pasture, and farmland management in controlling unwanted eucalypts; and because very little work of this nature has been done with this genus. E. viminalis and E. polyanthemos were chosen because of their availability, and because they represent two distinct eucalypt leaf types: E. viminalis is a relatively mesomorphic species with non-glaucous foliage; E. polyanthemos is a relatively heteromorphic species with bluish glaucous foliage.

To reduce intraspecific variability, experimental material was collected from the same single adult tree of each species for the duration of this work. Care was taken to collect healthy material of the same
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To reduce intraspecific variability, experimental material was collected from the same single adult tree of each species for the duration of this work. Care was taken to collect healthy material of the same
visual appearance and stage of development. Collections were carried out at the same time of the day (9.30 a.m. - 10.30 a.m.), and only on sunny days. In any one experiment, material was collected at the same time thereby making results directly comparable, but caution is required in interpreting data between experiments because the material was collected on different occasions.

Leaf discs were used to allow stricter control of the factors under study. They are better suited to laboratory scale operations, and particularly suited to the radioassay technique used in this work. They enable attention to be confined to the actual absorbing environment, and eliminate any influence of plant parts remote from it. As interest will be focussed on surface penetration, the use of discs is considered justified. Leaf discs have been used by other workers for measuring uptake phenomena (Sargent and Blackman 1962, 1965, 1969, 1970a, b, Sargent et al. 1969a, b).

Picloram (4-amino-3,5,6-trichloropicolinic acid) was chosen as the test herbicide because it is an auxin type herbicide (Kefferd and Caso 1966, Eisinger 1968) and physiologically interesting; because it is a relatively recent discovery and has not been studied intensively; because it is effective in the control of eucalypts (Bachelard et al. 1965, Bachelard and Sands 1968); and because labelled material was freely available from the Dow Chemical Company.
An examination of the effect of period of treatment on uptake was a logical starting point so that a reasonable time scale for future experiments could be determined.

2.2. Materials and Methods

(1) Preparation and Treatment of Leaf Discs: Two filter paper circles, 9 cm in diameter, were placed in 10 cm petri dishes and saturated with distilled water, any free water being poured off. One glass microscope slide was placed in each petri dish, and ten leaf discs of the one species, 9 mm in diameter, were placed on each slide. The leaf discs were cut from mature leaves of *E. viminalis* and *E. polyanthemos* using a cork borer.

Mature leaves of *E. viminalis* and *E. polyanthemos* are isobilateral, hang vertically from the branches, and have the same number of stomata on either side (see chapter 3). As a result, an upper and a lower side of the leaf cannot be distinguished macroscopically. However, the midvein appears level on one side and it protrudes a little on the other. Where this could be recognized, the former surface was placed uppermost on the glass slide.

After the leaf discs were placed on the slide, clear vinyl tubing 6 mm in length and 3 mm internal diameter was adhered to the centre of each disc with lanolin, making a liquid proof seal. Twenty microlitres of the herbicide solution were injected into this tubing using a micropipette, care being taken to
eliminate any captured air bubbles and to ensure complete contact of the liquid with the leaf surface. The liquid initially extended 2.8 mm up the tubing from the leaf surface.

The lid of the petri dish was replaced to enclose the experimental material in an atmosphere of high humidity and so prevent evaporation of the herbicide solution. The dish could now be placed in any desired external environment.

The herbicide solution in all experiments contained 0.2 per cent (w/v) $^{14}$C-labelled picloram (carboxyl labelled, specific activity 1 mCi/mM) dissolved in 0.05M triethanolamine. In this experiment, Tween 20 (polyoxyethylene sorbitan monolaurate) was added at a concentration of 0.7 per cent (v/v) as surfactant. Twenty microlitres of 0.2 per cent labelled picloram contained 0.166 microcuries of radioactivity, equivalent to $36.85 \times 10^4$ disintegrations per minute.

Petri dishes containing the leaf discs were placed in a controlled temperature room held at $20 \pm 1^\circ C$. One half of the dishes were placed in a cupboard where they received no light; the remaining dishes were exposed to 600 ft. candles of light provided by four 20 watt white fluorescent tubes held 48 cm above the petri dishes placed on aluminium foil. An electric fan directed between the lights and the dishes ensured there was no localized heating by the lights.

Dishes were removed at intervals of 0.1, 0.5, 1, 2, 8, and 24 hours after time of treatment.
Upon removal of the dishes, the vinyl tubing was removed from each disc and all material adhering to the disc was firmly wiped from the surface using facial tissue. The disc surface was then washed with 10 ml of distilled water discharged from a syringe, and the adhering water shaken off. The discs were oven dried at 80°C for 24 hours, and each disc was then broken up finely using a pair of tweezers, wrapped in half a cigarette paper, and stored in a drying cabinet at room temperature.

(2) Radioassay Technique: The technique involved the use of a combustion flask as shown in PLATE 2.1. The broken up disc in cigarette paper (a) was placed in a stainless steel mesh basket (b) under an electrode (c). The self sealing stopper (d) was removed, the 500 ml erlenmeyer flask (e) was flushed out with oxygen, and the stopper quickly replaced. A 12 volt car battery, coil, and condenser were connected to the terminals (f) and (g) to throw a spark between the electrode (c) and basket (b). This spark ignited the disc material in the cigarette paper (a). A bank of ten such combustion flasks on magnetic stirrers allowed ten discs (one treatment) to be handled simultaneously.

At normal temperature and pressure, 500 ml of oxygen is sufficient to convert 250 mg of carbon to carbon dioxide. As the combined weight of disc and

13 mm plug diameter - supplied by A.H. Thomas, P.O. Box 779, Philadelphia, Pennsylvania 19105.
PLATE 2.1. Combustion flask used in radioassay technique.
cigarette paper never exceeded 40 mg, complete combustion was effected and all carbon, including the labelled portion, was converted to carbon dioxide. The temperature increase upon combustion resulted in increased pressure within the flask, and the flask was allowed to stand for twenty minutes to ensure that internal pressure had returned to atmospheric. (For larger amounts of material it is advisable to extend this period, and, after combustion, to place the flask on ice for quick temperature reduction).

After the internal pressure had returned to atmospheric, a six inch needle held by a syringe was pushed through the stopper (d), and two ml of a carbon dioxide absorbent discharged into the counting vial (h). Upon withdrawal of the needle, the self sealing nature of the stopper ensured no gas leak, but vacuum grease was wiped over the puncture area as an additional precaution.

The absorbent was a one:one mixture of phenethylamine (2-phenylethylamine) and anhydrous methanol. The phenethylamine quantitatively absorbed the carbon dioxide to form a carbamate; the methanol increased the solubility of the product and, by reducing the vapour pressure of the amine, minimised the gas phase of this reaction which could give carbamate deposition outside the solution (Woeller 1961). The solution was injected after combustion because of its combustible nature. Woeller (1961) considered that three moles of phenethylamine per mole of carbon dioxide
allowed sufficient stoichiometric excess for the reaction to proceed rapidly. Accepting this proportion, the two ml of absorbent mixture were sufficient for 37 mg of carbon.

The solution in the counting vial (h) was stirred magnetically using a teflon-coated magnetic flea (i)\textsuperscript{1} for 24 hours to ensure complete absorption. The large stopper (j) was then removed, and the vial (h) screwed out of its holder (k). Twelve ml of 0.5 per cent P.P.O. (2,5-diphenyloxazole) in toluene were added to the vial as a scintillant and magnetically stirred for five minutes.

The contents of the vial, including the flea, were then counted for carbon-14 using a Beckman LS-100 scintillation counter. Quench was relatively constant and external standard readings showed little variability. Within the range recorded, differences in counting efficiency were negligible and a constant efficiency of 79 per cent was accepted. This meant that uptake figures from all experiments could be compared with a total possible uptake of $29.11 \times 10^4$ disintegrations per minute.

2.3. Results and Discussion

The uptake of picloram with time after treatment is shown in FIG. 2.1. Uptake was measured as counts per minute (c.p.m.) and shown as c.p.m. $\times 10^{-4}$. The vertical lines through the mean symbol represent twice

\footnote{10 mm without raised central rim – supplied by John Morris Pty Ltd, P.O. Box 80, Chatswood, N.S.W., 2067.}
FIG. 2.1. Effect of time after application on uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs in light and dark. (Surfactant: Tween 20).
the standard error of the mean, and where no lines are evident, the standard error lies within the dimensions of the symbol. (This convention was adopted for all graphical figures in this study).

Uptake of picloram by *E. viminalis* leaf discs occurred at a similar rate in light and dark for the first two hours, after which it became relatively less in the dark and reached a maximum after about eight hours. Uptake increased in light up to 24 hours after treatment when it was approximately 2.5 times that in the dark.

Uptake by *E. polyanthemos* in the light increased slightly over the first hour after which there was no evidence of greater uptake. Uptake in the dark was very poor throughout the 24 hour period and, at the conclusion of the experiment, uptake in light was over 20 times that in the dark.

Within each species, light stimulated uptake: this is examined in considerable detail later. Between species, uptake by *E. viminalis* was considerably greater than by *E. polyanthemos* in both light and dark under the conditions used in this experiment.

In three of the four situations examined, the exception being *E. viminalis* leaf discs in the light, uptake had reached its maximum value within the 24 hour treatment period. Even with *E. viminalis* in the light, the uptake after 24 hours reached a value equivalent to 64 per cent of its theoretical maximum, and a treatment period of 24 hours was chosen as the standard for all subsequent experiments.
EFFECT OF SURFACTANTS AND NATURE OF THE LEAF SURFACES

3.1. Surfactant Type

Surfactants may be effective in increasing both uptake and efficiency of foliar-applied herbicides. The effect of several surfactants on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs was examined.

Leaf discs from both species were cut and plated out as described previously, and 20 microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc. Treatments consisted of six surfactants and dimethyl sulfoxide (DMSO). DMSO is a solubilizer rather than a surfactant but was included here (also at 0.7 per cent) for convenience. The surfactants were (1) Arquad 12/50 (cationic, lauryl tri-methyl ammonium chloride), (2) Kemmat QC-23 (cationic, coco-dimethyl benzyl ammonium chloride), (3) Kemmat SC-15 (anionic, calcium dodecyl benzene sulphonate), (4) Decol T/70 (anionic, triethanolamine dodecyl benzene sulphonate), (5) Aerosol 909 (nonionic, nonyl phenol condensed with 2 moles of ethylene oxide per mole of phenol), and (6) Tween 20 (nonionic, polyoxyethylene sorbitan monolaurate).

*Note*: Tween 20 was purchased from Tokyo Kasei Kogyo Co. Ltd, Tokyo, Japan. The other surfactants were donated by Chemical Materials Pty Ltd, Federal Road, Glebe, N.S.W. 2037.
triethanolamine dodecyl benzene sulphonate), (5) Kemonic 909 (nonionic, nonyl phenol condensed with 9 moles of ethylene oxide per mole of phenol), and (6) Tween 20 (nonionic, polyoxyethylene sorbitan monolaurate). A control treatment containing no surfactant was included, and there were ten discs per treatment. The treatments were kept for 24 hours in the light (600 ft. candles) at 20°C, after which the discs were wiped, washed, oven dried, and radioassayed as described previously.

The results are shown in FIG. 3.1. The single line through the middle of the top of each bar represents twice the standard error of the mean. (This convention was adopted for all bar type figures used in this work).

In E. viminalis, Kemmat SC-15 gave the greatest increase in uptake of picloram (over 2 times that with no surfactant) followed closely by Kemonic 909 and Decol T/70. Arquad 12/50, Kemmat QC-23, and Tween 20 gave comparable uptakes (over 1.5 times that with no surfactant), and DMSO gave the same uptake as with no surfactant.

In E. polyanthemos, Decol T/70 gave the greatest increase in uptake (over 2.5 times that with no surfactant), followed closely by Kemmat SC-15. Arquad 12/50 and Kemonic 909 gave comparable uptakes (nearly 2 times that with no surfactant), and Kemmat QC-23 had a comparable value to that with no surfactant. Uptake with Tween 20 was less than with no surfactant, and uptake with DMSO was very much inhibited.
FIG. 3.1. Effect of surfactants on the uptake of picloram by E. viminalis and E. polyanthemos leaf discs in the light.
There was no consistent relationship between the ionic class of the surfactant and the uptake of picloram, except that Decol T/70 and Kemmat SC-15, both anionic surfactants, were among the most efficient in both species. In any case, too few surfactants were tested to allow generalizations of this kind.

3.2. Surfactants and Wettability

Possible mechanisms of surfactant action were reviewed in chapter 1. The general consensus of opinion is that the capacity of surfactants to lower surface tensions, and their ability to increase wettability (as determined by contact angle measurements) of solutions on leaf surfaces could not fully explain surfactant action, but that more complex species-herbicide-surfactant interactions were involved. It was of interest to determine to what extent uptake of picloram in various surfactant solutions by *E. viminalis* and *E. polyanthemos* was related to the surface tension of such solutions and their wettability on the leaf surfaces.

Picloram solutions containing surfactants, DMSO, and no surfactant, identical to those used in 3.1. were prepared, except that unlabelled picloram of equivalent purity was used. The surface tensions of the solutions were measured at 20°C with a Du Nuöy tensiometer.¹ Leaf material from both species representative of that used in 3.1. was collected, and a drop of herbicide-

surfactant solution was allowed to form on the end of a micropipette and was deposited on the leaf surface. For each solution, two contact angles per leaf, on each of five leaves from both species, were measured at 20°C. Except as mentioned later, contact angles were measured with a goniometer. The construction and application of this instrument was described by Fort and Patterson (1963); it depends on the geometrical properties of reflected light. It is based on the principle that the reflection of a point source of light directed at a droplet surface, and moving across it, is extinguished at the droplet-leaf surface junction. The angle between the vertical and the direction of the point source at the point at which this extinction occurs, is the contact angle. This is a simple and quick method for measuring contact angles with a satisfactory degree of reproducibility. However, the goniometer cannot measure contact angles greater than 90°, and therefore was unsuitable for the measurement of the contact angles of solutions containing no surfactant, Tween 20, and DMSO on E. polyanthemos, where angles in excess of 90° were observed.

For these situations a photographic technique was used. The droplets were photographed\(^1\), at 1:1 magnification, on a horizontal leaf surface with the principal axis of the lens horizontal and passing with a Nikon F Photomic 35 mm single lens reflex camera fitted with a Micro-Nikkor Auto F:3.5, f = 55 mm lens and M-ring.
through the vertex of the required contact angle. Enlargements of these photographs were printed and the contact angles were read using a protractor. It was observed that, with some solutions, the contact angle noticeably lessened with time. In the absence of further data on this phenomenon, all contact angles (both measured with the goniometer and by photography) were measured at an arbitrarily chosen 50 seconds after the deposition of solution on the leaf surface, this being a satisfactory time period for the efficient use of the goniometer.

Surface tension (mean of five), contact angle (mean of ten), and uptake of picloram (from data in FIG. 3.1.) corresponding to the various surfactants used are shown, for both species, in TABLE 3.1. These data are arranged, by species, in ascending order of magnitude of uptake of picloram. In general as uptake increased, surface tension and contact angle decreased in both species. The relationship between contact angle and uptake is shown graphically in FIG. 3.2. where a trend towards an inverse linear correlation of uptake on contact angle, particularly in E. viminalis, is apparent. Coefficients of linear correlation of uptake on surface tension, of uptake on contact angle, and of contact angle on surface tension calculated using the means values in TABLE 3.1., are shown in TABLE 3.2.
TABLE 3.1. Effect of surfactants on the surface tension of picloram solutions, the wettability of *E. viminalis* and *E. polyanthemos* leaf discs, and uptake of picloram.

### E. VIMALINIS

<table>
<thead>
<tr>
<th>SURFACANT</th>
<th>SURFACE TENSION ± S.E. (dynes cm.⁻¹)</th>
<th>CONTACT ANGLE ± S.E. (degrees)</th>
<th>UPTAKE ± S.E. (c.p.m. x 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No surfactant</td>
<td>66.90 ± 0.03</td>
<td>54.5 ± 2.8</td>
<td>10.66 ± 2.67</td>
</tr>
<tr>
<td>DMSO</td>
<td>63.10 ± 0.17</td>
<td>58.1 ± 2.6</td>
<td>10.68 ± 2.84</td>
</tr>
<tr>
<td>Tween 20</td>
<td>40.10 ± 0.04</td>
<td>28.3 ± 2.2</td>
<td>17.01 ± 0.90</td>
</tr>
<tr>
<td>Arquad 12/50</td>
<td>34.22 ± 0.02</td>
<td>29.0 ± 1.3</td>
<td>17.08 ± 0.76</td>
</tr>
<tr>
<td>Kemmat QC-23</td>
<td>35.14 ± 0.06</td>
<td>34.9 ± 1.6</td>
<td>17.37 ± 1.73</td>
</tr>
<tr>
<td>Decol T/70</td>
<td>31.80 ± 0.05</td>
<td>21.4 ± 1.1</td>
<td>20.81 ± 0.57</td>
</tr>
<tr>
<td>Kemonic 909</td>
<td>32.02 ± 0.02</td>
<td>19.7 ± 1.7</td>
<td>21.54 ± 0.56</td>
</tr>
<tr>
<td>Kemmat SC-15</td>
<td>29.10 ± 0.03</td>
<td>16.2 ± 1.5</td>
<td>22.85 ± 0.51</td>
</tr>
</tbody>
</table>

### E. POLYANTHEMOS

<table>
<thead>
<tr>
<th>SURFACANT</th>
<th>SURFACE TENSION ± S.E. (dynes cm.⁻¹)</th>
<th>CONTACT ANGLE ± S.E. (degrees)</th>
<th>UPTAKE ± S.E. (c.p.m. x 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>63.10 ± 0.17</td>
<td>117.1 ± 1.2</td>
<td>0.63 ± 0.12</td>
</tr>
<tr>
<td>Tween 20</td>
<td>40.10 ± 0.04</td>
<td>115.2 ± 2.2</td>
<td>3.67 ± 1.25</td>
</tr>
<tr>
<td>Kemmat QC-23</td>
<td>35.14 ± 0.06</td>
<td>62.2 ± 3.2</td>
<td>8.22 ± 0.98</td>
</tr>
<tr>
<td>No surfactant</td>
<td>66.90 ± 0.03</td>
<td>114.4 ± 1.7</td>
<td>9.70 ± 3.75</td>
</tr>
<tr>
<td>Arquad 12/50</td>
<td>34.22 ± 0.02</td>
<td>56.0 ± 3.8</td>
<td>18.38 ± 1.16</td>
</tr>
<tr>
<td>Kemonic 909</td>
<td>32.02 ± 0.02</td>
<td>54.6 ± 2.2</td>
<td>18.87 ± 1.19</td>
</tr>
<tr>
<td>Kemmat SC-15</td>
<td>29.10 ± 0.03</td>
<td>15.5 ± 2.0</td>
<td>22.08 ± 0.80</td>
</tr>
<tr>
<td>Decol T/70</td>
<td>31.80 ± 0.05</td>
<td>15.8 ± 1.7</td>
<td>24.45 ± 0.50</td>
</tr>
</tbody>
</table>
FIG. 3.2. Relation between leaf wettability (contact angle) and uptake of picloram by E. viminalis and E. polyanthemos leaf discs. No surfactant (△), DMSO (Ο), Arquad 12/50 (Ο), Kemmat QC-23 (●), Kemmat SC-15 (□), Decol T/70 (■), Kemonic 909 (△), Tween 20 (▲).
TABLE 3.2. Coefficients of linear correlation of $Y$ on $X$ calculated from surfactant data in TABLE 3.1.

**E. VIMINALIS**

<table>
<thead>
<tr>
<th>$X$</th>
<th>$Y$</th>
<th>CORRELATION COEFFICIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface Tension</td>
<td>Uptake</td>
<td>-0.94***</td>
</tr>
<tr>
<td>Contact Angle</td>
<td>Uptake</td>
<td>-0.98***</td>
</tr>
<tr>
<td>Surface Tension</td>
<td>Contact Angle</td>
<td>0.95***</td>
</tr>
</tbody>
</table>

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

**E. POLYANTHEMOS**

<table>
<thead>
<tr>
<th>$X$</th>
<th>$Y$</th>
<th>CORRELATION COEFFICIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface Tension</td>
<td>Uptake</td>
<td>-0.68*</td>
</tr>
<tr>
<td>Contact Angle</td>
<td>Uptake</td>
<td>-0.91**</td>
</tr>
<tr>
<td>Surface Tension</td>
<td>Contact Angle</td>
<td>0.81*</td>
</tr>
</tbody>
</table>

In Decol T/70 was a particularly effective surfactant in both species, though not nearly as impressive, has been widely in laboratory research. For these reasons, and in the hope of better understanding the opposing nature of the species-surfactant interactions mentioned above, Decol T/70 and Tween 20 were used as surfactants for the rest of this work.
Uptake and contact angle were highly negatively correlated in both species. This means that uptake was highly positively correlated with wettability (which increases with decreasing contact angle). Uptake was highly negatively correlated with surface tension in *E. viminalis*, but was less so in *E. polyanthemos*. Similarly contact angle was highly positively correlated with surface tension in *E. viminalis*, but was less so in *E. polyanthemos*.

Though uptake correlated very well with wettability in both species yet, consistent within this relationship, there was evidence of a surfactant-species interaction in that some surfactants behaved differently in both species. This was reflected in the fact that uptake was better correlated with contact angle than with surface tension, and is best illustrated in FIG. 3.2. An example is that of Tween 20 where with *E. viminalis*, the contact angle was low and the uptake high, but with *E. polyanthemos* the contact angle was high and the uptake low. The opposite situation occurred with Decol T/70 where the contact angle was low and the uptake high in *E. polyanthemos*, but the contact angle was higher and the uptake lower in *E. viminalis*.

Decol T/70 was a particularly effective surfactant in both species, and Tween 20, though not nearly as impressive, has been used widely in herbicide research. For these reasons, and in the hope of better understanding the opposing nature of the species-surfactant interactions mentioned above, Decol T/70 and Tween 20 were used as surfactants for the rest of this work.
3.3. - Surfactant Concentration

The effect of surfactant concentration on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs was examined.

Leaf discs of both species were cut and plated out as described previously. Twenty microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with added surfactant, were applied to each disc. Treatments consisted of five surfactant concentrations (0.2%, 0.4%, 0.7%, 1.0%, and 1.8%) for each of three surfactant types (Arquad 12/50, Decol T/70, Tween 20), chosen as representative of cationic, anionic, and nonionic classes respectively. A control treatment having no surfactant was included, and there were ten discs per treatment. Treatments were for 24 hours in the light (600 ft. candles) at 20°C, after which the discs were wiped, washed, oven dried, and radioassayed as described previously.

The results are shown in FIG. 3.3. The data for *E. viminalis* showed the control (no surfactant) had quite a high uptake value, and no definite surfactant effect when compared to control was obvious. Variability was high and there was no consistent trend in uptake with increasing surfactant concentration. However, results with *E. polyanthemos* were much more consistent over the range, and stimulation of uptake in the presence of all surfactants over that of the control was marked.
FIG. 3.3. Effect of surfactant concentration on uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs in the light. Tween 20 (■), Arquad 12/50 (▲), Decol T/70 (●).
Maximum surfactant efficiency was reached in the concentration range 0.4% to 0.7% for Arquad 12/50 and Tween 20, and 0.2% for Decol T/70. Further surfactant additions caused little effect, and a surfactant concentration of 0.7% was chosen for all future experiments in this work. Uptake, in general, decreased in the order Decol T/70, Arquad 12/50, and Tween 20.

3.4. Surfactant Concentration and Wettability

It was observed (section 3.2.) that contact angle correlated very well with uptake: differences in wettability here were achieved by using different surfactants all at a concentration of 0.7 per cent. It was decided to see to what extent the variation in uptake caused by different concentrations of a particular surfactant was related to differences in wettability. Differences in uptake of picloram by E. polyanthemos from solutions containing Arquad 12/50 at concentrations between 0.0 per cent and 1.0 per cent (v/v) were quite marked (FIG. 3.3.), and this provided a suitable uptake range for this experiment.

Contact angles of solutions containing 0.2 per cent unlabelled picloram in 0.05M triethanolamine with 0.0%, 0.2%, 0.4%, 0.7%, and 1.0% Arquad 12/50 on E. polyanthemos leaves were determined using the photographic technique described in section 3.2. One angle on each of ten leaves for each surfactant concentration was measured 50 seconds after droplet formation and at 20°C. The surface tension of each of the solutions was also measured at 20°C using a Du Nuoy tensiometer.
These results and the corresponding uptake data are shown in TABLE 3.3. The surface tensions of the picloram solutions containing 0.2%, 0.4%, 0.7%, and 1.0% Arquard 12/50 were similar and much lower than that of the solution containing no surfactant. Contact angle decreased from its initially high value with no surfactant to a minimum at 0.7 per cent after which it increased again. This corresponded with an increase in uptake up to 0.7 per cent surfactant after which it decreased. The coefficient of linear correlation of uptake on surface tension was -0.83, whereas that of uptake on contact angle was -0.91* and significant at the 0.05 probability level. The observed differences in uptake were therefore well correlated with wettability and less so with surface tension.

3.5. Contact Angle Variation with Time

It was observed in section 3.2. that contact angles of some solutions noticeably lessened with time. In a wettability experiment to be described later (section 3.6.), contact angle measurements of picloram solutions containing no surfactant, Decol T/70, and Tween 20 on leaf surfaces of _E. viminalis_ and _E. polyanthemos_ were required, and therefore a preliminary investigation of the variation of such contact angles with time after droplet formation was carried out. Such an investigation could establish a sensible time period after droplet formation at which to measure the contact angle, and perhaps could give further insight into the mechanism of surfactant action and the nature of the species-surfactant interactions involved.
TABLE 3.3. Effect of different concentrations of Arquad 12/50 on the surface tension of picloram solutions, the wettability of *E. polyanthemos* leaf discs, and uptake of picloram.

<table>
<thead>
<tr>
<th>CONCENTRATION (per cent)</th>
<th>SURFACE TENSION ± S.E. (dynes cm(^{-1}))</th>
<th>CONTACT ANGLE ± S.E. (degrees)</th>
<th>UPTAKE ± S.E. (c.p.m. x 10(^{-4}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>66.90 ± 0.03</td>
<td>133.4 ± 0.3</td>
<td>1.93 ± 0.55</td>
</tr>
<tr>
<td>0.2</td>
<td>33.48 ± 0.10</td>
<td>72.5 ± 2.0</td>
<td>11.39 ± 2.57</td>
</tr>
<tr>
<td>0.4</td>
<td>32.38 ± 0.04</td>
<td>66.8 ± 3.0</td>
<td>22.12 ± 0.53</td>
</tr>
<tr>
<td>0.7</td>
<td>34.22 ± 0.02</td>
<td>56.7 ± 2.1</td>
<td>25.52 ± 0.32</td>
</tr>
<tr>
<td>1.0</td>
<td>34.20 ± 0.03</td>
<td>66.2 ± 4.1</td>
<td>19.36 ± 1.37</td>
</tr>
</tbody>
</table>
Three solutions were prepared each containing 0.2 per cent unlabelled picloram in 0.05M triethanolamine and with (1) no surfactant, (2) 0.7 per cent Tween 20, and (3) 0.7 per cent Decol T/70. Contact angles at 20°C of these solutions on each of two leaves of both species were photographed (as described in section 3.2.) at intervals of 5 seconds up to 100 seconds, then of 10 seconds up to 180 seconds, and thereafter of 30 seconds up to 300 seconds after the formation of the droplet on the leaf surface.

The results are shown in FIGS 3.4. and 3.5. The greatest contact angles on both species were with solutions containing no surfactant, and the least with those containing Decol T/70; those with Tween 20 had intermediate values.

All contact angles on E. viminalis decreased with time, and had a relatively greater rate of decrease up to about 30-50 seconds after which the rate of decrease was slower. After 300 seconds, the initial contact angle of solutions with no surfactant was reduced by 13° and 15°30', with Tween 20 by 30°30' and 38°, and with Decol T/70 by 26°30' and 10° on leaves A and B respectively.

Contact angles of solutions containing no surfactant and Tween 20 on E. polyanthemos were very high (considerably higher than on E. viminalis), were practically the same on leaves A and B, and were constant with time up to 300 seconds. Contact angles of the solution containing Decol T/70 fell rapidly with time, and after 300 seconds the initial contact angle value
FIG. 3.4. Variation with time of contact angle of picloram solutions containing no surfactant (••••••), Tween 20 (oooooo), and Decol T/70 (----), on two leaves (A. and B.) of *E. viminalis*. 
FIG. 3.5. Variation with time of contact angle of picloram solutions containing no surfactant (●●●●●), Tween 20 (○○○○○), and Decol T/70 (-----), on two leaves (A. and B.) of E. polyanthemos.
was reduced by 43° and 55°30' on leaves A and B respectively. Initially, the contact angle of the solution containing Decol T/70 was higher on *E. polyanthemos* than *E. viminalis*, but this difference became less with time and at 300 seconds the contact angle was less on *E. polyanthemos*. Other than this, the relativity between treatments was maintained over the time range.

Although the contact angle of the solution containing Decol T/70 on *E. polyanthemos* was still falling slightly after 100 seconds, contact angles were measured 100 seconds after droplet formation in all future work. Times in excess of this caused the leaf material to be used late in the experiment to become wilted and unrepresentative.

3.6. Leaf Surface Wax and Wettability

In chapter 1 it was reviewed how the amount, the physical and chemical nature, and particularly the configuration of the leaf surface wax could affect the wettability of solutions on leaf surfaces. The extent to which the surface wax of the eucalypts used in this study was responsible for wettability and uptake behaviour was of interest. The effect of mechanical and chemical (chloroform) removal of surface wax on the wettability of picloram solutions containing no surfactant, Tween 20, and Decol T/70 on *E. viminalis* and *E. polyanthemos* leaf surfaces, and on the uptake of picloram, was therefore examined.
Leaves from both species were collected. A single leaf was divided into three sections, and each subjected to one of the following three treatments:

1. the leaf section surface was left untreated (control);
2. the leaf section surface was gently but firmly wiped ten times with cotton wool in an attempt to remove some of the surface wax (mechanical removal); and
3. the leaf section was immersed in chloroform for three seconds to extract some or all of the surface wax (CHCl₃ extraction). A leaf disc was cut from each section and plated out as described previously, and the remainder of the section was used for contact angle determinations.

Ten leaves of each species were so treated for each of three subsequent picloram solution treatments. These solutions contained 0.2 per cent picloram in 0.05M triethanolamine and with (1) no surfactant, (2) 0.7 per cent Tween 20, and (3) 0.7 per cent Decol T/70. Labelled and unlabelled picloram were used for the uptake and contact angle determinations respectively. Twenty microlitres of the appropriate solution were added to each disc and the treated discs kept for 24 hours in the light (600 ft. candles) at 20°C, after which they were wiped, washed, oven dried, and radioassayed. Two contact angles of the appropriate solution on the remaining portion of each leaf section were measured using the photographic technique described in section 3.2. These angles were photographed 100 seconds after their formation on the leaf surface.
The uptake (mean of 10) and contact angle (mean of 20) corresponding to each treatment is shown in TABLE 3.4. Chloroform extraction did not reduce the contact angle on *E. viminalis*, but significantly reduced it on *E. polyanthemos*. Except for *E. viminalis* with no surfactant and *E. polyanthemos* with Decol T/70 where no difference was recorded, uptake of picloram after chloroform extraction of surface wax was markedly reduced compared with that following mechanical wax removal.

The data on mechanical removal compared to control are shown diagramatically in FIG. 3.6.

With *E. viminalis*, mechanical removal did not alter contact angle or uptake. Contact angle decreased and uptake increased in the order no surfactant, Tween 20, and Decol T/70. The coefficient of linear correlation of individual uptake values on the corresponding contact angle values (mean of two) for the control and mechanical removal treatments was $-0.40^{**}(p<0.01)$.

With *E. polyanthemos*, mechanical removal decreased contact angle and there was a corresponding increase in uptake. As with *E. viminalis*, contact angle decreased and uptake increased in the order no surfactant, Tween 20, and Decol T/70, but this difference was more marked in *E. polyanthemos*. The contact angle reduction caused by surfactants was greater than that caused by mechanical removal (or chloroform extraction) of surface wax. The coefficient of linear correlation of individual uptake values on the corresponding contact
TABLE 3.4. Effect of mechanical (mech.) removal and chloroform extraction of wax on wettability (contact angle) of E. viminalis and E. polyanthemos leaf surfaces, and on the uptake of picloram.

**E. VIMINALIS**

<table>
<thead>
<tr>
<th>CONTACT ANGLE ± S.E.</th>
<th>UPTAKE ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Degrees)</td>
<td>(c.p.m. x 10^-4)</td>
</tr>
<tr>
<td><strong>NO SURFACTANT</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>65.6 ± 1.2</td>
</tr>
<tr>
<td>Mech. Removal</td>
<td>61.2 ± 2.9</td>
</tr>
<tr>
<td>CHCl₃ Extraction</td>
<td>64.1 ± 2.7</td>
</tr>
<tr>
<td><strong>TWEEN 20</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40.1 ± 1.6</td>
</tr>
<tr>
<td>Mech. Removal</td>
<td>43.1 ± 2.3</td>
</tr>
<tr>
<td>CHCl₃ Extraction</td>
<td>47.9 ± 1.7</td>
</tr>
<tr>
<td><strong>DECOL T/70</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.8 ± 0.9</td>
</tr>
<tr>
<td>Mech. Removal</td>
<td>24.7 ± 1.2</td>
</tr>
<tr>
<td>CHCl₃ Extraction</td>
<td>20.3 ± 1.9</td>
</tr>
</tbody>
</table>

**E. POLYANTHEMOS**

<table>
<thead>
<tr>
<th>CONTACT ANGLE ± S.E.</th>
<th>UPTAKE ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Degrees)</td>
<td>(c.p.m. x 10^-4)</td>
</tr>
<tr>
<td><strong>NO SURFACTANT</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>135.1 ± 1.1</td>
</tr>
<tr>
<td>Mech. Removal</td>
<td>124.0 ± 1.9</td>
</tr>
<tr>
<td>CHCl₃ Extraction</td>
<td>121.9 ± 1.7</td>
</tr>
<tr>
<td><strong>TWEEN 20</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>123.5 ± 1.3</td>
</tr>
<tr>
<td>Mech. Removal</td>
<td>101.5 ± 2.2</td>
</tr>
<tr>
<td>CHCl₃ Extraction</td>
<td>96.0 ± 3.0</td>
</tr>
<tr>
<td><strong>DECOL T/70</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.1 ± 1.4</td>
</tr>
<tr>
<td>Mech. Removal</td>
<td>10.3 ± 0.9</td>
</tr>
<tr>
<td>CHCl₃ Extraction</td>
<td>complete wettability</td>
</tr>
</tbody>
</table>
FIG. 3.6. Effect of mechanical (mech.) removal of wax on wettability (contact angle) of E. viminalis and E. polyanthemos leaf surfaces, and on the uptake of picloram.
angle values (mean of two) for the control and mechanical removal treatments was \(-0.90^{***}(p<0.001)\).

3.7. The Nature of the Leaf Surfaces

The nature of the leaf surfaces, particularly their surface wax and stomatal characteristics, was examined to determine (1) the morphological differences between the species, and the nature and extent of the mechanical removal and chloroform extraction of surface wax as performed in section 3.6., and (2) whether or not the different picloram-surfactant solutions used in 3.5. and 3.6. altered the nature of the leaf surfaces.

(1) Leaf Surface Morphology and Wax Removal Treatments: Leaves from both species were collected and subjected to one of the three wax removal treatments (control, mechanical removal, and chloroform extraction) as described in 3.6., and then prepared for observation by transmission and scanning electron microscopy.

For transmission electron microscopy, carbon replicas of the leaf surfaces were prepared using the technique described by Armstrong (1969) which was a simplification of the original technique described by Juniper and Bradley (1958). A film of 50 per cent (v/v) bedacryl 122x\(^1\) in benzene was spread over a glass slide with a glass rod, and immediately, leaf sections approximately 15 mm x 5 mm were adhered to this film with the treated side up. The slide was stored in a dust

\(^1\) manufactured by I.C.I. Dyestuffs Division, 1 Nicholson Street, Melbourne, 3000.
free environment for 24 hours to allow the film to dry completely. The sections on the slide were shadowed under vacuum at an angle of 45 degrees with platinum/carbon, and then coated under vacuum by carbon evaporation from directly above. The sections on the slide were then flooded with 15 per cent (v/v) bedacryl 122x in benzene and the slides allowed to drain. After this film had dried (24 hours plus), grid size sections were scored through the plastic and into the leaf tissue with a razor blade. This section of plastic with the adhering carbon film was removed from the leaf section with a pair of fine tweezers, and placed carbon side down on a grid placed on top of a series of filter papers kept continually saturated with acetone. After two minutes, when most of the plastic had dissolved, the film was floated off the grid into A.R. acetone for a further two minutes. This was repeated in a second acetone wash to remove the final traces of plastic, and the replica was picked up on a fresh 200 mesh supporting grid and examined at 60 KV under the electron microscope.

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1 coated using a 'Speedivac' coating unit, Model 12E6/1128, manufactured by Edwards High Vacuum Ltd, Manor Royal, Crawley, Sussex.


3 Japan Electron Optics Laboratory, model JEM/T6, 70 KV.
For each treatment at least eight leaf sections from each of ten leaves of both species were prepared. Carbon replicas from most sections and from all leaves were observed, and many electron micrographs were taken. Electron micrographs were taken on Ilford N.50 plates. The carbon replicas were a negative of the leaf surface, and therefore the developed plates were a positive. The plates were reversed by contact printing on Ilford fine grain ordinary N5.31 film to form negatives from which the final prints were made. The variation within each treatment was small, and an average electron micrograph for each treatment was subjectively chosen.

These are shown in PLATE 3.1. The difference in surface wax between *E. viminalis* and *E. polyanthemos* was quite marked. *E. viminalis* had a scattered distribution of plate-like wax structures, whereas *E. polyanthemos* was much more waxy and had a continuous dense distribution of rod-like wax structures overlying plate-like formations. This basic difference is not surprising in view of the fact that, macroscopically, *E. viminalis* has green non-glaucous foliage, and *E. polyanthemos* has bluish glaucous foliage. Surface wax on *E. viminalis* was removed mechanically and chemically, though the amount of removal was slight there being little wax there to start with. Large amounts of wax were removed both mechanically and chemically from the surface of *E. polyanthemos*. 
PLATE 3.1. Transmission electron micrographs of carbon replicas of *E. viminalis* (V) and *E. polyanthemos* (P) leaf surfaces. (6500 x).

1 = control (wax untouched), 2 = mechanical removal of wax, 3 = chloroform extraction of wax.
Scanning electron microscopy had some advantages over the carbon replica technique for the purpose of this work in that, as well as being an inherently simple technique, the actual surface was seen, larger and more grossly structured areas could be examined, stomata could be faithfully reproduced, and stero pairs could be obtained for three dimensional study. Nine mm diameter discs of treated leaves were cut with a cork borer, quickly dipped into liquid nitrogen, and freeze dried. This reduced the likelihood of the leaf surface, particularly the stomata, changing form during the drying process. The discs were then adhered with Tarzan's Grip\(^1\) to a microscopic specimen stub with the treated side up, coated under vacuum with gold/palladium, and viewed through a scanning electron microscope.\(^2\)

Because of the very considerable hire charge on this instrument, only two discs per treatment were prepared and observed. The nature of the leaf surface appeared to be uniform over the area of any one disc and, within a treatment, there was no discernible difference between the two discs.

Representative scanning electron micrographs for each treatment are shown in PLATE 3.2. These showed the same differences in surface wax between the species as shown in the transmission electron micrographs. They

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\(^1\) manufactured by Tarzan's Grip Manufacturing Co. Pty Ltd, 451 Willoughby Road, Willoughby, N.S.W., 2068.

\(^2\) JSM-U3 Scanning Electron Microscope, manufactured by Japan Electron Optics Laboratory Co. Ltd.
PLATE 3.2. Scanning electron micrographs of *E. viminalis* (V) and *E. polyanthemos* (P) leaf surfaces. (1200 x).

1 = control (wax untouched), 2 = mechanical removal of wax, 3 = chloroform extraction of wax.
also showed that leaf surfaces of both species were undulating and consisted of 'ridges' and 'gullies' with a relief of greater scale than the configurational nature of the wax components. Most of the plate-like wax on *E. viminalis* was found in the gullies and, in *E. polyanthemos*, there was some abrasion of wax on the ridge tops, even in the control treatments.

The scanning electron micrographs again showed large amounts of wax were removed mechanically and chemically from the leaf surface of *E. polyanthemos*, but not from the leaf surface of *E. viminalis*. In *E. polyanthemos*, mechanical removal of wax was observed principally on the ridge areas with the wax in the gullies often being untouched, whereas chloroform removed large amounts of wax from both the ridges and gullies. Wax was mechanically and chemically removed from the area surrounding the stomata, the chemical removal here being particularly efficient.

Although it is obvious from the transmission and scanning electron micrographs that the chloroform treatment may dissolve much of the wax, it is not completely clear whether or not all the dissolved wax was removed, or whether some was redeposited as a fine film continuous over the leaf surface. Such a surface could be more wettable but would also be less penetrable by hydrophilic molecules. This could account for the lowered uptake following the chloroform treatment of some leaf discs. This was examined using the dye Sudan IV.
Sudan IV (Scarlet Red) is used as a fat stain (Gurr 1963) and can stain the hemihydrophilic cutin, but it cannot stain the extremely apolar surface and cuticular waxes (Sitte and Rennie 1963). The use of this stain therefore should give some indication of the amount of surface wax present on a leaf, and the extent to which it hinders the staining of the cutin in the underlying cuticle. Leaves of both species were selected and divided into three sections. Each section was treated with one of each of the three wax removal treatments described previously (i.e. control, mechanical removal, and chloroform extraction). These leaf sections were then immersed in 0.2 per cent (w/v) Sudan IV in 70 per cent ethanol in water for 15 minutes, after which the differential degree of staining was observed. A representative leaf of each species is shown in colour in PLATE 3.3.

In *E. viminalis*, all treatments were well stained by Sudan IV. The control and mechanical removal treatments had an equal intensity of staining, and the chloroform extraction treatment was stained a little more. In *E. polyanthemos*, there was a gradation in intensity of staining from lightly stained in the control, through a medium intensity in the mechanical removal, to heavily stained in the chloroform extraction. As the chloroform extraction treatments were stained most heavily, this indicates that there was no barrier, such as a fine continuous wax film, to the penetration of the stain to the cutin in the cuticle.
PLATE 3.3. *E. viminalis* (V) and *E. polyanthemos* (P) leaf surfaces stained with Sudan IV. (actual size)
1 = control (wax untouched), 2 = mechanical removal of wax, 3 = chloroform extraction of wax.
The intensity of staining in the chloroform extraction was comparable between the species, but the intensity of the control and mechanical removal treatments was less in _E. polyanthemos_ than _E. viminalis_. This suggests that _E. polyanthemos_ was initially more waxy than _E. viminalis_, and that mechanical removal and particularly chloroform extraction of surface wax was much more obvious in _E. polyanthemos_. The staining therefore supports what is shown in the electron micrographs.

The nature and distribution of the stomata were of interest because of their possible effect on overall wettability, and their possible role as portals of entry for applied solutions.

Stereo pairs of scanning electron micrographs of representative stomata on untreated leaf surfaces of both species are shown in PLATE 3.4. for observation with a stereoscope. In the right hand photographs the leaf surface was untilted in the microscope, but in the left hand photographs it was tilted ten degrees.

The stomata and surrounds were morphologically different between the species. _E. viminalis_ stomata were surrounded by a massive doughnut shaped raised rim. This encroached on to the stomatal area to the extent that only a portion of the guard cells adjacent to the aperture was visible, and either end of the slit like aperture was often hidden. _E. polyanthemos_ had slightly sunken stomata with no rim like structure as in _E. viminalis_, and a greater area of the guard cells adjacent to the aperture was visible.
PLATE 3.4. Stereo pairs of scanning electron micrographs of *E. viminalis* and *E. polyanthemos* stomata. (1250 x).
Stomatal densities on both sides of *E. viminalis* and *E. polyanthemos* leaves were determined using a replica technique described by Zelitch (1961). Silicon rubber\(^1\) was mixed with sufficient catalyst\(^2\) to set about ten minutes after being spread over the leaf surface as a film. The rubber was then peeled off the leaf surface, and cellulose acetate (clear nail polish) brushed thinly but continuously over the negative impression of the leaf surface on the rubber. The acetate film, when dry, was peeled off the rubber, mounted in a water mounting medium\(^3\) replica side up on a glass slide, observed through a light microscope at 40x magnification, and the number of stomata per field of 0.36 mm diameter recorded. Stomata were counted in five different fields on each side of ten leaves of both species. The results, shown as stomata per mm\(^2\), are given in TABLE 3.5. Side A represented the side with no midvein protruding, and side B the side with the midvein slightly protruding, as discussed in chapter 2. The stomatal density on *E. polyanthemos* leaves was approximately 75 per cent of that on *E. viminalis* leaves. In both species the stomatal densities on either side of the leaf were equal, and variation in stomatal density between leaves of the same species was small.

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1 Silastic 583 RTV Silicone Rubber, manufactured by Dow Corning Aust. Pty Ltd, Sydney, N.S.W.

2 Dow Corning RTV Catalyst F, manufactured by Dow Corning Corporation, Midland, Michigan, U.S.A.

TABLE 3.5. Stomatal densities on both sides of E. viminalis and E. polyanthemos leaf discs.

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>E. VIMINALIS</th>
<th>E. POLYANTHEMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Side A</td>
<td>Side B</td>
</tr>
<tr>
<td>1</td>
<td>204 ± 19 181 ± 10</td>
<td>104 ± 7 122 ± 8</td>
</tr>
<tr>
<td>2</td>
<td>179 ± 10 228 ± 15</td>
<td>110 ± 5 132 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>212 ± 12 179 ± 8</td>
<td>126 ± 10 124 ± 7</td>
</tr>
<tr>
<td>4</td>
<td>177 ± 12 200 ± 9</td>
<td>120 ± 12 96 ± 7</td>
</tr>
<tr>
<td>5</td>
<td>200 ± 12 220 ± 14</td>
<td>130 ± 8 153 ± 12</td>
</tr>
<tr>
<td>6</td>
<td>210 ± 16 204 ± 11</td>
<td>136 ± 8 157 ± 8</td>
</tr>
<tr>
<td>7</td>
<td>187 ± 16 191 ± 6</td>
<td>120 ± 11 128 ± 18</td>
</tr>
<tr>
<td>8</td>
<td>200 ± 8 212 ± 8</td>
<td>179 ± 10 193 ± 7</td>
</tr>
<tr>
<td>9</td>
<td>230 ± 15 194 ± 17</td>
<td>208 ± 13 212 ± 20</td>
</tr>
<tr>
<td>10</td>
<td>210 ± 14 193 ± 10</td>
<td>183 ± 8 187 ± 13</td>
</tr>
<tr>
<td>MEAN ± S.E.</td>
<td>201 ± 5 200 ± 4</td>
<td>141 ± 6 150 ± 6</td>
</tr>
</tbody>
</table>
(2) **Effect of Surfactants on Leaf Surface Morphology:** Three solutions were prepared each containing 0.2 per cent unlabelled picloram in 0.05M triethanolamine, and with (1) no surfactant, (2) 0.7 per cent Tween 20, and (3) 0.7 per cent Decol T/70. A petri dish lined with filter paper was prepared as in all the uptake experiments and as described in chapter 1, except that three 15 mm x 15 mm midvein-free leaf segments were cut and placed same side up on the glass slide instead of a series of leaf discs. A 6 mm length of 10 mm internal diameter clear vinyl tubing was adhered to each segment with lanolin, into which 0.22 ml of one of each solution was added by a pipette. The liquid initially extended 2.8 mm up the tubing from the leaf surface. This was repeated for 12 leaves of both species, and the closed petri dishes were held at a temperature of 20°C under a light intensity of 600 ft. candles for 8 hours.

With the exception of the wider tubing on larger leaf areas and the 8 hours exposure time, these were identical experimental conditions to the uptake phase of the experiment described in 3.5. The wider tubing was needed to give adequate sized treated areas for subsequent preparation for electron microscopy, and 8 hours was considered a suitable time period at which any solution effect on the leaf surface that could affect uptake over a 24 hour period should be noticeable. After the 8 hours, the leaves were gently blotted dry. The treated surfaces of ten of the leaves were then
prepared for transmission electron microscopy of carbon replicas, and the remaining two leaves for scanning electron microscopy, as described previously.

The transmission electron micrographs are shown in PLATE 3.5. *E. polyanthemos* was again shown to have considerably more surface wax than *E. viminalis*. In both species, Tween 20 and particularly Decol T/70 appeared to reduce the amount of wax compared to the no surfactant treatment, the effect being relatively more pronounced in the more waxy *E. polyanthemos*. The greatest effect was with the Decol T/70 treatment on *E. polyanthemos* where large amounts of wax were removed, the wax appearing as though it was being dissolved.

The scanning electron micrographs (PLATE 3.6.) showed very little difference between treatments on *E. viminalis*, except for a slight dissolved appearance of the wax with the Decol T/70 treatment. With the Tween 20 treatment on *E. polyanthemos*, there was some removal of surface wax, and the remaining wax was changed in its physical form to globular formations giving the surface a pocked appearance. This alteration was evident right up to the stomatal edge, and the visible parts of the guard cell just adjacent to the stomatal aperture had a fibrous and contorted appearance. Considerably more wax was removed with the Decol T/70 treatment on *E. polyanthemos* than with Tween 20 and, with Decol T/70, wax was also removed right to the stomatal edge.
PLATE 3.5. Transmission electron micrographs of carbon replicas of *E. viminalis* (V) and *E. polyanthemos* (P) leaf surfaces treated with picloram solutions containing no surfactant (1), Tween 20 (2), and Decol T/70 (3). (6500 x).
PLATE 3.6. Scanning electron micrographs of *E. viminalis* (V) and *E. polyanthemos* (P) leaf surfaces treated with picloram solutions containing no surfactant (1), Tween 20 (2), and Decol T/70 (3). (1200 x).
3.8. Discussion

Surfactants in general increased the uptake of picloram, and sometimes very much so. Uptake of picloram was very dependent on the wettability of the leaf surface by the applied solution when different surfactants or different concentrations of the same surfactant were used. It is suggested that the surfactants largely affected uptake by their effects on wetting the leaf surface, and that the observed species-surfactant interactions were no more complex than the interfacial energy relationships as expressed by contact angle. This is contrary to the consensus of opinion as reviewed in chapter 1, where more sophisticated interactions than explicable in terms of simple wetting have been proposed, but not elucidated.

Picloram solutions containing no surfactant wet *E. viminalis* leaves better than *E. polyanthemos*. This could have been due to differences in the physical, chemical, and topographical nature of the leaf surfaces modifying interfacial energy relationships and thereby wettability. The most obvious differences between the leaf surfaces were in their surface wax and stomatal characteristics. In view of the reported inhibitory nature of surface wax on wettability (chapter 1), *E. polyanthemos* might be naturally less wettable because of its very waxy surface.

Effects of mechanical treatment in removing wax were much more obvious in *E. polyanthemos* than in *E. viminalis*. (This conforms to Hallam and Chambers
(1970) observation that eucalypt plate waxes were difficult, and tube waxes easy, to remove by rubbing). The same was essentially true with chloroform extraction.

The wax removal treatments had no effect on the wettability of the leaf surfaces of E. viminalis by solutions. This is not surprising because E. viminalis had relatively little wax to start with and the degree of removal was consequently small. It appears that surface wax does not play a significant role in wetting E. viminalis leaf surfaces. Differences in the wetting ability of picloram solutions containing different surfactants did occur, but these were independent of any effect on surface wax.

The wax removal treatments slightly increased the wettability of E. polyanthemos leaf surfaces. Chloroform extraction of wax was more efficient than mechanical removal, and a corresponding greater efficiency in wetting after chloroform extraction was recorded. The leaf surface wax on E. polyanthemos therefore had some inhibiting effect on wettability. However, the increase in wettability after wax removal was not as great as that caused by surfactant addition.

The wax-dissolving effect of the picloram-surfactant solutions was more pronounced in E. polyanthemos, and particularly with Decol T/70. A component of the increased ability of solutions containing surfactants to wet E. polyanthemos could be due to this dissolving effect of the surfactant on the wax. The rapid and
large decrease in contact angle with time after droplet formation observed with the picloram solution containing Decol T/70 on E. polyanthemos, could be caused partly by a continuous process of wax dissolution thereby continuously increasing wettability.

Though wax removal by chloroform extraction increased wettability on E. polyanthemos, uptake of picloram both by this species and by E. viminalis was, in general, inhibited by chloroform treatment. Use of the stain Sudan IV showed that this inhibition was not due to the redeposition of a fine continuous layer of surface wax on the leaf surface. Darlington and Barry (1965) found that prior soaking of discs of isolated apricot leaf cuticle in chloroform greatly increased their permeability to acetamide. Fogg stated that leaves were 'killed' by solvent (ether and benzene) removal of wax (1948a), but that this death did not alter their surface properties (1947). If metabolism were involved in uptake, then this could explain the marked inhibition of uptake after chloroform extraction even though wettability was increased. If penetration via stomata were significant, then any impairment by chloroform extraction of metabolism responsible for the maintenance of stomatal opening could also be an explanation. These aspects of metabolism and stomatal involvement will be discussed later.

Uptake of picloram correlated better with contact angle than with surface tension. Contact angle is an expression of the interfacial energy relationships
between solution, surface, and atmosphere, and as such may be regarded as a reliable index of wettability. The surface tension of a solution, on theoretical grounds, could not be expected to be an entirely reliable index of the ability of that solution to wet a surface, as it takes no account of the nature of this other surface and any solution-surface interactions that may be involved.

A theoretical equation often used to describe the balance of forces at equilibrium at a solid-liquid-air interface (see Bikerman 1958) is:

\[ \gamma_S = \gamma_L + \gamma_{SL} \cos \theta \]

where
- \( \gamma_S \) = surface tension of the solid
- \( \gamma_L \) = surface tension of the liquid
- \( \gamma_{SL} \) = surface tension of the solid-liquid interface, i.e. interfacial tension.
- \( \theta \) = contact angle within the liquid

\[ \therefore \cos \theta = \frac{\gamma_S - \gamma_L}{\gamma_{SL}} \]

These theoretical considerations help to explain the data presented.

(i) Contact angles of the same solution on *E. viminalis* and *E. polyanthemos* varied considerably, and contact angles on *E. polyanthemos* were reduced after mechanical and chemical removal of the surface wax. This is a measure of the contribution of the surface to the overall wettability. With the same surfactant solution (and therefore constant \( \gamma_L \)) on different leaf
surfaces, $\gamma_s$, and therefore $\gamma_{SL}$, may vary giving different contact angles. Therefore the same surfactant solution may not wet different surfaces to the same extent. The data showed that picloram solutions containing Tween 20 wet *E. polyanthemos* leaves very poorly but wet *E. viminalis* leaves quite satisfactorily.

(ii) With different surfactant solutions or with different concentrations of the same surfactant solution on the same surface, the resultant contact angle was not necessarily predictable using the surface tension of the applied liquid alone. Under these conditions $\gamma_s$ is constant, but both $\gamma_{SL}$ and $\gamma_L$ may vary and not in the same manner. Therefore the ability of different solutions to wet the same surface can not necessarily be predicted from the surface tension values of the applied solution. Nevertheless, as reviewed in chapter 1, this has often been done. It has often been observed also, that surfactants are most effective at concentrations well above that where the maximum lowering of surface tension occurs, and from this it has been implied that interactions other than wettability are involved. However, the substitution of wettability for surface tension in this context is unjustified on theoretical grounds, and is challenged by the data given here. Maximum lowering of the surface tension of picloram solutions containing Arquad 12/50 occurred at the lowest surfactant concentration used (0.2 per cent), whereas the maximum wettability (minimum contact angle) of these solutions on *E. polyanthemos* occurred at a surfactant concentration around 0.7 per cent.
If penetration via stomata were significant, then, under conditions of equal wettability, the slightly sunken and more exposed *E. polyanthemos* stomata could be more accessible to applied solutions than the less exposed *E. viminalis* stomata with their bulky surrounding rims. There are, however, less stomata per unit area on leaves of *E. polyanthemos* than on those of *E. viminalis*. The stomatal morphology, in its own right, may also be a component of the total leaf surface effect on wettability. The role of stomata in uptake will be studied in more depth later.

---

Leaf discs of both species were cut and placed out as described previously, and 20 microlitres of 0.1 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc. Treatments consisted of five light intensities (2000, 1000, 500, 250, 125, and 62.8 ft. candles) for each of two surfactants (Tween 20 and Decol 7/79), and there were ten discs per treatment. The different light intensities were achieved by the use of arena shadecloth frames over the petri dishes in an illuminated general constant environment chamber.

---

*measured with a Weston Sunlight Illumination Meter, Model 756, supplied by Warrington Franki Industries, Box 1523, G.P.O. Sydney, 2009.*
4.1. Light Intensity

In chapter 2 it was observed that, for both *E. viminalis* and *E. polyanthemos*, uptake in the light was greater than that in the dark. In this chapter the effect of light will be considered in more detail. Initially, the effect of light intensity on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs was examined.

Leaf discs of both species were cut and plated out as described previously, and 20 microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc. Treatments consisted of five light intensities (2000, 1000, 500, 250, 125, and 62.5 ft. candles) for each of two surfactants (Tween 20 and Decol T/70), and there were ten discs per treatment. The different light intensities were achieved by the use of sarlon shadecloth frames over the petri dishes in an illuminated Sherer constant environment chamber.

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1 measured with a Weston Sunlight Illumination Meter, Model 756, supplied by Warburton Franki Industries, Box 1523, G.P.O. Sydney, 2000.
Treatments were for 24 hours of continuous light at a constant temperature of 20°C. Upon termination of treatment, the discs were wiped, washed, oven dried, and radioassayed as described previously.

The results are shown in FIG 4.1. Except for *E. polyanthemos* in the presence of Tween 20, uptake decreased initially with increasing light intensity, and reached a minimum at light intensities of 125 to 250 ft. candles. Light at intensities greater than 250 ft. candles stimulated uptake, and uptake values at 2000 ft. candles were considerably more than the initial 62.5 ft. candle and minimum values. Uptake by *E. polyanthemos* in the presence of Tween 20 increased with all increases in light intensity, but the absolute increase was always slight, and the uptake over the range was small. Except for *E. viminalis* in the presence of Tween 20, where uptake was still increasing steeply at 2000 ft. candles, the rate of increase of uptake slowed down toward 2000 ft. candles suggesting a saturation light intensity was being approached. There was no obvious effect of surfactant on the response to light intensity: the surfactants seemed simply to determine a level of uptake efficiency which was maintained over the whole light intensity range.

4.2. Light Quality

The effect of light of different spectral compositions on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs was examined. Any effect of light quality on uptake might help in understanding the light-stimulated component of uptake.
FIG. 4.1. Effect of light intensity on uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs.
Leaf discs of both species were cut and plated out, and 20 microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc. Treatments consisted of five light qualities (white, far red, red, green, and blue) and darkness, for each of two surfactants (Tween 20 and Decol T/70), and there were ten discs per treatment.

The lights were set up with materials provided by, and on recommendations from, research workers in the Research School of Biological Sciences at the Australian National University, who also provided data on their spectral composition. Filters were clear cinemoid\(^1\) no. 30 for white, primary red cinemoid no. 6 for red, primary green cinemoid no. 39 for green, deep blue (primary) cinemoid no. 20 for blue, and 3 mm black plexiglas\(^2\) for far red. Light sources were two Philips 20 watt fluorescent tubes of the appropriate colour for white, red, green, and blue\(^3\), and four 60 watt clear incandescent bulbs for far red.

In all cases the filter formed the top panel of a bottomless box, which was placed over the petri dish supplied by Strand Electric (Aust.) Pty Ltd, Theatre Equipment Supplies, 19 Trent Street, Burwood, Victoria, 3125.

 manufactured by Röhm and Haas, Darmstadt, W. Germany.

one MCFER 20W white, and one MCFA 20W daylight for white.
two TL 20W/15 (L5) for red.
two TL 20W/17 (H7) for green.
two TL 20W/18 (A8) for blue.
containing the leaf discs. The filter was 23 cm above the leaf discs, and the sides of the box were of black cloth. Dimensionally, the cinemoid filters were 61 cm x 25 cm and the plexiglas was 30 cm x 30 cm. The white, red, green, and blue light sources were 16 cm above their appropriate filter, and were centrally located with the length of the tubes parallel to the long dimension of the filter. The bulbs for the far red source were located at the corners of a 19 cm square, and were centrally positioned 75 cm above the black plexiglas. Each filter box plus light source was completely isolated from any external contaminating light. The spectral composition of these lights is shown in TABLE 4.1. The white light contained radiation continuous over the whole experimental range. These data were obtained with the use of a laboratory designed and assembled spectroradiometer, and a Carey 14 recording spectrophotometer. At this stage there was no instrument available to measure the power of the light under each filter.

Treatments were carried out in a 25 ± 1°C constant temperature room, the dark treatment being placed in a light proof cupboard. The temperature range encountered by the treatments under each filter and in darkness was monitored by a maximum and minimum recording thermometer, and is shown in TABLE 4.2. Treatments were for 24 hours, after which the discs were wiped, washed, oven dried, and radioassayed.
TABLE 4.1. Wavelength range and intensity peaks under light qualities*.

<table>
<thead>
<tr>
<th>LIGHT QUALITY</th>
<th>RANGE</th>
<th>PEAK</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>continuous</td>
<td>825</td>
</tr>
<tr>
<td>Far Red</td>
<td>697 - 725</td>
<td>660</td>
</tr>
<tr>
<td>Red</td>
<td>620 - 660</td>
<td>580</td>
</tr>
<tr>
<td>Green</td>
<td>470 - 510</td>
<td>530</td>
</tr>
<tr>
<td>Blue</td>
<td>400 - 510</td>
<td>436</td>
</tr>
</tbody>
</table>

* Data of D.J. Crossley and R. Menhenett.
Research School of Biological Sciences, Australian National University.

TABLE 4.2. Temperature range recorded under light qualities.

<table>
<thead>
<tr>
<th>LIGHT QUALITY</th>
<th>MINIMUM</th>
<th>MAXIMUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>23.5°C</td>
<td>31.5°C</td>
</tr>
<tr>
<td>Dark</td>
<td>24.0°C</td>
<td>26.0°C</td>
</tr>
<tr>
<td>Far Red</td>
<td>23.0°C</td>
<td>27.5°C</td>
</tr>
<tr>
<td>Red</td>
<td>23.0°C</td>
<td>28.0°C</td>
</tr>
<tr>
<td>Green</td>
<td>24.0°C</td>
<td>30.0°C</td>
</tr>
<tr>
<td>Blue</td>
<td>24.0°C</td>
<td>32.0°C</td>
</tr>
</tbody>
</table>
The results are shown in FIG. 4.2. With *E. viminalis*, white light stimulated uptake over that in dark with both surfactants, as observed earlier (FIGS. 2.1. and 4.1.). Red, green, and blue light also stimulated uptake slightly, but uptake rarely equalled that under white light. Far red light, however, stimulated greater uptake than white light, particularly when Decol T/70 was the surfactant used. With *E. polyanthemos*, light of any quality had very little effect, and uptake was excellent in the presence of Decol T/70 but poor in the presence of Tween 20.

The temperature ranges under each light quality differed (TABLE 4.2.), but these differences were not large; and since the temperatures recorded under the far red filters were generally lower than the others, the tendency would be for uptake to be depressed under far red light (see data on temperature given later in this chapter). Thus, the observed far red stimulation of uptake by *E. viminalis* could not be explained in terms of a temperature effect.

The power of the different light qualities at the leaf disc surface was measured when instrumentation became available. Power was measured by a thermopyle in a solarimeter\(^1\) and recorded on a millivolt potentiometer.\(^2\) These results are shown in TABLE 4.3. Though white, red, green, and blue...

---

1 Direct Reading Solarimeter manufactured by Swissteco Pty Ltd, 26 Miami Street, E. Hawthorne, Victoria, 3122.

FIG. 4.2. Effect of light quality on uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs.
blue, and green light had comparable values, far red light had four times and more the power of the other light qualities. This raises the question of whether the far red stimulated uptake of picloram by *E. viminalis* was an increased power effect rather than a light quality effect.

By reducing the light source to two bulbs at diagonally opposite corners of the square and decreasing its distance to 70 cm above the black plexiglas, the power of the far red light was reduced from 1.52 mW cm\(^{-2}\) (far red - high power) to 0.38 mW cm\(^{-2}\) (far red - low power) the same as that of white and red light. This is shown in TABLE 4.4. The uptake of picloram by *E. viminalis* and *E. polyanthemos* with Decal T/70 as surfactant under white light and high and low power far red light was examined. This was done under identical experimental conditions to those described for the previous experiment where all light qualities were involved.

These results are shown in FIG. 4.3. Uptake of picloram by *E. viminalis* was stimulated by far red light irrespective of power: low power was more stimulatory than high. Uptake by *E. polyanthemos* in high power far red was equal to, and in low power far red less than that of white light. The initial far red stimulation of uptake by *E. viminalis* was therefore not due to the higher power.
**TABLE 4.3.** Power under light qualities in FIG. 4.2. and TABLE 6.3.

<table>
<thead>
<tr>
<th>LIGHT QUALITY</th>
<th>POWER (mW. cm.(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Far Red</td>
<td>1.52</td>
</tr>
<tr>
<td>White</td>
<td>0.38</td>
</tr>
<tr>
<td>Red</td>
<td>0.38</td>
</tr>
<tr>
<td>Blue</td>
<td>0.34</td>
</tr>
<tr>
<td>Green</td>
<td>0.30</td>
</tr>
</tbody>
</table>

**TABLE 4.4.** Power under light qualities in FIG. 4.3. and TABLE 6.4.

<table>
<thead>
<tr>
<th>LIGHT QUALITY</th>
<th>POWER (mW. cm.(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>0.38</td>
</tr>
<tr>
<td>Far Red - High Power</td>
<td>1.52</td>
</tr>
<tr>
<td>Far Red - Low Power</td>
<td>0.38</td>
</tr>
</tbody>
</table>
FIG. 4.3. Effect of power of far red light on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs. (Surfactant: Decol T/70).
4.3. Test for Phytochrome Activity

The differential effects of red and far red light on uptake of picloram by *E. viminalis* leaf discs suggest phytochrome might be involved. The classical test for phytochrome activity involves subjecting the material under test to alternating short irradiations of red and far red light (see Hendricks 1964). If the last irradiation was red, most phytochrome would be in its active form ($P_{730}$) resulting in phytochrome activity; but if the last irradiation was far red, most phytochrome would be in its inactive form ($P_{660}$) resulting in phytochrome inactivity. Phytochrome activity may express itself as either a positive (Toole et al. 1955, Mohr 1966) or negative (Laetsch and Briggs 1962, Mohr 1966) photoresponse.

If far red stimulation of uptake by *E. viminalis* is phytochrome activated, it is unlikely to be a negative photoresponse, since uptake in light is greater than in dark and increases with light intensity. Wagner and Mohr (1966) described typical phytochrome responses induced by short irradiations of red light. Exactly the same response was achieved by 'long-time irradiations' (6 hr plus) of far red light but not red light. They argued that both the long- and short-time responses were phytochrome activated. This aspect of phytochrome behaviour is further discussed by Mohr (1969).

Accepting this hypothesis, stimulation of uptake by *E. viminalis* under 24 hours of continuous far red light could be completely compatible with its being a
phytochrome activated positive photoresponse. The rationale for suspecting phytochrome activity having been established, it was experimentally tested for by the classical short irradiation method.

Leaf bearing twigs of both species, with their stems in water, were stored for 24 hours in the dark in a 25°C constant temperature room. After this dark pretreatment, discs were cut and plated out under illumination from a weak green safelight. Twenty microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc immediately prior to irradiation treatments. There were four irradiation treatments (5 minutes of red light; 5 minutes of far red; 5 minutes of red followed by 5 minutes of far red; and 5 minutes of far red followed by 5 minutes of red) for each of two surfactants (Tween 20 and Decol T/70). There were ten discs per treatment, and red light and high power far red light as in the initial light quality experiments were used. After the irradiations, the discs were kept in the dark in the constant temperature room for a further 24 hours, after which they were wiped, washed, oven dried, and radioassayed.

The results are shown in FIG. 4.4. Phytochrome activity requires a last irradiation of far red to be inhibitory and of red to be stimulatory: no such effect was evident. Except for *E. polyanthemos* with Decol T/70, the 10 minute irradiations (red and far red combinations) gave better uptake than the 5 minute
**FIG. 4.4.** Effect of initial irradiations (for five minutes) of red and far red lights on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs in the dark.
irradiations (one colour only). This suggests a possible effect of period of illumination on uptake. However, this possibility was not examined further.

4.4. Temperature

The effect of temperature on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs in light and dark was examined.

Treatments consisted of five temperatures (8°C, 15°C, 20°C, 25°C, and 30°C) for each of two surfactants (Tween 20 and Decol T/70) in both light (600 ft. candles) and dark. There were ten discs per treatment, and 20 microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc. Because of the size of this experiment, and because only one 600 ft. candle light source was available, the experiment was carried out over five consecutive days, one temperature on each day. Treatments were for 24 hours in constant temperature rooms of the appropriate value, after which the discs were wiped, washed, oven dried, and radioassayed.

The results are shown in FIG. 4.5. In the light, uptake increased with temperature in both species and with both surfactants. Except for *E. polyanthemos* in the presence of Tween 20, the increase was slight but steady over the temperature range. With *E. polyanthemos* and Tween 20, uptake increased greatly as the temperature increased from 20°C to 30°C.

In the dark, and in the presence of Tween 20, uptake was unaffected by temperature except possibly
FIG. 4.5. Effect of temperature on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs in light and dark. Tween 20 - light (---○---), Tween 20 - dark (---■---), Decol T/70 - light (---△---), Decol T/70 - dark (---■---).
for an increase at 30°C with *E. polyanthemos*. In the dark, and in the presence of Decol T/70, uptake by both species decreased as the temperature was raised from 8°C to 20°C after which temperature stimulated uptake. The overall result of increasing temperature from 8°C to 30°C in the dark was a slight increase in uptake.

$Q_{10}$ values for the whole temperature range are given in TABLE 4.5. These values were higher in the light than in the dark; were approximately the same for *E. viminalis* with both surfactants; and were higher with Tween 20 as surfactant on *E. polyanthemos* leaf discs than when Decol T/70 was used.

4.5. Discussion

Although there were inconsistencies in the data such as decreases in uptake with increasing light intensity up to 250 ft. candles (FIG. 4.1.), and a decrease in uptake in some cases with increases in temperature from 8°C to 20°C (FIG. 4.5.), the overall trends were clear:

(i) Uptake in light was greater than in the dark and increasing the light intensity up to at least 2000 ft. candles stimulated uptake.

(ii) Far red light, of equal power, stimulated greater uptake by *E. viminalis* leaf discs than white light. This did not appear to be a phytochrome effect.

(iii) Uptake increased with temperature, the overall increase being greater in light than in the dark. The magnitude of the temperature effect
TABLE 4.5. \( Q_{10} \) values between 8°C and 30°C calculated from data in FIG. 4.5.

<table>
<thead>
<tr>
<th></th>
<th>TWEEN 20</th>
<th>DECOL T/70</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. VIMINALIS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>1.97</td>
<td>1.93</td>
</tr>
<tr>
<td>Dark</td>
<td>1.46</td>
<td>1.65</td>
</tr>
<tr>
<td><strong>E. POLYANTHEMOS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>2.59</td>
<td>1.60</td>
</tr>
<tr>
<td>Dark</td>
<td>2.03</td>
<td>1.37</td>
</tr>
</tbody>
</table>
in *E. polyanthemos* depended on the surfactant used, it being greater with Tween 20 than Decol T/70.

Possible explanations of these results will be considered here and will be developed in later chapters.

The light-stimulated component of uptake could be caused by (1) a direct effect of light on stomatal aperture thereby increasing penetration via stomata, or (2) a direct or indirect effect of photometabolism other than by modification of the stomatal aperture.

With the constant energy conditions approximated over the range of light qualities used in these experiments, there would be more quanta with a lower energy per quanta at the far red end of the spectrum, grading to less quanta with a higher energy per quanta at the blue end. It might be more significant physiologically if light quality effects could be examined at constant quantum flux levels over the experimental range of wavelengths. As quantum flux varies with wavelength, this would involve the establishment of narrow wavelength bands (perhaps 20 nm wide) over the range, each being individually adjusted to the constant quantum flux level. This was not possible with the unsophisticated equipment used here where a relatively wide range of wavelengths was transmitted through each filter. Nevertheless, comparison at constant power (energy per unit time) can still be meaningful, and may contribute to an understanding of the light stimulated component of uptake.
The most interesting aspect of the light quality experiments was that far red light could stimulate uptake of picloram, and then only in one species, *E. viminalis*. The test for phytochrome activity proved to be negative. Tanada (1965) found far red light increased, and red light decreased the rate of rubidium absorption by dark grown mung bean seedlings. He was able to reverse the far red effect with red light, but was unable to reverse the red effect with far red light.

White light and far red light stimulated uptake by *E. viminalis* leaf discs more than blue light or red light. This suggests that photosystem 2 of photosynthesis, was not involved. However, in addition to phytochrome activation, far red light is specifically involved in photosystem 1 of photosynthesis.

Photosystem 1, which contains pigments absorbing above 690 nm, would be most active under the far red filter and not operative under the green and blue. The smaller component of 690 nm plus radiation in white and red could support photosystem 1, but at a much lower level than the far red. Therefore, photosystem 1 and its associated cyclic photophosphorylation could be related to the light-stimulated component of uptake.

It appears, therefore, that light most likely influenced uptake by a direct effect on stomata, or by photometabolic effects, particularly those associated with photosystem 1 of photosynthesis.

Uptake generally increased with temperature. The greater increase in the light compared with that in the dark could be due to (1) increased stomatal opening
with increased temperature in the light and thereby increased penetration via stomata, or (2) increased rates of photochemical reactions.

Though increase in uptake with temperature was usually less in the dark than in the light, high $Q_{10}$ values were recorded in both light and dark. If active uptake were involved, the necessary energy could be provided by photosynthesis and associated processes (perhaps photophosphorylation) in the presence of light, and respiration and associated processes (perhaps oxidative phosphorylation) in light and dark. The high $Q_{10}$ values, however, are just as explicable in terms of facilitated diffusion, a process dependent on thermal agitation (see chapter 1).

It appears therefore that photosynthetic or respiratory metabolism, or direct effects on stomata, were likely factors involved in the results observed. Attention will now be focussed on these.
CHAPTER 5

EFFECT OF METABOLISM ON UPTAKE

5.1. Respiratory Metabolism

The effect of respiratory (oxygen-dependent) metabolism on the uptake of picloram by *E. viminalis* and *E. polyanthemos* leaf discs was examined by comparing uptake in atmospheres of oxygen, nitrogen, and air. If respiration or associated oxidative phosphorylation is necessary or stimulatory to uptake, then uptake in an atmosphere of oxygen should exceed that in air, and uptake in an atmosphere of nitrogen should be least.

Leaf discs of both species were cut, placed on glass slides, and clear vinyl tubing segments adhered to them as described previously, but they were not placed in petri dishes. Twenty microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc. The ten discs per treatment on a slide were then placed on top of an inverted 50 ml beaker inside a 2.5 litre clear glass jar, the bottom of which was lined with saturated filter papers (see PLATE 5.1.). The lid of the jar was then screwed on tightly, a cardboard gasket ensuring a gas proof seal.
PLATE 5.1. Jar used for atmosphere experiments.
The lid had two glass rods passing through it. One of these (the gas inlet) extended almost to the bottom of the jar, while the other (the gas outlet) protruded only a few centimetres below the lid. The glass rods were connected externally to vinyl tubing which could be sealed off with screw clamps. The desired gas was introduced through the inlet and the displaced gas expelled through the outlet. When the jar was completely filled with the gas, the system was isolated as a gas proof entity by applying both the screw clamps.

Treatments consisted of three atmospheres (oxygen, nitrogen, and air) for two surfactants (Tween 20 and Decol T/70) in both light (600 ft. candles) and dark. Treatments were maintained for 24 hours at 20°C, after which the discs were wiped, washed, oven dried, and radioassayed as described previously.

The results are shown in FIG. 5.1. Uptake by both species with Decol T/70 as surfactant was good, E. polyanthemos having better uptake than E. viminalis. Uptake in oxygen (in both light and dark) was greater than in air with E. viminalis leaf discs, and equal to that in air with E. polyanthemos, but nitrogen (in both light and dark) stimulated uptake by both species to an even greater extent overall. The only exception was uptake in the dark by E. viminalis. Uptake in the light was generally greater than in the dark, the difference being most marked in the nitrogen atmosphere. Uptake
FIG. 5.1. Effect of oxygen \((O_2)\), nitrogen \((N_2)\), and air atmospheres on the uptake of picloram by E. viminalis and E. polyanthemos leaf discs in light (□) and dark (■).
by both species with Tween 20 as surfactant was poor, and there was little difference in uptake between treatments.

These results indicate that uptake does not depend on any oxidative metabolism.

5.2. Photosynthetic Metabolism

The effect of photosynthetic (light-dependent) metabolism, and photophosphorylation in particular, on the uptake of picloram by E. viminalis and E. polyanthemos leaf discs was examined using the metabolic inhibitors 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and p-trifluoromethoxyphenylhydrazone (F-CCP).

In order to show why these inhibitors were used and how they act in the plant, a brief description of the process of photosynthesis is necessary. This will be a very simplified treatment of a complicated process about which a great deal is still unknown. A more comprehensive review of the electron-transport pathways involved is given by Hind and Olson (1968) and Bishop (1971) and of their relation to phosphorylation by Schwartz (1971).

A diagramatic representation of the electron-transport chain in photosynthesis given by Levine (1969) is reproduced in FIG. 5.2. Water is oxidised to oxygen and the released electrons are passed along by various electron acceptors and donors until they are finally used in the reduction of NADP to NADPH. The energy required to move the electrons against the electro-chemical gradient is provided by light which activates
"From 'The Mechanism of Photosynthesis' by R.P. Levine. Copyright (c) 1969 by Scientific American, Inc. All rights reserved."

FIG. 5.2. Electron-transport chain in photosynthesis
two photosystems, photosystem 1 (PS1) and photosystem 2 (PS2). These photosystems are connected in series in the electron-transport pathway. Photosystem 2 is activated by blue and red light, but photosystem 1 is activated by far red light (690 nm+).

The production of the high energy phosphate compound ATP is coupled with the electron-transport flow. Photosystem 1, besides contributing in the non-cyclic electron flow which is initiated by photosystem 2, can stimulate a cyclic electron flow which is not involved in NADP reduction. ATP production coupled with this cyclic electron flow is called cyclic photophosphorylation. ATP production coupled with the non-cyclic electron flow between Q (an electron acceptor and donor) and photosystem 1 is called non-cyclic photophosphorylation. Both ATP and NADPH are needed to reduce carbon dioxide to carbohydrate, a reaction which still occurs in the chloroplast but does not require light.

DCMU blocks electron transport somewhere between Q and the cytochromes. This means that non-cyclic photophosphorylation and oxygen evolution are inhibited, and NADP and therefore carbon dioxide are not reduced. However, cyclic electron flow, and therefore cyclic photophosphorylation, can continue. On the other hand, carbonyl cyanide phenylhydrazones are total uncouplers of phosphorylation, both oxidative and photo. This means both cyclic and non-cyclic photophosphorylation (as well as oxidative phosphorylation) are inhibited and carbon
dioxide is not reduced. However, photosynthetic electron flow can continue, and oxygen evolution is unimpaired. F-CCP is the most potent of the carbonyl cyanide phenylhydrazones, exhibiting uncoupling effects at concentrations as low as $10^{-8}$M (Heytler and Pritchard 1962).

DCMU was used at $10^{-5}$M, and F-CCP at $10^{-6}$M, both in 0.66 per cent ethanol in water. A control treatment of 0.66 per cent ethanol in water was included. The ethanol was needed to bring the F-CCP into solution. DCMU was used only in the light (600 ft. candles) since this is a specific inhibitor of non-cyclic photophosphorylation. F-CCP and control were used in both the light (600 ft. candles) and the dark since F-CCP blocks both photo and oxidative phosphorylation.

Leaf discs of both species were cut and plated out, and five microlitres of inhibitor (or control) were added to each of ten discs per treatment. The discs were kept at 25°C for two hours, after which 20 microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent Decol T/70 were added to each disc. The discs were then kept under the same conditions of light and dark for a further 24 hours. Addition of picloram in the dark treatments was done under illumination from a weak green safelight. At completion of the treatment, the discs were wiped, washed, oven dried, and radioassayed.

The results are shown in FIG. 5.3. In *E. polyanthemos* uptake in the dark was less than in the light, and the DCMU and F-CCP treatments in the light
FIG. 5.3. Effect of inhibitors on the uptake of picloram by E. viminalis and E. polyanthemos leaf discs in light (■) and dark ( ).
(Surfactant: Decol T/70).
reduced uptake to the control dark level, i.e. by an amount equal to the light-stimulated component of the uptake. F-CCP in the dark did not further reduce uptake. Variability within treatments was high in *E. viminalis*, and there was no evidence of any inhibition of uptake by either DCMU or F-CCP.

5.3. Discussion

There was no evidence that oxidative phosphorylation (or any other oxygen-dependent metabolism) was necessary for uptake. On the contrary, uptake in nitrogen was generally greater than in air and at least as great as in oxygen. The lack of a requirement for oxidative phosphorylation was supported by the fact that there was no inhibition by F-CCP of uptake by both species in the dark.

Air is a mixture of approximately one part oxygen to four parts nitrogen, but contains a small proportion (approximately 330 volumes per million) of carbon dioxide. Therefore it is possible that the lesser uptake in air compared to that in oxygen and nitrogen could be caused by carbon dioxide, the non-common component in the air mixture. Carbon dioxide could possibly be exerting an effect via the stomata.

Stomata may open more widely in the light (Fischer 1968), and may open in the dark, in carbon dioxide-free air (Mouravieff 1965). It is possible that under the carbon dioxide-free conditions of the oxygen and nitrogen, the stomata on the leaf discs were more open than in the air.
If penetration via stomata were a significant factor in uptake, then this could explain why, with Decol T/70, the uptake in air was less than that in oxygen and nitrogen. DCMU and F-CCP both reduced uptake by *E. polyanthemos* by an amount equal to the light-stimulated component. DCMU inhibits non-cyclic photophosphorylation but allows cyclic photophosphorylation to continue; F-CCP inhibits both forms of photophosphorylation. Therefore, if photophosphorylation is required as a source of energy for the stimulation of uptake under the light regime used here, non-cyclic photophosphorylation was necessary for this stimulation, and cyclic photophosphorylation alone could not provide sufficient energy. No inhibition, however, was observed in *E. viminalis*.

An interpretation of these results in terms of penetration via stomata is also possible. Both DCMU and F-CCP inhibit carbon dioxide utilization in photosynthesis. As a result, the carbon dioxide concentration in the intercellular spaces could build up creating a carbon dioxide-enriched atmosphere, a condition which Pallas (1965) and Jones and Mansfield (1970) have shown can cause partial stomatal closure. DCMU (Willmer and Mansfield 1970) and its analogue 3-((4-chlorophenyl)-1,1-dimethylurea (CMU) (Allaway and Mansfield 1967) can cause partial stomatal closure in the light, and this effect can be reversed under carbon dioxide-free conditions. Allaway and Mansfield
(1967) suggested CMU produced this stomatal response because of the build up in carbon dioxide concentration mentioned above. Consequently DCMU and F-CCP may have inhibited uptake by *E. polyanthemos* by causing partial stomatal closure and thereby reduced penetration via stomata. Evidence suggests that cyclic photophosphorylation may provide the energy for stomatal opening in the light (Willmer and Mansfield 1970, Humble and Hsiao 1970). Inhibition of cyclic photophosphorylation by F-CCP might therefore contribute to partial stomatal closure.

Since DCMU and far red light both block photosystem 2 and therefore non-cyclic electron flow, but allow the cyclic electron flow associated with photosystem 1 to continue, they could both have the same effect on uptake. Though this similarity was not immediately obvious, there is a consistency in the effects of the two treatments on the two species. Under both treatments, *E. polyanthemos* had a relative reduction in uptake when compared to *E. viminalis*: this expressed itself as a far red stimulation in *E. viminalis* compared to no effect or a slight inhibition in *E. polyanthemos* (FIGS. 4.2. and 4.3.), and no or little effect of DCMU in *E. viminalis* compared to an inhibition in *E. polyanthemos* (FIG. 5.3.). If stomata were involved in the uptake, then it could be that *E. polyanthemos* stomata were more sensitive to the effect of carbon dioxide concentration on closure than *E. viminalis* stomata.
In this and previous chapters, the results have led to interpretations that are compatible with the principle of penetration via stomata. The role of stomata in uptake will now be discussed.

CHAPTER 6

ROLE OF STOMATA IN UPTAKE

6.1. Introduction

The nature and distribution of the stomata on leaves of both species was examined in chapter 3, and suggestions on the possible role of stomata as a partial factor in determining wettability and uptake differences between the species was introduced. It was further suggested that observed light and temperature effects (chapter 4) and metabolic effects (chapter 5) could be explained in terms of the effect of these treatments on the stomata. Penetration via stomata has not been enthusiastically supported in the literature (chapter 1), and this chapter seeks to determine whether or not the stomata were major portals of entry, and whether, or not the observed effects of light, temperature, different atmospheres, and metabolic inhibitors on uptake could be explained in terms of penetration via stomata.

This was approached experimentally in two ways: (1) the uptake of picloram by both sides of P. virosa juvenile leaves (which have more stomata on the lower than the upper surface) in both light and dark was examined, and the corresponding degree of stomatal opening was monitored; and (2) the degree of stomatal
CHAPTER 6

ROLE OF STOMATA IN UPTAKE

6.1. Introduction

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This was approached experimentally in two ways: (1) the uptake of picloram by both sides of \textit{E. viminalis} juvenile leaves (which have more stomata on the lower than the upper surface) in both light and dark was examined, and the corresponding degree of stomatal opening was monitored; and (2) the degree of stomatal
opening corresponding to treatments in previous experiments was examined.

6.2. Uptake by *E. viminalis* juvenile leaves

*E. viminalis* and *E. polyanthemos* adult leaves have an equal number of stomata on either side of the leaf (chapter 3). The juvenile *E. polyanthemos* leaves are morphologically similar to the adult, but *E. viminalis* has a juvenile leaf form morphologically quite different to that of the adult. The *E. viminalis* juvenile is typically dorsiventral, and oriented at right angles to gravity. A preliminary investigation showed that the lower surface had significantly more stomata than the upper.

Half sib *E. viminalis* seed was sown in a one:one mixture of vermiculite and perlite in 13 cm diameter plastic pots. After seed germination the seedlings were grown under controlled environment conditions of 16 hour days (8 hours natural light plus 8 hours incandescent light) and a light/dark temperature regime of 21°C/16°C. The pots were watered with nutrient solution daily. Seedlings were subsequently thinned to one per pot and, when four months old, seedlings of similar development were chosen for use in this experiment. The seedlings had juvenile leaves in opposite sessile pairs on the main stem, and minimum branching.

Upper and lower surfaces of leaf discs were treated in both light (600 ft. candles) and dark with picloram solutions containing each of two surfactants (Tween 20
and Decol T/70). Two leaves (not an opposite pair) were selected from the middle of the seedling, one for each surfactant. From each leaf, a disc was cut for each of the upper and lower surface treatments in both light and dark, i.e. four discs per leaf. This was repeated for ten seedlings giving ten discs per treatment. The discs were plated out as described previously, and 20 microlitres of 0.2 per cent labelled picloram in 0.05M triethanolamine with 0.7 per cent surfactant were added to each disc. The treatments were kept for 24 hours at 20°C, after which the discs were wiped, washed, oven dried, and radioassayed.

The leaves opposite those from which the discs were cut were used for obtaining stomatal densities using the replica technique described in chapter 3. This could not be done on the leaves providing the treated discs, as the total leaf area of any one leaf was too small. Stomata were counted in one field on each side of all twenty leaves, and the results recorded as the number of stomata per mm² of leaf surface.

In order to determine whether or not any observed differences in uptake by upper and lower surfaces could be due to differences in wettability, contact angles were measured by the photographic technique described in chapter 3. The same picloram-surfactant solutions as used in the uptake phase of the experiment were prepared using unlabelled picloram. One leaf pair from the middle of each of ten seedlings was taken, and contact angles of the solution containing Tween 20 were
measured on one leaf, and of that containing Decol T/70 on the other. Two contact angles on each of the lower and upper surfaces were measured.

The degree of stomatal opening in discs treated identically to those in the uptake experiment was monitored. In a preliminary experiment, the feasibility of measuring stomatal apertures on freeze killed and freeze dried leaf material through a scanning electron microscope was examined. The stomatal apertures, however, visibly altered under the beam and this technique could not be used. The technique selected was the measurement of stomatal resistances using a porometer. This had the advantage of giving a single reading proportional to both the number of stomata open and the degree of opening.

One leaf from the middle of each of ten seedlings was taken and four discs (an upper and a lower surface exposed to both light and dark) were cut from each leaf. The discs were plated out in the normal way but no tubing was adhered to them. These discs (with no added solution) were kept for 8 hours under identical conditions to those in the uptake phase of the experiment. It was considered that, if penetration via stomata were significant, stomatal resistances at 8 hours should be related to the uptake of picloram over 24 hours.

After 8 hours the stomatal resistances were measured using an aspirated diffusion porometer. The principle of this porometer depends on measuring the
rate of change in the relative humidity of an initially dried volume of air in contact with the required leaf surface. The instrument used in this experiment was designed and described by Byrne et al. (1970), and was particularly suited to the measurement of stomatal resistances of small leaf areas such as the discs used in this study. The time required for the water vapour stream from a known area (a 7.5 mm diameter circle) of the appropriate leaf surface to increase the relative humidity of a small volume of dried air through a known range was measured. The time in seconds was converted to stomatal resistance in seconds cm\(^{-1}\) by reference to conversion graphs for a particular temperature.

Care was taken to ensure that the temperature of the disc and instrument were the same at the time of measurement, this being a prerequisite for its accurate use (Morrow and Slatyer 1971). Measurements on the discs kept in the dark were carried out under illumination from a weak green safelight. Stomatal resistances were measured on discs without added solution because, since an aspirated diffusion porometer measures the diffusive resistance to water vapour, measurements on wet surfaces would be unrepresentative. Wiping off the excess liquid and lanolin with facial tissue could disturb the leaf surface and alter the stomatal resistance.

The uptake of picloram by upper and lower surfaces of juvenile leaf discs of *E. viminalis* in light and dark is shown in FIG. 6.1. Within any one surfactant
FIG. 6.1. Uptake of picloram by upper and lower sides of *E. viminalis* juvenile leaf discs in light and dark.
treatment, uptake was greatest by the lower surface in the light. Light also stimulated uptake by the upper surface in the presence of Decol T/70, but not with Tween 20. In the dark, the lower surface had a slightly greater mean uptake than the upper. In all cases Decol T/70 gave greater uptake than Tween 20.

The stomatal counts, contact angles, and stomatal resistances corresponding to the uptake data are shown in TABLE 6.1. The lower leaf surfaces had 2.5 times more stomata than the upper surfaces. The contact angle with Tween 20 was higher (less wettable) on the lower than on the upper surface: there was no difference in contact angle with Decol T/70. The stomatal resistance of the lower surface in the light was approximately one-third of that of the other treatments which showed relatively little difference. The coefficient of linear correlation of uptake of picloram on stomatal resistance was -0.96* with Tween 20 and -0.95* with Decol T/70 (TABLE 6.8).

6.3. Degrees of Stomatal Opening

Stomatal resistances (an inverse measure of the degree of stomatal opening) of leaf discs were measured under the treatment conditions of previous experiments, where it was considered that stomata could have been involved. These experiments were light intensity (4.1.), light quality (4.2.), temperature (4.4.), respiratory metabolism (5.1.), and photosynthetic metabolism (5.2.). Stomatal resistances corresponding to the wax removal treatments in 3.5. were also measured to see whether the
TABLE 6.1. Relation between stomatal resistance and uptake of picloram by upper and lower sides of *E. viminalis* juvenile leaf discs in light and dark. (Standard errors shown).

<table>
<thead>
<tr>
<th>SIDE OF LEAF DISC REGIME</th>
<th>LIGHT</th>
<th>NO. OF STOMATA (mm.(^{-2}))</th>
<th>RESISTANCE (sec. cm.(^{-1}))</th>
<th>UPTAKE (c.p.m. x 10(^{-4}))</th>
<th>CONTACT ANGLE (Degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
<td>Light</td>
<td>419 ± 26</td>
<td>8.4 ± 1.0</td>
<td>8.41 ± 2.96 (Tween 20)</td>
<td>71.3 ± 3.2 (Tween 20)</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>26.1 ± 4.5</td>
<td>1.34 ± 0.63 (Tween 20)</td>
<td>5.58 ± 0.65 (Decol T/70)</td>
<td>43.5 ± 4.0 (Decol T/70)</td>
</tr>
<tr>
<td>Upper</td>
<td>Light</td>
<td>169 ± 10</td>
<td>22.4 ± 2.1</td>
<td>9.62 ± 1.84 (Decol T/70)</td>
<td>40.5 ± 2.7 (Decol T/70)</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>24.3 ± 3.2</td>
<td>0.66 ± 0.47 (Tween 20)</td>
<td>3.46 ± 1.43 (Decol T/70)</td>
<td></td>
</tr>
</tbody>
</table>
observed inhibition of uptake after chloroform
treatments could be explained in terms of stomatal
aperture. In some cases the resistances were measured
at a later time than the corresponding uptake phase of
the experiment, in which case absolute values would not
necessarily be comparable but relative trends could
still be established. However as the work progressed
and the involvement of stomata appeared more likely, the
resistances were measured at the same time as, and on
similar leaf material to, the corresponding uptake
phase.

(1) **Light Intensity**: Leaf discs of both species
were cut, but tubing was not adhered to them and no
solution was added. These discs were then subjected
to identical experimental conditions to the corresponding
uptake phase of the light intensity experiment as
described in 4.1., except that after 8 hours their
stomatal resistances were measured using an aspirated
diffusion porometer as described previously. The
stomatal resistances were not measured at the same time
as the uptake data.

The results are shown in **TABLE 6.2**. In both species
the stomatal resistance steadily decreased as the light
intensity increased. This corresponded to a general
increase in the uptake of picloram with increasing light
intensity with both surfactants. In other words, both
the degree of stomatal opening and the uptake of picloram
increased with increasing light intensity, with the
exception that the decreased uptake at the lower light
TABLE 6.2. Effect of light intensity on the stomatal resistance of *E. viminalis* and *E. polyanthemos* leaf discs, and on the uptake of picloram.

**E. Viminalis**

<table>
<thead>
<tr>
<th>LIGHT INTENSITY (foot candles)</th>
<th>STOMATAL RESISTANCE ± S.E. (sec. cm.(^{-1}))</th>
<th>UPTAKE ± S.E. (c.p.m. (\times 10^{-4}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.5</td>
<td>11.2 ± 2.3</td>
<td>1.05 ± 0.79</td>
</tr>
<tr>
<td>125</td>
<td>9.4 ± 1.5</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>250</td>
<td>6.3 ± 0.2</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>500</td>
<td>5.6 ± 1.1</td>
<td>3.87 ± 2.46</td>
</tr>
<tr>
<td>1000</td>
<td>4.2 ± 0.4</td>
<td>5.08 ± 2.03</td>
</tr>
<tr>
<td>2000</td>
<td>2.6 ± 0.2</td>
<td>11.27 ± 3.32</td>
</tr>
</tbody>
</table>

**E. Polyanthemos**

<table>
<thead>
<tr>
<th>LIGHT INTENSITY (foot candles)</th>
<th>STOMATAL RESISTANCE ± S.E. (sec. cm.(^{-1}))</th>
<th>UPTAKE ± S.E. (c.p.m. (\times 10^{-4}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.5</td>
<td>19.4 ± 3.0</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>125</td>
<td>13.0 ± 1.6</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>250</td>
<td>10.8 ± 2.8</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>500</td>
<td>9.0 ± 1.5</td>
<td>0.46 ± 0.09</td>
</tr>
<tr>
<td>1000</td>
<td>4.0 ± 0.4</td>
<td>1.74 ± 1.33</td>
</tr>
<tr>
<td>2000</td>
<td>2.7 ± 0.2</td>
<td>2.09 ± 0.60</td>
</tr>
</tbody>
</table>
intensities in some cases was not reflected in the stomatal resistance values. The coefficient of linear correlation of uptake of picloram by *E. viminalis* on the stomatal resistance was -0.80* with Tween 20 and -0.82* with Decol T/70. The correlation coefficient of uptake by *E. polyanthemos* on stomatal resistance was -0.88* with Tween 20 and -0.62 with Decol T/70 (TABLE 6.8.).

(2) **Light Quality**: Leaf discs of both species (without adhered tubing and added solution) were subjected to identical experimental conditions to that in both light quality experiments as described in 4.2. The results of which were given in FIGS. 4.2. and 4.3., except that after 8 hours their stomatal resistances were measured using an aspirated diffusion porometer. The stomatal resistances were not measured at the same time as the uptake data.

The results for the first light quality experiment (all light qualities included) are shown in TABLE 6.3. The light qualities have been ranged in the increasing order of stomatal resistances recorded on *E. viminalis*. The observed far red stimulation of uptake by *E. viminalis* (with both surfactants) corresponded with a far red stimulated increase in the degree of stomatal opening (decrease in stomatal resistance). There was no far red stimulation of uptake by *E. polyanthemos*, and no corresponding far red stimulation of stomatal opening. The stomatal resistance of *E. polyanthemos* under far red was only exceeded by that in the dark. The coefficient of linear correlation of uptake of picloram by
TABLE 6.3. Effect of light quality on the stomatal resistance of *E. viminalis* and *E. polyanthemos* leaf discs, and on the uptake of picloram.

**E. VIMINALIS**

<table>
<thead>
<tr>
<th>LIGHT QUALITY</th>
<th>STOMATAL RESISTANCE ± S.E. (sec. cm.⁻¹)</th>
<th>UPTAKE ± S.E. (c.p.m. x 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tween 20</td>
</tr>
<tr>
<td>Far red</td>
<td>2.9 ± 0.1</td>
<td>25.67 ± 0.80</td>
</tr>
<tr>
<td>White</td>
<td>3.6 ± 0.8</td>
<td>23.58 ± 0.53</td>
</tr>
<tr>
<td>Red</td>
<td>4.1 ± 0.8</td>
<td>18.93 ± 0.71</td>
</tr>
<tr>
<td>Blue</td>
<td>4.2 ± 0.7</td>
<td>20.69 ± 0.68</td>
</tr>
<tr>
<td>Green</td>
<td>5.5 ± 1.4</td>
<td>17.18 ± 0.55</td>
</tr>
<tr>
<td>Dark</td>
<td>7.0 ± 1.2</td>
<td>15.58 ± 0.67</td>
</tr>
</tbody>
</table>

**E. POLYANTHEMOS**

<table>
<thead>
<tr>
<th>LIGHT QUALITY</th>
<th>STOMATAL RESISTANCE ± S.E. (sec. cm.⁻¹)</th>
<th>UPTAKE ± S.E. (c.p.m. x 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tween 20</td>
</tr>
<tr>
<td>Far red</td>
<td>9.8 ± 2.2</td>
<td>2.97 ± 1.84</td>
</tr>
<tr>
<td>White</td>
<td>3.2 ± 0.2</td>
<td>3.61 ± 1.74</td>
</tr>
<tr>
<td>Red</td>
<td>4.6 ± 0.6</td>
<td>6.42 ± 3.02</td>
</tr>
<tr>
<td>Blue</td>
<td>7.0 ± 1.5</td>
<td>3.91 ± 2.93</td>
</tr>
<tr>
<td>Green</td>
<td>4.1 ± 0.4</td>
<td>3.31 ± 1.81</td>
</tr>
<tr>
<td>Dark</td>
<td>11.4 ± 1.1</td>
<td>1.72 ± 0.99</td>
</tr>
</tbody>
</table>
E. viminalis on stomatal resistance was -0.92** with Tween 20 and -0.74* with Decol T/70. The correlation coefficient of uptake by E. polyanthemos on stomatal resistance was -0.62 with Tween 20 and -0.09 with Decol T/70 (Table 6.8).

The results for the second light quality experiment (comparison of powers of far red light) are shown in TABLE 6.4. The stomatal resistances were measured at the same time as the uptake data. A far red stimulation of uptake by E. viminalis (particularly with low power) was observed when compared with white light. In this case, the stomatal resistances were low in each treatment, and no differences were observed between treatments. There was no far red stimulation of uptake by E. polyanthemos (high power far red had uptake equal to, and low power far red less uptake than, uptake under white light), and the stomatal resistances under far red light of both powers were greater than under white light.

(3) Temperature: Leaf discs of both species (without adhered tubing and added solution) were subjected to identical experimental conditions to the corresponding uptake phase of the temperature experiment as described in 4.4., except that after 8 hours their stomatal resistances were measured using an aspirated diffusion porometer. The stomatal resistances were not measured at the same time as the uptake data.

The results are shown in TABLE 6.5. Though uptake of picloram by both species generally increased with increasing temperature in both light and dark with both
TABLE 6.4. Effect of power of far red light on the stomatal resistance of *E. viminalis* and *E. polyanthemos* leaf discs, and on the uptake of picloram. (Surfactant: Decol T/70).

<table>
<thead>
<tr>
<th></th>
<th><em>E. VIMINALIS</em></th>
<th></th>
<th><em>E. POLYANTHEMOS</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STOM. RESISTANCE ± S.E.</td>
<td>UPTAKE ± S.E.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(sec. cm.⁻¹)</td>
<td>(c.p.m. × 10⁻⁴)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1.7 ± 0.02</td>
<td>9.73 ± 1.54</td>
<td></td>
</tr>
<tr>
<td>Far Red - High Power</td>
<td>1.6 ± 0.04</td>
<td>12.51 ± 1.64</td>
<td></td>
</tr>
<tr>
<td>Far Red - Low Power</td>
<td>1.7 ± 0.04</td>
<td>15.40 ± 1.78</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2.6 ± 0.2</td>
<td>24.11 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>Far Red - High Power</td>
<td>5.3 ± 0.6</td>
<td>24.67 ± 0.62</td>
<td></td>
</tr>
<tr>
<td>Far Red - Low Power</td>
<td>4.3 ± 0.3</td>
<td>21.73 ± 0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>STOMATAL RESISTANCE ± S.E. (sec. cm.⁻¹)</td>
<td>UPTAKE ± S.E. (c.p.m. x 10⁻⁴)</td>
<td>Tween 20</td>
</tr>
<tr>
<td>------------------</td>
<td>----------------------------------------</td>
<td>-------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>E. VIMINALIS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Light</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8°C</td>
<td>5.1 ± 1.0</td>
<td>4.24 ± 2.07</td>
<td>9.35 ± 0.82</td>
</tr>
<tr>
<td>15°C</td>
<td>4.7 ± 0.4</td>
<td>4.79 ± 2.00</td>
<td>14.83 ± 1.31</td>
</tr>
<tr>
<td>20°C</td>
<td>4.4 ± 1.0</td>
<td>8.08 ± 2.96</td>
<td>15.06 ± 1.35</td>
</tr>
<tr>
<td>25°C</td>
<td>5.1 ± 0.8</td>
<td>6.47 ± 3.21</td>
<td>17.99 ± 2.70</td>
</tr>
<tr>
<td>30°C</td>
<td>4.8 ± 0.8</td>
<td>11.29 ± 3.54</td>
<td>22.83 ± 2.15</td>
</tr>
<tr>
<td><strong>Dark</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8°C</td>
<td>15.8 ± 3.2</td>
<td>2.10 ± 1.08</td>
<td>8.25 ± 0.59</td>
</tr>
<tr>
<td>15°C</td>
<td>9.6 ± 2.1</td>
<td>2.48 ± 0.89</td>
<td>6.82 ± 1.25</td>
</tr>
<tr>
<td>20°C</td>
<td>10.4 ± 1.6</td>
<td>2.26 ± 1.07</td>
<td>3.95 ± 0.89</td>
</tr>
<tr>
<td>25°C</td>
<td>8.8 ± 0.4</td>
<td>0.37 ± 0.06</td>
<td>8.51 ± 1.82</td>
</tr>
<tr>
<td>30°C</td>
<td>9.6 ± 1.4</td>
<td>2.83 ± 1.14</td>
<td>13.15 ± 2.49</td>
</tr>
<tr>
<td><strong>E. POLYANTHEMOS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Light</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8°C</td>
<td>5.9 ± 0.9</td>
<td>5.27 ± 1.76</td>
<td>18.59 ± 1.13</td>
</tr>
<tr>
<td>15°C</td>
<td>4.8 ± 0.5</td>
<td>1.82 ± 0.88</td>
<td>22.39 ± 0.50</td>
</tr>
<tr>
<td>20°C</td>
<td>5.1 ± 0.6</td>
<td>5.08 ± 2.17</td>
<td>22.90 ± 1.86</td>
</tr>
<tr>
<td>25°C</td>
<td>3.6 ± 0.4</td>
<td>10.48 ± 3.94</td>
<td>27.77 ± 0.55</td>
</tr>
<tr>
<td>30°C</td>
<td>4.7 ± 0.6</td>
<td>22.65 ± 1.25</td>
<td>28.26 ± 0.87</td>
</tr>
<tr>
<td><strong>Dark</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8°C</td>
<td>18.0 ± 2.2</td>
<td>1.95 ± 0.86</td>
<td>19.95 ± 0.79</td>
</tr>
<tr>
<td>15°C</td>
<td>10.3 ± 0.5</td>
<td>0.61 ± 0.30</td>
<td>17.33 ± 0.62</td>
</tr>
<tr>
<td>20°C</td>
<td>12.3 ± 0.6</td>
<td>1.62 ± 0.79</td>
<td>15.79 ± 0.85</td>
</tr>
<tr>
<td>25°C</td>
<td>14.0 ± 1.4</td>
<td>1.16 ± 0.55</td>
<td>21.40 ± 0.44</td>
</tr>
<tr>
<td>30°C</td>
<td>12.8 ± 2.1</td>
<td>5.97 ± 1.90</td>
<td>25.53 ± 0.52</td>
</tr>
</tbody>
</table>
surfactants, there was no apparent effect of increasing temperature on the stomatal resistance of both species. Stomatal resistances in the dark were noticeably higher than in the light. The coefficient of linear correlation of uptake of picloram by _E. viminalis_ on stomatal resistance was -0.67* with Tween 20 and -0.64* with Decol T/70. The correlation coefficient of uptake by _E. polyanthemos_ on stomatal resistance was -0.51 and -0.46 (TABLE 6.8.).

(4) Respiratory Metabolism (Oxygen, Nitrogen, and Air Atmospheres): Leaf discs of both species (without adhered tubing and added solution) were placed in the 2.5 litre jars as described in 5.1. These were then subjected to identical experimental conditions to the corresponding uptake phase of the atmospheres experiment as described in 5.1., except that after 8 hours the stomatal resistances were measured using an aspirated diffusion porometer. The oxygen and nitrogen atmospheres would equilibrate with the atmosphere when the jars were opened, and therefore the resistances would need to be measured quickly. In an attempt to reduce this measurement period to an acceptable limit, there were five discs per jar (instead of ten as in the uptake phase) and two jars per treatment. The stomatal resistances were not measured at the same time as the uptake data.

The results are shown in TABLE 6.6. Uptake of picloram by both species with Tween 20 as surfactant was poor, and no consistent trend with the different
TABLE 6.6. Effect of oxygen, nitrogen, and air atmospheres on the stomatal resistance of *E. viminalis* and *E. polyanthemos* leaf discs in light and dark, and on the uptake of picloram.

### E. VIMALINALS

<table>
<thead>
<tr>
<th></th>
<th>STOMATAL RESISTANCE ± S.E.</th>
<th>UPTAKE ± S.E. (c.p.m. \times 10^{-4})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(sec. cm.(^{-1}))</td>
<td>Tween 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decol T/70</td>
</tr>
<tr>
<td><strong>LIGHT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>2.4 ± 0.2</td>
<td>2.48 ± 2.05</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.8 ± 0.4</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td>Air</td>
<td>3.7 ± 0.4</td>
<td>0.85 ± 0.57</td>
</tr>
<tr>
<td><strong>DARK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>11.0 ± 3.4</td>
<td>0.95 ± 0.48</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>15.5 ± 4.5</td>
<td>0.37 ± 0.11</td>
</tr>
<tr>
<td>Air</td>
<td>3.9 ± 0.5</td>
<td>1.89 ± 1.29</td>
</tr>
</tbody>
</table>

### E. POLYANTHEMOS

<table>
<thead>
<tr>
<th></th>
<th>STOMATAL RESISTANCE ± S.E.</th>
<th>UPTAKE ± S.E. (c.p.m. \times 10^{-4})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(sec. cm.(^{-1}))</td>
<td>Tween 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decol T/70</td>
</tr>
<tr>
<td><strong>LIGHT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>14.6 ± 3.7</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>10.6 ± 1.7</td>
<td>4.02 ± 2.58</td>
</tr>
<tr>
<td>Air</td>
<td>19.4 ± 3.5</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td><strong>DARK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>19.5 ± 2.2</td>
<td>2.04 ± 0.88</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>15.6 ± 2.6</td>
<td>0.49 ± 0.32</td>
</tr>
<tr>
<td>Air</td>
<td>24.6 ± 5.6</td>
<td>0.17 ± 0.07</td>
</tr>
</tbody>
</table>

---

Note: The data in Table 6.6 show the effect of different atmospheres (oxygen, nitrogen, air) on stomatal resistance and uptake of picloram in the leaves of *E. viminalis* and *E. polyanthemos* in both light and dark conditions. The values presented are the mean ± standard error (S.E.) for each condition.
atmospheres was evident. Uptake by both species with Decol T/70 as surfactant was good, and the uptake (in light and dark) in oxygen and particularly nitrogen was superior to that in air. Except for *E. viminalis* leaf discs in the dark, the corresponding stomatal resistances were lower in oxygen and nitrogen than in air. In *E. polyanthemos* the stomatal resistances in nitrogen were less than in oxygen. However, the stomatal resistance of *E. viminalis* in air in the dark was almost as low as that in the light, and considerably less than that in oxygen and nitrogen in the dark. This result was contrary to the general trend, and was inconsistent with stomatal resistance data obtained in earlier experiments (TABLES 6.3. and 6.5.) where resistances in air in the dark were much higher than those in the light. No explanation can be offered for this seemingly aberrant result.

The coefficients of linear correlation of uptake of picloram by *E. viminalis* on stomatal resistance was -0.45 with Tween 20 and -0.28 with Decol T/70. The contrary stomatal resistances in the dark contributed to the low correlation with Decol T/70. The correlation coefficient of uptake by *E. polyanthemos* on stomatal resistance was -0.60 with Tween 20 and -0.94** with Decol T/70 (TABLE 6.8.).

(5) Photosynthetic Metabolism (Metabolic Inhibitors):
Leaf discs of both species were cut, tubing was adhered to them, and they were plated out as previously described. Metabolic inhibitors were added to these
discs in exactly the same manner as in the uptake phase of the metabolic inhibitor experiment described in 5.2. However, no picloram solution was added to the discs after 2 hours, and the discs were left for a further 8 hours (i.e. 10 hours after addition of the inhibitors), after which they were gently blotted dry and their stomatal resistances were measured using an aspirated diffusion porometer. The stomatal resistances were measured at the same time as the uptake data.

The results are shown in TABLE 6.7. In *E. viminalis*, where no effect of the inhibitors on the uptake of picloram was evident, the inhibitors caused no difference in the stomatal resistances: the stomatal resistances were only slightly less in the light than in the dark. In *E. polyanthemos*, where both inhibitors reduced uptake in the light to the level of that in the dark, both inhibitors increased the stomatal resistance in the light: the stomatal resistances in the dark were unaffected by inhibitor treatment and were greater than in all the light treatments. The coefficient of linear correlation of uptake of picloram on stomatal resistance was -0.63 in *E. viminalis* and -0.68 in *E. polyanthemos* (TABLE 6.8.).

(6) Correlation: Coefficients of linear correlation of uptake of picloram by both species on the stomatal resistances for the various experiments are shown in TABLE 6.8. Meaningful coefficients suffer markedly from lack of degrees of freedom, but the exercise serves to show that, in general, uptake was more closely
TABLE 6.7. Effect of inhibitors on the stomatal resistance of *E. viminalis* and *E. polyanthemos* leaf discs in light and dark, and on the uptake of picloram.
(Surfactant: Decol T/70).

**E. VIMINALIS**

<table>
<thead>
<tr>
<th></th>
<th>STOMATAL RESISTANCE ± S.E. (sec. cm.⁻¹)</th>
<th>UPTAKE ± S.E. (c.p.m. x 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LIGHT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.5 ± 0.2</td>
<td>9.53 ± 2.70</td>
</tr>
<tr>
<td>F-CCP</td>
<td>2.5 ± 0.1</td>
<td>9.35 ± 3.15</td>
</tr>
<tr>
<td>DCMU</td>
<td>2.6 ± 0.2</td>
<td>7.36 ± 2.39</td>
</tr>
<tr>
<td><strong>DARK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.3 ± 0.2</td>
<td>6.04 ± 1.66</td>
</tr>
<tr>
<td>F-CCP</td>
<td>3.0 ± 0.4</td>
<td>9.34 ± 1.82</td>
</tr>
</tbody>
</table>

**E. POLYANTHEMOS**

<table>
<thead>
<tr>
<th></th>
<th>STOMATAL RESISTANCE ± S.E. (sec. cm.⁻¹)</th>
<th>UPTAKE ± S.E. (c.p.m. x 10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LIGHT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.4 ± 0.3</td>
<td>21.19 ± 0.64</td>
</tr>
<tr>
<td>F-CCP</td>
<td>5.8 ± 0.8</td>
<td>16.03 ± 1.14</td>
</tr>
<tr>
<td>DCMU</td>
<td>5.0 ± 0.4</td>
<td>16.36 ± 0.98</td>
</tr>
<tr>
<td><strong>DARK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.6 ± 1.8</td>
<td>16.14 ± 1.57</td>
</tr>
<tr>
<td>F-CCP</td>
<td>8.4 ± 1.8</td>
<td>16.46 ± 0.51</td>
</tr>
</tbody>
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TABLE 6.8. Coefficients of linear correlation of uptake of picloram on stomatal resistance of E. viminalis and E. polyanthemos leaf discs for various experiments.

**E. VIMINALIS**

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>CORRELATION COEFFICIENT</th>
<th>TABLE REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIGHT INTENSITY</td>
<td>- Tween 20 -0.80*</td>
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<td></td>
<td>- Decol T/70 -0.82*</td>
<td></td>
</tr>
<tr>
<td>LIGHT QUALITY</td>
<td>- Tween 20 -0.92**</td>
<td>6.3.</td>
</tr>
<tr>
<td></td>
<td>- Decol T/70 -0.74*</td>
<td></td>
</tr>
<tr>
<td>TEMPERATURE</td>
<td>- Tween 20 -0.67*</td>
<td>6.5.</td>
</tr>
<tr>
<td></td>
<td>- Decol T/70 -0.64*</td>
<td></td>
</tr>
<tr>
<td>ATMOSPHERES</td>
<td>- Tween 20 -0.45</td>
<td>6.6.</td>
</tr>
<tr>
<td></td>
<td>- Decol T/70 -0.28</td>
<td></td>
</tr>
<tr>
<td>INHIBITORS</td>
<td>- Decol T/70 -0.63</td>
<td>6.7.</td>
</tr>
<tr>
<td>JUVENILE LEAVES</td>
<td>- Tween 20 -0.96*</td>
<td>6.1.</td>
</tr>
<tr>
<td></td>
<td>- Decol T/70 -0.95*</td>
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**E. POLYANTHEMOS**

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>CORRELATION COEFFICIENT</th>
<th>TABLE REFERENCE</th>
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</thead>
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<tr>
<td>LIGHT INTENSITY</td>
<td>- Tween 20 -0.88*</td>
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<td>- Decol T/70 -0.62</td>
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<tr>
<td>LIGHT QUALITY</td>
<td>- Tween 20 -0.62</td>
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</tr>
<tr>
<td></td>
<td>- Decol T/70 -0.09</td>
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</tr>
<tr>
<td>TEMPERATURE</td>
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<td></td>
<td>- Decol T/70 -0.46</td>
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<td>ATMOSPHERES</td>
<td>- Tween 20 -0.60</td>
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<tr>
<td></td>
<td>- Decol T/70 -0.94**</td>
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</tr>
<tr>
<td>INHIBITORS</td>
<td>- Decol T/70 -0.68</td>
<td>6.7.</td>
</tr>
</tbody>
</table>

*p < 0.05  **p < 0.01  ***p < 0.001
correlated to stomatal resistance (and thus the degree of stomatal opening) in *E. viminalis* than in *E. polyanthemos*.

(7) Wax Removal: Ten leaves of both species were selected, and each leaf was divided into three segments. These segments received one of each of the three wax removal treatments (i.e. control, mechanical removal, and chloroform extraction) as described in 3.5., and discs were punched from each section. These leaf discs (without adhered tubing and added solution) were subjected to identical experimental conditions to the corresponding uptake and contact angle measurement phase of the wax removal experiment as described in 3.5., except that after 8 hours their stomatal resistances were measured using an aspirated diffusion porometer. The stomatal resistances were not measured at the same time as the uptake and contact angle data.

The results are shown in **TABLE 6.9**. In *E. viminalis*, the stomatal resistance of the control treatment was slightly less than those of the mechanical removal and chloroform extraction treatments. In *E. polyanthemos*, there were no differences in stomatal resistances between the three treatments. Therefore the observed inhibition of uptake of picloram after chloroform extraction of surface wax (see chapter 3) could not be explained in terms of stomatal opening: neither could the observed increase in uptake by *E. polyanthemos* after mechanical removal of surface wax.
TABLE 6.9. Effect of mechanical removal and chloroform extraction of surface wax on the stomatal resistance of *E. viminalis* and *E. polyanthemos* leaf discs.

<table>
<thead>
<tr>
<th></th>
<th><em>E. VIMINALIS</em></th>
<th></th>
<th><em>E. POLYANTHEMOS</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STOMATAL RESISTANCE ± S.E. (sec. cm.(^{-1}))</td>
<td>STOMATAL RESISTANCE ± S.E. (sec. cm.(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.1 ± 0.6</td>
<td>Control</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Mechanical Removal</td>
<td>7.1 ± 0.9</td>
<td>Mechanical Removal</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Chloroform Extraction</td>
<td>7.8 ± 0.5</td>
<td>Chloroform Extraction</td>
<td>4.0 ± 0.2</td>
</tr>
</tbody>
</table>

**Discussion**

*E. viminalis* juvenile leaves have many more stomata on their under (abaxial) surface (TABLE 6.1.). If penetration of herbicide via stomata is slightly greater than by the upper (adaxial) surface, particularly under conditions corresponding to the different treatments. These conditions were observed.

Some workers (see chapter 1) have suggested that increased uptake by the upper leaf surfaces is due to increased penetration through guard and/or subsidiary cells. The present data because of the small differences in uptake by the upper and lower surfaces could not be adequately explained in terms of differences in cuticle thickness and wettability between the two surfaces. In any case, the wettability of the lower surface was less than (with Trach 20) or equal to (with Decol 5/70) the wettability of the upper surface, which would tend to decrease the uptake by the lower surface relative to the upper rather than increase it.
6.4. **Discussion**

Most of the data presented in this chapter supports the possibility that stomata are major portals of entry of herbicide into eucalypt leaf discs. *E. viminalis* juvenile leaves have many more stomata on their under (abaxial) than their upper (adaxial) surface (TABLE 6.1.). If penetration of herbicide via stomata is significant, then uptake by the lower surface should be greater than by the upper surface, particularly in the light, and uptake should correlate well with the total amount of stomatal opening (i.e. a composite measure of the number and aperture of stomata) corresponding to the different treatments. These conditions were observed.

Some workers (see chapter 1) have suggested that increased uptake by lower compared to upper leaf surfaces is due to increased penetration through guard and/or accessory cells. This could not adequately explain the present data because of the much greater differences in uptake between upper and lower surfaces in the light than in the dark. For the same reason, the observed differences in uptake by the upper and lower surfaces could not be adequately explained in terms of differences in cuticle thickness and wettability between the two surfaces. In any case, the wettability of the lower surface was less than (with Tween 20) or equal to (with Decol T/70) the wettability of the upper surface, which would tend to decrease the uptake by the lower surface relative to the upper rather than increase it.
It is concluded that, under these experimental conditions, the uptake of picloram by leaf discs from *E. viminalis* juvenile leaves was closely related to, and probably largely determined by, the degree of stomatal opening.

Although there are some conflicting measurements of uptake at the lower light intensities, the correlation between uptake and stomatal aperture with increasing light intensity was generally good (TABLES 6.2. and 6.8.). One of the most convincing pieces of evidence for the involvement of stomata in uptake was the effect of far red light on uptake by *E. viminalis* leaf discs. Far red light stimulated uptake by this species better than any other single light quality tested, and at least as well as white light. There was no far red stimulation of uptake by *E. polyanthemos*. No involvement of the phytochrome system could be shown, and the possible effect of far red light on photosystem 1 of photosynthesis and on stomatal aperture was speculated on (see chapters 4 and 5). Actual measurements of stomatal resistances (TABLES 6.3. and 6.4.) showed that far red light increased stomatal opening in *E. viminalis* to at least that under white light. There was no such increase in *E. polyanthemos* where the stomatal aperture was, in fact, reduced. Support for a far red effect on stomatal opening is given by Humble and Hsiao (1970) who demonstrated that far red light could open the stomata in epidermal strips of *Vicia faba* L.

A different response between the species was also observed in the effect of the metabolic inhibitors
DCMU and F-CCP. Neither DCMU nor F-CCP affected stomatal aperture or uptake in *E. viminalis* leaf discs, but both inhibitors decreased stomatal aperture and uptake in the light in *E. polyanthemos*.

Therefore far red light, DCMU, and F-CCP did not reduce stomatal apertures in *E. viminalis*, but did in *E. polyanthemos*. A possible reason for this difference is that *E. viminalis* stomata are less sensitive than *E. polyanthemos* stomata to reduction in aperture from increased carbon dioxide concentrations.

Mention was made in chapter 5 of how DCMU, F-CCP, and far red light inhibit carbon dioxide utilization in photosynthesis, thereby creating a carbon dioxide-enriched atmosphere in the intercellular spaces which would induce partial stomatal closure. This partial closure was observed in *E. polyanthemos*, but not in *E. viminalis* where the chemical inhibitors had no effect and far red light sometimes had a stimulatory effect on stomatal aperture. The greater amount of 690 nm + radiation under far red than under white light of equal power would support the cyclic electron flow associated with photosystem 1, and thereby cyclic photophosphorylation, at an enhanced level. Cyclic photophosphorylation may provide the necessary energy for stomatal opening in the light. An increase in cyclic photophosphorylation under far red light could therefore be working against the tendency toward stomatal closure caused by a build up in carbon dioxide concentration. This could explain the stimulatory effect of far red light on stomatal
aperture in *E. viminalis* which has stomata suggested to be less sensitive to changes in carbon dioxide concentration.

With the exception of *E. viminalis* in the dark, the stomata were more widely open in oxygen and nitrogen than in air (TABLE 6.6.). With the same exception, uptake of picloram (particularly with Decol T/70) corresponded quite well with stomatal apertures. The requirement for oxygen in stomatal opening is by no means clear. Louguet (1968) considered oxygen was necessary for stomata to open in leaves. Humble and Hsiao (1970) found oxygen was not necessary for stomatal opening in epidermal strips of *Vicia faba*, but that opening in leaf discs of the same species was markedly inhibited by nitrogen. Raschke (1967), in contrast, found stomatal opening in *Vicia faba* leaves to be increased under anaerobic conditions. Walker and Zelitch (1963) considered external oxygen to be necessary for the opening of stomata, but not for the maintenance of opening or closing.

Though the effects of different gaseous atmospheres on stomatal opening are far from clear, it is generally accepted that high carbon dioxide concentrations induce stomatal closure, and low concentrations (or complete absence) favour opening (see chapter 5). It has been suggested that *E. viminalis* stomata are less sensitive to increased carbon dioxide concentrations than *E. polyanthemos* stomata.
It was therefore of interest to observe the effects of carbon dioxide concentration on stomatal aperture and picloram uptake by the eucalypt leaf discs. The leaf discs were exposed to atmospheres of carbon dioxide-free air (1.9 volumes per million of carbon dioxide), normal air (approximately 330 volumes per million of carbon dioxide), and carbon dioxide-enriched air (5000 volumes per million of carbon dioxide) in both light and dark. Stomatal resistances measured were highly inconsistent within treatments and between experiments done at different times. Probably a continuous flow of the desired atmosphere over the leaf discs was required rather than the static atmospheric conditions used. The high cost of carbon dioxide-free air prevented tests using continuous flow of atmospheres, and this experiment could not be concluded satisfactorily.

Uptake of picloram increased with increasing temperature in the light, and less so in the dark. In view of the generally reported increase in stomatal aperture with increasing temperature in the literature, it seemed possible that the observed increases in uptake would correlate with stomatal apertures. However, measurement of stomatal resistance at the various temperatures (TABLE 6.5.) showed no effect of temperature on stomatal opening. The reasonable correlation shown between uptake and stomatal aperture at different temperatures in *E. viminalis* (TABLE 6.8.) was due to the differences between light and dark. Other possible effects of temperature on uptake include effects on
membrane permeability or influences on a facilitated diffusion process (see chapter 1).

In this work, relationships between stomatal opening and uptake have been shown in a variety of experiments. Uptake by upper and lower surfaces of leaf discs from *E. viminalis* juvenile leaves was closely related to stomatal aperture, and effects of light intensity were largely related to stomatal apertures in both *E. viminalis* and *E. polyanthemos*. Far red stimulation of uptake by *E. viminalis* was related more closely to stomatal aperture than any other factor examined, and the differing effects of the metabolic inhibitors DCMU and F-CCP on uptake by *E. viminalis* and *E. polyanthemos* corresponded with their effects on stomatal aperture. Relationships between stomatal aperture and uptake under different gaseous atmospheres were observed, but effects of temperature on uptake did not correspond to changes in stomatal opening.

The relationships observed were by no means perfect, and in many instances a statistically significant correlation could not be shown. This is probably due, in part, to the insufficient number of samples compared (and therefore low degrees of freedom in the analyses), but because of the inconsistencies, the role of stomata in uptake has not been proved beyond doubt.

Nevertheless, in every comparison made, whether statistically significant or not, the relationship between uptake and stomatal aperture was positive. This is unlikely to have occurred by chance.
The relationship between uptake and stomatal aperture was generally more significant with *E. viminalis* than with *E. polyanthemos*. In chapter 3, the structure of the leaf surfaces and the stomata of *E. viminalis* and *E. polyanthemos* were described, and it was suggested that *E. polyanthemos* stomata could be more easily wet and penetrated once extra-stomatal factors inhibiting wettability were removed. The early experiments with surfactants indicated that *E. polyanthemos* leaf discs were much more dependent on a suitable surfactant for wettability and uptake than *E. viminalis* leaf discs. It is quite possible that in *E. polyanthemos*, a solution that wets poorly, such as one containing Tween 20, has difficulty in entering stomata, whereas a solution that wets well, such as one containing Decol T/70, could enter stomata, and in significant amounts provided they are slightly open. Such a possibility is supported by the occasion when uptake of solutions containing Decol T/70 by *E. polyanthemos* was as good in the dark as in the light (TABLE 6.3.).

Though stomatal resistances in the dark were higher than those in the light, their values were still relatively low and not as high as would be expected if cuticular resistance only (stomata closed) were measured (Slatyer 1967). This indicates that the stomata were not completely closed in the dark. This was supported by scanning electron microscopy of leaf discs of both species which were kept in the dark. The high
humidity conditions under which the leaf discs were treated throughout this work would favour partial opening in the dark.

The evidence presented appears consistent with the following hypothesis of foliar penetration. Stomata are major portals of entry of herbicides into the eucalypt leaf discs examined. The amount penetrating via the stomata depends on stomatal morphology, and degree of wetting of the leaf surface by the applied solution, as well as on stomatal aperture. These are interacting factors which cannot be considered in isolation.

In view of the controversy associated with the penetration of herbicides via stomata and the inconclusiveness of the data presented here, the arguments against stomatal penetration will be discussed fully in the next chapter.
CHAPTER 7

DISCUSSION

7.1. Introduction

The data presented suggest that a significant amount of uptake occurred via the stomata, and that differences in uptake recorded were largely due to changes in wettability or in stomatal aperture. Nevertheless, both the concept of uptake being strictly related to wettability, and penetration via stomata being important in uptake have not been well received in the literature. Also, though uptake via stomata in the presence of a suitable surfactant is conceded, little attention has been given to considering any interrelationships between wettability and stomatal entry.

Wettability was considered fully in chapter 3. In this chapter theoretical and practical aspects of stomatal entry by solutions and its interrelationship with wettability will be discussed.

7.2. Penetration via Stomata

Sargent (1966) referring to stomata concluded that 'their role as sites for entry of applied solutions is without doubt small'. In other reviews, Sargent (1965) and Franke (1967) also doubt the importance of stomatal
entry. It is suggested that this viewpoint is largely ill-founded, and that the role of stomata in uptake may have been under-rated. To substantiate this, the basic premises on which some published viewpoints have been founded will be questioned, and some related experimental work critically examined.

Stomatal cavities are lined with cuticle which has to be penetrated to achieve foliar uptake. However, it cannot be concluded from this that penetration via stomata is unimportant. It was shown in chapter 1 that, provided solutions could enter stomatal cavities, the cuticular surface of the cavity and its microenvironment were better suited than the external surface for penetration by solutions.

Sargent (1965, 1966) quoted the work of Adam (1948) as proving that solutions with a surface tension close to that of water cannot penetrate pores similar in dimensions to those of stomata, and this has been accepted in subsequent literature. However, this does not seem to be a reasonable conclusion from the work of Adam.

Adam discussed the theory of penetration of liquids into solids based on the rise of liquid against gravity in a capillary tube. In this connexion he quoted the equation:

\[ P = \frac{2\gamma \cos \theta}{r} \]  

where \( P \) = pressure spontaneously driving the liquid into the capillary 
\( \gamma \) = surface tension of the liquid 
\( \theta \) = contact angle of the liquid on the capillary surface 
\( r \) = radius of the capillary tube
Turrell (1947) used the same theory when he discussed the penetration of liquids into citrus stomata. He quoted the equation:

\[ h = \frac{2 \gamma \cos \theta}{rg} \]  

where \( h \) = height of rise in the capillary tube
\( g \) = gravitational force

The derivation of this relationship and the reasoning behind it was shown by Morgan (1969) who expressed it in the form:

\[ h = \frac{2 \gamma \cos \theta}{\rho rg} \]  

where \( \rho \) = density of the liquid

As \( P = \rho gh \), this shows the relationship between equation (1) of Adam and equation (3) of Morgan. Presumably Turrell omitted \( \rho \) from his equation (2) because he dealt specifically with water.

Of interest also, though not mentioned by Adam or Turrell, is the equation for calculating the rate of capillary rise. This is a substituted form of Poiseuille's equation and is given by Bikerman (1958):

\[ \frac{dh_t}{dt} = \frac{r^2}{8n\eta_t} \left( \frac{2 \gamma \cos \theta}{r} - \rho gh_t \right) \]  

where \( h_t \) = height of rise in time \( t \)
\( \eta \) = viscosity of the liquid

In order to relate the physical system to which these equations apply to stomatal entry of solutions, an idealized situation must be accepted. This is one where spray droplets are adhering to the lower surface of a horizontally held leaf; where the stomatal pores
are cylindrical, open at both ends, and with non-porous walls; and where the pore walls are lined with a similar material to that on the leaf surface.

If this idealized system is accepted as practically meaningful, then the following general observations can be made:

(i) The contact angle $\theta$ (wettability) is the only variable which can determine whether or not capillary rise occurs. Variation in the other parameters can only determine the extent of such rise. If $\theta$ is equal to or greater than $90^\circ$, then $\cos \theta$ is zero or negative respectively, and there can be no capillary rise. If $\theta$ is less than $90^\circ$, then $\cos \theta$ is positive, and as $\theta$ decreases, $h$ and $\frac{dh}{dt}$ (at a constant $h_t$) increase. It appears that contact angle is the most significant variable, but it is disregarded in arguments by others.

A major part of the effect of lowering of surface tension on penetration via stomata would be due to its effect on contact angle and not on the surface tension value in itself. If a leaf surface had a contact angle of greater than $90^\circ$ with water and less than $90^\circ$ after surfactant addition, then surfactant addition could induce stomatal entry that would otherwise not have occurred. If a leaf surface had a contact angle of less than $90^\circ$ with water, then stomatal entry could occur to some extent: the addition of surfactant could lower the contact angle even further and thereby increase the potential extent and rate of penetration.
Adam (1948) dealt with the principles of penetration of liquids into solids on theoretical grounds and did not mention stomata. His only reference to practical applications was how the orderly arrangement of pores in a surface could give very high contact angles on cloth and ducks' feathers, thereby resulting in non-penetration and water repellancy. It is unlikely that Adam would have agreed with the generalizations about the role of stomata based on surface tension and pore dimensions, which have been made on the basis of his work. Turrell (1947) considered that the resinous plug which characterizes the entrance to citrus stomata would give a high contact angle in the stomatal pore. This formed a major part of his argument suggesting that water could not enter citrus stomata.

(ii) If $\theta$ is less than 90°, then as $r$ decreases, $h$ increases and $\frac{dh}{dt}$ decreases. Therefore the height of rise in a partially closed stoma (still assuming a circular cross section) would be greater than in the same stoma when fully open, but the fully open stoma would permit more solution to enter in a given time (by a function of $r^3$).

(iii) For a constant value of $\theta$ less than 90°, then as $\gamma$ increases, both $h$ and $\frac{dh}{dt}$ (at a constant $h_t$) increase. It is difficult therefore to understand the argument which excludes from stomatal pores liquids with surface tensions close to that of water without any qualifying statement on the degree of wettability. If water made a contact angle $\theta_1$ (less than 90°) on an
easily wetted leaf surface, and a surfactant solution with a lower surface tension made the same angle $\theta_1$ on a difficult to wet surface, then the extent and rate of stomatal penetration would be greater with the water.

In TABLE 7.1., values of $h$ and $\frac{dh}{dt}$ are given, calculated from equations (3) and (4), for various values of $r$, $\gamma$, and $\theta$. Stomatal pores are not circular in cross section but approximate an ellipse when fully open. Curtis and Clark (1950) gave a range of stomatal pore dimensions and pore cross sectional areas for a range of species. Assuming the cross sectional area to be circular, then the calculated radii range from approximately 1$\mu$ to 10$\mu$ with an average of 5$\mu$, and these three $r$ values have been used in the table. Surface tension values of 75 dynes cm$^{-1}$ (representing 'a solution with a surface tension value close to that of water') and 30 dynes cm$^{-1}$ (representing a solution containing a surfactant efficient in surface tension lowering) were used. No contact angles greater than 90° were used because no height rise is possible under these circumstances. Angles of 70° and 40°, as representative of angles that have been recorded on leaves with both water and surfactant solutions, were included.

The table shows that the potential height rise in all cases far exceeded the height that would need to be penetrated in the stomatal situation. Turrell (1947) gave a range of 2$\mu$ to 14$\mu$ for stomatal pore depths in citrus. In camera lucida reproductions of eucalypt leaf cross sections given by Pryor (1957), stomatal
TABLE 7.1. Height of rise ($h$) and rate of rise ($\frac{dh}{dt}$) at height $50\mu$ calculated from equations (3) and (4) respectively, for various values of $r$, $\gamma$, and $\theta$.

<table>
<thead>
<tr>
<th>$\theta$</th>
<th>$\gamma$ (dynes cm$^{-1}$)</th>
<th>$r$ (µ)</th>
<th>$h$ (cm)</th>
<th>$\frac{dh}{dt}$ (cm sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70°</td>
<td>75</td>
<td>1µ</td>
<td>523</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5µ</td>
<td>105</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10µ</td>
<td>52</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1µ</td>
<td>209</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5µ</td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10µ</td>
<td>21</td>
<td>51</td>
</tr>
<tr>
<td>40°</td>
<td>75</td>
<td>1µ</td>
<td>1172</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5µ</td>
<td>234</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10µ</td>
<td>117</td>
<td>287</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1µ</td>
<td>469</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5µ</td>
<td>94</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10µ</td>
<td>47</td>
<td>115</td>
</tr>
</tbody>
</table>

$g = 980$ cm sec$^{-2}$  
$\rho = 1.0$ gm cm$^{-3}$  
$\eta = 0.01$ poise
pore depths (i.e. depth of the sub-stomatal chamber) ranged from 45µ to 75µ. \( \frac{dh}{dt} \) is strictly monotonic decreasing to an asymptote at 0 where \( h_t = h \). The rate was calculated at a height rise of 50µ which would be a realistic level for relating to the depth of stomatal pores. The rates were very fast under all conditions.

It is therefore concluded that, if the specified idealized situation is accepted, the argument supporting a general exclusion of solutions with surface tensions close to that of water from pores similar in dimensions to those of stomata is not supported by the theoretical considerations of the rise of a liquid against gravity in a capillary tube. However, it does not automatically follow that penetration via stomata of such solutions may occur. This is because the idealized situation necessary for the application of the capillary rise equations is far from the actual.

Stomatal pores are not open at both ends but are closed internally. If the stomatal cavity were airtight, then any entering solution would have to compress the air thereby creating a pressure that would resist entry. However, as stomata are the inlet and outlet ports for the gaseous exchange system of the leaf, it is unlikely that this is so.

The contact angle of stomatal pore walls may be different from that on the external surface. Stomatal pore walls are porous and probably more so than the external surface because of the thinner and more hydrated cuticle. The contact angle on porous surfaces
should be smaller than on non-porous surfaces (Adam 1948), and this should assist penetration (Adam and Jessop 1925). Stomatal pore walls are also less waxy and smoother than the external surface, which should also mean a smaller contact angle on the walls. If the surface of the cavity walls is wet, or movement of the applied solution along the walls precedes the mass flow of solution into the cavity, then the advancing contact angle would be moving over a wet surface and would therefore approach its receding value, which is generally less.

If the droplet is on the upper surface of a horizontally held leaf, stomatal entry would be gravity assisted. As r increases, capillary forces would decrease and the component of gravity assistance would increase. For the values of r approximating that of stomata, the gravity assisted component of the penetrating force would be small compared to that of the capillary forces. However, in situations where \( \theta \) is close to 90°, the gravity assisted component may be sufficient to give penetration that would not have occurred on the under surface.

Stomatal pores are neither circular in cross section nor uniform in bore: also they may change in shape and dimensions during the penetration process. Adam (1948) considered that 'the theory of penetration into capillaries of complex shape is difficult'. He mentioned that extra pressure was needed for a solution to pass a constriction in a bore, and showed in a
geometrically idealized situation, that if the contact angle on the bore walls on the approach side of a constriction were less than the angle between the bore wall on the other side of the constriction and the cross sectional direction, then penetration could proceed. It seems therefore that stomatal shape is another factor that could prevent the entry of solutions into stomata. Martin and Juniper (1970) suggested that if stomatal cavities were infiltrated by rain, then they would be waterlogged for much of the year thereby greatly reducing gaseous exchange. Therefore, it could be to the advantage of the plant if water were unable to enter stomatal cavities. However, if water is excluded from the stomata of a particular species, it is likely to be due to a combination of wettability and stomatal shape, rather than surface tension and stomatal dimensions.

This highlights the lack of knowledge concerning the three dimensional shape of stomata, how it varies with different degrees of stomatal opening, and its relation to stomatal entry of solutions. Like wettability, stomatal shape varies markedly between species, and this emphasizes the danger in making generalizations on whether or not stomatal entry of a particular solution will occur. Stomatal shape can also vary on a particular leaf surface, which could result in some stomata being infiltrated while others are not.

Most workers concede that penetration via stomata of aqueous solutions containing a suitable surfactant can occur. This largely follows from the work of
Dybing and Currier (1959, 1961) and Currier et al. (1964) who demonstrated the entry of a fluorescent tracer into stomatal pores in the presence of a suitable surfactant. Such work prompted Franke (1967) to comment 'By using detergents this passage may, of course, be experimentally induced'. His general attitude, however, is reinforced by the immediately following statement 'Thus, under natural conditions absorption of solutes must take its regular course by the penetration of cuticles'. This infers that the use of surfactants is an unnatural condition in any foliar application of solutions. This is difficult to understand. The incorporation of surfactants in solutions for foliar application is the usual commercial practice, and it seems logical that the acknowledged most efficient use should be that subject to experimental investigation. Further, although surfactant action has been extensively examined, very little attention has been given to examining the role of stomata in this action.

A recent review by Robertson and Kirkwood (1969) suggested that the work of Weaver and de Rose (1946), Fogg (1948b), Bennett and Thomas (1954), and Teubner et al. (1957) showed that aqueous solutions (because of their high surface tensions) do not penetrate open stomata. This work will be critically examined.

Weaver and de Rose (1946) sprayed potted Nasturtium and Coleus plants (which have astomatous upper leaf surfaces) with 0.1 per cent of the ammonium salt of 2,4-D. Some were sprayed such that the upper
leaf surface only received the spray droplets, and the others sprayed so that the lower leaf surface only received the spray droplets. After 23 days the plants were harvested, and the fresh weight of the leaf blades was determined. They found the degree of inhibition of growth to be about the same between the upper and lower treatments, and on this basis they suggested that stomata were not important portals of entry. However, the degree of inhibition of growth and not uptake was measured, and the relationship between uptake and inhibition (which cannot be assumed) was not given. The amounts of liquid retained on the upper and lower leaf surfaces after spraying were unspecified. Similarly, the nature of the upper and lower leaf surfaces and their relative wettabilities were unspecified. In fact, no quantitative results of this experiment were presented. The data given were not sufficiently conclusive to merit a general acceptance of the principle that stomata are not entered by solutions with surface tensions close to that of water.

Bennett and Thomas (1954) measured the absorption of schradan by upper and lower surfaces of Coleus, broad bean, apple, and chrysanthemum leaves. Absorption by intact leaves was measured over a period of 3 to 7 days, and by detached leaves over a period of 2.5 hours. There was no difference in absorption between upper and lower surfaces of Coleus and broad bean leaves, but the lower surfaces of apple and chrysanthemum leaves absorbed considerably more than the upper. With the exception of
Coleus, no information on the stomatal densities on the upper and lower surfaces was given. The authors claimed that the results from the detached leaves 'in which the stomata are probably closed' suggested entry was through the cuticle. However, this assumption was not tested under the prevailing experimental conditions.

They stated that Coleus had no stomata on the upper surface, and that the similar absorption rates by both surfaces of Coleus leaves indicated the unimportance of vapour phase entry through the stomata. However, in contrast, Sargent and Blackman (1962) stated that Coleus had one stoma adaxially for every eleven stomata abaxially, and they found absorption of an aqueous solution of 2,4-D to be greater by the abaxial than the adaxial surface, particularly in the light.

Teubner et al. (1957) reported greater absorption of nutrients by upper than lower surfaces of bean leaves (which have more stomata on the lower surface). However, this is hardly evidence that the stomata were not infiltrated under these circumstances, as retention, wettability, and cuticular penetration could differ between the surfaces.

Fogg (1948b) claimed that solutions within sub-stomatal chambers should be visible under a direct-illumination microscope. He found no such injection of 3,5-dinitro-o-cresol solutions and suspensions into Sinapis arvensis L. stomata. Additionally, in experiments with epidermal strips, Fogg found the majority of open stomata remained uninjected. However,
no experimental details or data were given here. Presumably stomatal shape and wettability of the surfaces prevented entry of the solutions. Fogg cited Adam (1948) in support of his findings but, unlike others, he appeared to recognize that it was Adam's theoretical treatment of shape ('the effect of a constriction') which was applicable.

Herrett and Linck (1961) found amitrole penetrated leaves of Canada thistle and field bindweed at different rates. On the grounds that these species have a similar number of stomata per unit leaf area but cuticles of a different nature, it was suggested that penetration was primarily cuticular. To justify this conclusion, the stomata of both species should be morphologically and physiologically similar, they should have the same degree of opening at the same time, the gross leaf surface should contribute to their wettability to the same extent, and the different cuticular surfaces in the absence of stomata should be penetrated at different rates. As none of these conditions was established, the suggestion has very little experimental support.

Sargent (1965, 1966) questioned the significance of stomatal entry. The relevant experimental work with which he has been associated will be critically examined.

Sargent and Blackman (1962) concluded that penetration of Phaseolus leaf discs by 2,4-D largely took place through the guard and/or accessory cells. They concluded this because the ratio between the rates of penetration into the abaxial and adaxial surfaces, in
both light and dark, was similar to the ratio between the stomatal densities (approximately 5:1). An underlying assumption for this conclusion was that the stomata were closed in the dark, but no experimental data were presented to support this. In fact, their basic experimental technique (i.e. application of herbicide to leaf discs in petri dishes under high humidity) was very nearly identical to that used in the present work where stomatal resistance measurements indicated that eucalypt stomata were not completely closed in the dark.

Sargent and Blackman (1962) also presented data on the uptake of 2,4-D by Phaseolus leaf discs under different pre- and post-treatment illumination schedules. The experiment appeared to have been designed to create different degrees of stomatal opening, and the uptake recorded seemed to be related to the theoretically expected (but not experimentally determined) degree of stomatal opening. However, a relationship between uptake and stomatal opening was not conceded. In a further experiment they found that 2,4-D penetrated the abaxial and adaxial surfaces of Coleus leaf discs at an equal rate in the dark, but that in the light, this rate doubled on the adaxial surface and increased 27 fold on the abaxial surface. These data strongly suggest that penetration via stomata occurred. Sargent and Blackman conceded that 'At first sight these findings might be regarded as substantial evidence in support of physical penetration of a solution of 2,4-D through stomatal pores'.
However, they dismissed this possibility by referring to Adam (1948) who, they claimed, showed that liquids with a surface tension close to that of water cannot penetrate open stomatal pores. As discussed previously, such a conclusion does not necessarily follow from Adam's work, particularly as penetration into the inverted abaxial surfaces as used in these experiments was gravity assisted.

Sargent and Blackman (1962) further reasoned that if the increased penetration of 2,4-D in the light were due to penetration via stomatal pores, then surfactant addition should enhance this movement. They compared the uptake of 2,4-D solutions, containing either Tween 20 or no surfactant, by the abaxial and adaxial surfaces of Phaseolus leaf discs in both light and dark. They found that increases in the rate of penetration of 2,4-D with Tween 20 over that with no surfactant addition ranged from 46 per cent to 251 per cent. The proportional increase in penetration of the abaxial surface was greater, and of the adaxial surface less, in the light than in the dark. However, the absolute increase in penetration of both surfaces was greater in the light (approximately 2.5 times). These data appear to support rather than contradict the principle of stomatal entry as implied by Sargent and Blackman. They suggested that, as Tween 20 promoted penetration in the dark, this supported the proposed relationship between penetration and stomatal density, thereby strengthening the case for penetration through guard
and/or accessory cells. However, this again assumed that the stomata were closed in the dark under the prevailing experimental conditions, an assumption that was not experimentally verified.

Sargent and Blackman (1965) examined the rate of penetration of 2,4-D into leaf discs of Ligustrum, a species having abundant stomata on the abaxial surface but none on the adaxial surface. Uptake by the abaxial surface was greater than by the adaxial surface in both light and dark. Light increased the uptake by both surfaces, and the absolute increase in uptake by the abaxial surface was greater than by the adaxial surface. Light obviously increased cuticular uptake as there are no stomata on the upper surface. However, this does not deny the possibility that the greater increase in uptake by the abaxial surface in the light was due to the contribution of penetration via stomata.

Sargent (1966) mentioned that although stomata are usually plentiful on the under surface of leaves, they are often less, and sometimes absent, on the upper surface which would receive more droplets in a spray application under field conditions. However, penetration via stomata could still be important under these conditions, and could make considerable difference to the economy, efficiency, and consistency of a particular herbicidal application. (Ormrod and Renney (1968) examined the stomatal distribution on 35 weed species and found that, while none had astomatous upper surfaces, twenty had significantly more stomata on their
lower than their upper surface). Then there are those leaves (as in the eucalypts used in this study) where stomata are present in equal numbers on both sides of the leaves. Such leaves often hang vertically, and each side would have the same opportunity of retaining droplets after spray applications. Under these circumstances, penetration via stomata could form a very significant part of total foliar uptake. It is difficult to reconcile Sargent's (1966) opinion that penetration of under surfaces was unimportant under field conditions, with his concentration in experimental work on measuring penetration into inverted abaxial surfaces (where penetration would be gravity assisted).

The aim of this discussion is not to prove that aqueous solutions do in general enter stomata: in fact it is possible that more often they will not (see Fogg 1948b, Dybing and Currier 1959, 1961, Foy 1962, Currier et al. 1964). The aim is rather to discuss the physical factors involved in penetration via stomata, and to suggest that generalizations on whether stomatal entry will or will not occur under particular circumstances is invalid without experimental support. In other words, the maxim that 'solutions with a surface tension close to that of water cannot penetrate pores similar in dimensions to those of stomata' is misleading in terms of the physical factors involved, and is a rule that may well have exceptions.

On the basis of the experiments and arguments presented, it is suggested that entry of solutions into leaves via stomata depends on:
(i) The wetting of the leaf surface and the stomatal pore walls by the applied solution.

(ii) The shape of the stomata and their immediate surrounds.

It is further suggested that the amount of penetration via stomata depends on:

(i) The volume rate of entry into the stomatal cavity.

(ii) The volume or 'reservoir capacity' of the stomatal cavity.

(iii) The volume rate of removal from the stomatal cavity by penetration through the cavity walls.

If the rate of removal is greater than, or equal to, the rate of entry, then the amount of penetration would be proportional to the volume rate of entry. However, this is unlikely as the rate of entry into stomatal pores would be very fast. If the rate of removal is the limiting factor then, in the presence of a continual supply of solution, the amount of penetration would depend on the surface area of the cavity walls (providing there are no preferential penetration sites in the wall). If stomata act mainly as reservoirs and contain the solution in a protected environment for subsequent penetration over an extended period, then the amount of penetration would depend on the volume of the stomatal cavity.

If changes in stomatal aperture are accompanied by changes in cavity wall area or cavity volume, then the amount of penetration via stomata in the above-mentioned
circumstances is likely to be correlated with stomatal aperture. However, there is a lack of knowledge of the changes in shape and dimensions of stomatal cavities throughout the opening or closing process. Observation of longitudinal sections through some stomata suggest that volume and wall area would not change a great deal during opening or closing. Under these circumstances, a correlation between uptake and stomatal aperture could be less evident. Also in the opening and closing process there could be shape changes which could dictate whether or not stomatal entry occurred. Therefore, significant penetration via stomata which is not correlated with stomatal aperture could occur. If shape is not considered, partially closed stomata would exert a greater capillary pull than fully open stomata, and would therefore be more likely to be entered by solutions. However, the acuteness of the angle of constriction may increase as stomata close thereby making penetration less likely on the basis of shape.

Increased wettability of a leaf surface and the stomatal pore walls by applied solutions can induce stomatal entry that otherwise would not have occurred, and increase the pressure and rate of entry of solutions that would have entered. The factors of wettability and penetration via stomata are therefore interrelated. There was a good correlation between uptake and wettability in the work presented here, and it is possible that at least part of the increase in uptake with increase in wettability reported was due to increased penetration via stomata.
Direct evidence of stomatal entry might best be obtained using micro-autoradiography. The micro-autoradiography of soluble labelled materials is a difficult procedure. Pickering (1965) developed a technique using emulsion which is satisfactory for foliar penetration studies using labelled herbicides. In conjunction with the work presented in this thesis, attempts were made to develop a technique using stripping film. Among various techniques attempted, a modification of that described by Wangermann (1970) showed most promise, but was not developed sufficiently for use in this work.

7.3. Conclusion

In this thesis, the results from many experiments could be explained in terms of penetration of picloram via stomata. The likelihood of this mode of entry was examined in some detail by measuring stomatal aperture under a variety of experimental conditions, and attempting to relate this to the relevant uptake data. In many instances, good correlations were found, and were at least sufficient to encourage the idea of penetration via stomata. Critical appraisal of the literature questioning stomatal penetration suggested that much of this is based on false premises. It is considered that uptake of herbicides via stomata could be an important component of herbicide action, and one which should receive considerably more attention.
REFERENCES


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<table>
<thead>
<tr>
<th>COMMON NAME</th>
<th>CHEMICAL NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>amitrole</td>
<td>3-amino-1,2,4-triazole</td>
</tr>
<tr>
<td>atrazine</td>
<td>2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine</td>
</tr>
<tr>
<td>dalapon</td>
<td>2,2-dichloropropionic acid</td>
</tr>
<tr>
<td>diquat</td>
<td>6,7-dihydrodipipyrido[1,2-a:2',1'-c] pyrazinedium ion</td>
</tr>
<tr>
<td>diuron (=DCMU)</td>
<td>3-(3,4-dichlorophenyl)-1,1-dimethylurea</td>
</tr>
<tr>
<td>DNBP (=dinoseb)</td>
<td>2-sec-butyl-4,6-dinitrophenol</td>
</tr>
<tr>
<td>EPTC</td>
<td>S-ethyl dipropylthiocarbamate</td>
</tr>
<tr>
<td>MCPA</td>
<td>[(4-chloro-o-tolyl)oxy]butyric acid</td>
</tr>
<tr>
<td>paraquat</td>
<td>1,1'-dimethyl-4,4'-bipyridinium ion</td>
</tr>
</tbody>
</table>
| phenmedipham | methyl m-hydroxycarbanilate  
|             | m-methylcarbanilate |
| picloram    | 4-amino-3,5,6-trichloropicolinic acid |
| simazine    | 2-chloro-4,6-bis(ethylamino)-s-triazine |
| TCA         | trichloroacetic acid |
| 2,4-D       | 2,4-dichlorophenoxyacetic acid |
| 2,4,5-T     | 2,4,5-trichlorophenoxyacetic acid |