THE DISTRIBUTION AND ECOLOGY OF *BUDDLEIA DAVIDII* FRANCH. IN BRITAIN, WITH PARTICULAR REFERENCE TO CONDITIONS SUPPORTING GERMINATION AND THE ESTABLISHMENT OF SEEDLINGS.

by

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BSc.

A thesis submitted in partial
fulfilment of the requirements of the
Council for National Academic Awards
for the award of Doctor of Philosophy

Department of Biology

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June 1984

In collaboration with the
Department of Forestry
University of Oxford

CONTENTS.

		PAGE
ABSTRACT		i
ACKNOWLEDGEMENTS		ii
1: INTRODUCTION		1
1:1	Plant structure and floral morphology	2
1:2	The history of the name *Buddleia*	14
1:3	The classification and evolutionary history of the genus *Buddleia*	16
1:4	The introduction of *Buddleia* in Europe	21
1:5	The spread of naturalised buddleia through Britain	25
1·6	Buddleia as a component of the wall community	30
1:7	Secondary compounds and herbivory in buddleia	33
1:8	Aims of these investigations	35
2: SOIL ANALYSES AND DEMOGRAPHIC SURVEYS		
2:1	Introduction	38
2:2	Soil analyses: materials and methods	
2:2·1	Sample sites	38
2:2:2	Mechanical analysis of the soils using sedimentation techniques	39
2:2:3	Soil pH	42
2:2:4	The measurement of exchangeable calcium and potassium ion content and the estimation of the cation exchange capacity	42
2 3	Results and discussion of soil analyses	43

2:4 Survey of natural populations: the site 56

2:5 Materials and methods of measurement 60

2:6 Analysis and interpretation of the distribution data 64

3: SEED PRODUCTION AND DISPERSAL

3:1 Introduction 76

3:2 Self-pollination 77

3:3 Seed production 79

3:4 Seed viability and innate dormancy 80

3:5 Seed dispersal: the timing of release 87

3:6 Seed dispersal patterns in space 94

4· LABORATORY SEED TESTING

4·1 Introduction 104

4:2 The process of germination 105

4·3 Germination and development of buddleia seeds 107

4:4 Temperature requirements for germination 108

4:5 Light and temperature interactions 113

4:6 Low temperature effects on imbibed seeds· the impact on viability 117

4:7 The maintenance of viability of seed stored on the bush 120

4:8 The influence of oxygen supply on germination 123

4:9 The effect of pH on germination of buddleia seeds 129

4:10 The effect of depth of burial of seed on the emergence of seedlings 132

4.11 Allelopathy 133

4:12 A summary of the findings on germination 138

5· THE EFFECT OF WATER STRESS ON GERMINATION AND SEEDLING SURVIVAL

5 1 Introduction 140

5 2 The choice of medium used for studies of water stress effects 141

5·3 The germination of buddleia seeds under moisture stress 144

5:4 The effect of water stress on pre-emergent seedling growth 150

5:5 The effect of drought on post-emergent seedlings 155

5:6 Conclusions about the effects of water stress on seeds and seedlings 170

6: THE FATE OF THE SEEDS AND SEEDLINGS IN THE FIELD

6:1 Field investigations 172

6:2 The existence of a soil seed bank incorporating buddleia 172

6 2·1 Testing for a persistent buddleia seed bank in the field 173

6:2:2 The fate of buddleia seeds in an experimental seed bank 174

6:3 Conclusions: the fate of buddleia seeds in soil and the kind of seed bank formed 186

6:4 Survey of seedling emergence and survival on 2 experimental sites

6:4:1 The design and techniques used in the survey 188

6:4:2 Discussion of observations at site D 204

6:4:3 A comparison of the results from the 2 sites 212

6:4:4 Conclusions 219

7: DISCUSSION OF RESULTS IN RELATION TO THE ECOLOGY AND DISTRIBUTION OF BUDDLEIA IN BRITAIN

7:1 The ecological role of external factors 222

7·2 Suggested reasons for the patterns of growth observed in the field 229

7:3 A comparison with demographic studies of other shrub species 234

7:4 The growth strategy adopted by buddleia 237

7·5 Conclusions and predictions of future spread in Britain 244

REFERENCES 251

APPENDIX 1 I

ABSTRACT.

The distribution and ecology of Buddleia davidii Franch. in Britain, with particular reference to conditions supporting germination and the establishment of seedlings, by A. Miller.

The history of the introduction of buddleia to Britain and its subsequent spread as a self-established species is reviewed, together with the current classification of the genus Buddleia. Its distribution in Britain and its place in the eu-atlantic mural community is described.

Analysis of soil from beneath many self-established plants reveals that buddleia is not an obligate calcicole and can colonise soils of varied nutrient status. Surveys of natural populations show that adults can grow at high densities with little evidence of self-thinning; however, the difficulties of deciding what constitutes an individual are discussed in relation to shrubs.

To identify the life history strategies of buddleia, annual seed production, and spatial and temporal patterns of seed dispersal, are monitored and combined with data from extensive laboratory tests. These tests indicate that the only significant factors influencing germination are the depth of seed burial and the water supply available for imbibition and post-germinative growth. The existence of a persistent soil seed bank is demonstrated and the rate of depletion of seeds from experimental banks is recorded.

A survey of seedling emergence and survival over fifteen months from a series of monthly sowings is described, and the data related to the results of the laboratory studies. The survey results confirm that the seedling needs approximately 4 weeks of plentiful water supply before it develops significant drought resistance.

The strategies of buddleia in the established and regenerative phases are discussed in relation to the small amount of work published on other shrubs. The need for more life history studies on shrubs and novel urban colonisers is noted. Finally, predictions are made of its potential for further spread in Britain.

ACKNOWLEDGEMENTS.

I would like to thank my supervisors in this research, Dr. Denis Owen and Dr. Ian Cumbus, for all their guidance, advice and patience over the past 4 years. I am also greatly indebted to many other staff, academic and technical, for assistance throughout my stay at Oxford Polytechnic, particularly to Dr. Peter Morris and Dr. Allister Smith for inspiration, many invaluable discussions, and constructive criticism, as well as friendly support! Among the technicians thanks are particularly due to Derek Whiteley and Barbara Southall for aid and advice with the photographs and graphics, and to Di Cox and Judy Barrow for technical help along the way.

Numerous past and present students and post-graduates have contributed in multifarious ways, creating a congenial and stimulating atmosphere for research (and relaxation!), including Paul Walker, Tim Shreeve, Carol Parsons and Dave Duthie: I thank them all sincerely. I am particularly grateful to Olly Watts for field assistance (sweated labour!) on the hottest summer days, and to Ian Bingham for providing information and a listening ear on many occasions.

Thanks are due to Dr. Phillip Grime of the Sheffield N.E.R.C. Unit and Dr. Steven Prince of Queen Mary College, London, who were generous with their time and advice in the early stages of this work, and to Dr. Stan Woodell and Dr. Mike Koziol of the Botany School, Oxford, for ideas and technical advice. Similarly, I must thank the staff and members of B.B.O.N.T., the local naturalist's trust, who allowed me extensive use of their nature reserve at Dry Sandford. Mr. Mather and the staff at British Rail (Oxford) were most helpful in

locating suitable sites for several studies and kindly granted me access to their land at various locations. I thank them, and the staff of Mainland Car Deliveries in Forest Hill, who were always interested in my experimental site on their land.

The members and the general secretary, Mrs. Mary Briggs, of the B.S.B.I. furnished me with records of naturalised buddleia, and I am especially grateful to them for introducing me to the site at Slindon in Sussex which proved to be very important for this work.

Finally, and most importantly, I must thank my parents, and Alison Norris and Barbara Beedham for their unflagging support, assistance in the field and sheer tolerance over a very long period: I would never have made it without them all!

CHAPTER 1: INTRODUCTION.

Buddleia davidii (hereinafter called buddleia) is one of the most striking plants found on wasteland in Britain. Its profuse, heavily scented, purple flowers immediately attract attention. It is also a large shrub that often grows out of old walls where other vegetation is generally small and stunted.

The plant was introduced to Europe from China at the end of the 19th century (Bean 1970), along with many other shrubs, including Rhododendron which has also successfully escaped into the wild in Britain. With its conspicuous flowers and rapid growth buddleia soon became popular, and plants were transported to various parts of the country, including Ireland, to be cultivated in private gardens. Its flowers are extremely attractive to all sorts of insects, including butterflies, hoverflies and bees. This, together with its readiness to grow in almost any soil, has made buddleia a favourite with gardeners and naturalists. It is now constantly replanted and introduced to new areas and has earned itself the common name "butterfly bush".

The shrub is a native of Hupeh and Szechwan, hilly regions in Central and Western China. Shrubs form the predominant vegetation on the slopes and species of Buddleia are found growing at altitudes of 3600 m, although B.davidii grows only up to 2700 m, and occurs chiefly on the shingle banks of rivers. Farrer, one of the early explorers, reported that buddleia thickets provided "famous harborage for leopards" (Coats 1969).

The family Buddleiaceae is unrepresented in Europe, which makes the successful spread of buddleia particularly interesting on two

accounts. Firstly, it has been able to find and exploit a niche without becoming a weed like so many other alien escapes. Secondly, it has acquired a small but distinctive fauna which feeds on it, which is remarkable for such an alien species (Southwood 1984).

This study is concerned wih the first of these and aims to account for the limited success in colonisation of Britain by buddleia, by investigating the range of conditions under which it germinates and grows, and to relate these to its occurrence in the wild.

1:1 Plant structure and floral morphology.

Individual plants may grow up to 5 m where conditions are good, though most are smaller than this: shrubs growing in guttering or walls are usually 1-2 m tall. It is difficult to define exactly what are "good" conditions for buddleia: it grows as vigorously on a pile of building rubble, a heap of limestone chippings, sand or gravel as on the well-balanced loam which Bean (1970) suggests is the ideal substrate for a transplanted seedling. Similarly, it grows on exposed areas such as sea cliffs as well as in more sheltered locations in towns and woodlands, where the largest shrubs occur.

Some individuals have a spreading habit, repeatedly shooting from the base so that the total trunk diameter is 40-50 cm, with the whole plant extending over an area of 2-3 m^2. Many others comprise only a few slender stems which spread over less than 1 m^2. The flowers are also variable, arranged in a cymose inflorescence, forming narrow terminal panicles 10-30 cm in length, some densely massed, others sparsely assembled around the stem. Each shoot normally bears one

large terminal panicle with two smaller lateral panicles arising directly behind it. A range of different phenotypes is often apparent in one stand of buddleia.

The flower colours of garden cultivars vary from white to yellow and red in addition to the more common lilac and purple, yet it is only these last two which occur widely in the wild. Each flower is made up of 4 petals which are fused for three-quarters of their length into a corolla tube. Once again, individual specimens show considerable variation: some plants bearing mostly 4-petalled flowers also have variants possessing 5-9 petals in one flower. These anomalies generally occur in the middle of the inflorescence. Figures 1.1 and 1.2 show normal and mutant flowers and a bilateral section through the normal flower.

A typical flower is zygomorphic, possessing 4 stamens with filaments fused to the corolla wall and the anthers situated about two-thirds along the length of the tube. The corolla tube is 5-8 mm long, opening at the top to form separate petals. This part of the petal is purple as is the outside of the corolla tube, but the interior is orange with a series of yellow nectar guides leading into it over the purple petals. The guides are also marked by a number of fine hairs which extend down the length of the corolla and are particularly dense around the top. The superior ovary is bilocular with a stigma and style that extend along one third of the tube, ending well short of the anthers. There is a small ring of sepals around the base: as the flowers form, the corolla tube elongates until it is 3 or 4 times the length of the sepals, before the petals finally open.

Flowering is not synchronous: those at the base of the



Figure 1.1 Section of a buddleia inflorescence showing normal 4-petalled flowers and mutants with 5-8 petals.



Figure 1.2 A bi-lateral section through a buddleia flower, showing the long hair-lined corolla tube, the short style and the longer stamens.

inflorescence open first, so that in any one panicle there may be dead flowers at the base while it is flowering in the middle, with unopened terminal buds. The lateral panicles generally flower after the terminal one. The flowering season on any bush extends over several weeks and variation between plants and sites means that the flowering season lasts from July to October. Both the flower and the seed capsule have short stalks and are consequently borne upright. This favours both the attraction of pollinating insects and later, the dissemination of seed from the open capsules.

After fertilisation seed formation and ripening are rapid, seeds being mature in October-November, although they are not usually shed until the following spring. They are borne in a cylindrical two-valved capsule which is 5-8 mm long. The arrangement of the seeds in the capsule is shown in Figure 1.3; they are tightly packed, with their long axes aligned with the axis of the capsule. The embryo of the seeds lies straight in a fleshy endosperm (Hutchinson 1959) which is cellular, not nuclear, as in the family Loganiceae (Bendre 1975), which is of some significance in the taxonomy of the genus. The ovule alone is about 1 mm long but the axile placentas which extend on either side form wings so that the total dimensions of the dispersal unit are about 3 x 1 mm. Figure 1.4 shows a typical seed, isolated from the capsule.

Seeds are released only when the capsules dry out and the distal ends open outwards exposing them to the air. Wind or other movement of the branches then shakes them free from the capsule and with the small seed and relatively large wings typical of wind-dispersed plants each propagule may travel a great distance before reaching the ground. Interestingly, in wet weather the ends of



Figure 1.3 Buddleia seeds tightly packed in an opened capsule.

(Magnification approximately x 25).



Figure 1.4 Buddleia seeds. (Magnification approximately x40).

the capsule rapidly move back to close it, preventing the seed from being shaken out in conditions where wind-dispersal is not effective. Since it takes a lot longer to dry out and re-open than it does to shut, the actual shedding of seed may take place over a prolonged period. Figure 1.5 shows an infructescence before ripening, and Figure 1.6 shows the appearance once it has dried out and is releasing seeds.

The leaves are lanceolate or oval lanceolate, 5-20 cm long and 1-7 cm wide, accuminate with serrated margins and arranged in opposite pairs, with a covering of fine hairs on both surfaces. The hairs are sparse and become glabrous on the upper surfaces, giving them a dark green appearance, but dense on the lower surface, making this whitish and tomentose (Clapham, Tutin and Warburg 1965). The calyx and stems of the flowers and the short pedicels are often tomentose: this is most conspicuous during the winter. The shrub puts out a small flush of leaves during the autumn which are smaller than the spring growth and densely covered with downy hairs. Although there is at least one evergreen species in the genus, Buddleia davidii is partly deciduous since it loses all its leaves in the autumn but replaces them immediately with a second growth which persists until the following autumn, and is added to during the spring. The hairs are also noticable on the new spring shoots and stems but they disappear from most of the leaves as the year progresses.

In fact there are two types of hairs present: stellate and glanduliferous hairs, features which are also important in the taxonomy of the plant. All over the stem and leaves there are tiny crystals borne on the glanduliferous hairs that secrete them: they give the characteristic sheen to the leaves that enables the seedlings to be distinguished readily from other species. The function of these



Figure 1.5 An unripe buddleia infructescence, with tightly closed seed capsules.



Figure 1.6 A buddleia infructescence in dry conditions, capsules open to release seeds.

crystals is not known: one possibility is that they enable the plant to tolerate high salinity levels at seaside localities. Another is that they confer some degree of drought resistance by creating an increased osmotic gradient from the leaves to the roots.

Segal (1969) claims that buddleia is a nanophyte, according to Raunkaier's system of classification, but a notable feature of the plant is that the main meristem is not carried aerially as in many woody species, but is situated below ground level. This means that there is no main trunk in the mature individual but a number of older stems which grow from underground. In favourable years the apical meristem of each of these continues to grow in successive springs, with little lateral branching and new growth concentrated behind the old seed heads. However, in a cold winter, such as that experienced in 1981-82, these aerial shoots die or are broken off and new shoots arise from underground in the spring. The wood is easily broken, particularly at the base of shoots, which snap readily in strong winds or under the weight of snow. Examples of such damage are shown in Figure 1.7. Horticulturalists recommend severe pruning of the bush in March, and removal of old wood leads to enhanced growth of new basal shoots, which may grow over 2 m annually. Pruning also produces denser and larger panicles, up to 50 cm long, and an extended flowering season.

Buddleia produces an extensive shallow rooting system in the first weeks after germination: the roots grow to lengths of several cm and branch several times before any shoot development occurs beyond the extension of the cotyledons. Subsequently a main root develops, extending down 4 m or more. For a plant which was originally introduced for garden cultivation it is remarkably tolerant of



Figure 1.7 Stems of buddleia, broken off at the base by winds and the weight of snow.

extensive disruption of its root system. Large, mature shrubs growing on spoil heaps have been moved from one part of a quarry to another, breaking away much of the fine root system in so doing, yet the plants continue to flourish in the new location, losing only those shoots that are supplied by the portions of root no longer in any contact with the soil. Apparently the roots like the shoots are capable of withstanding considerable damage without obvious effects on the life of the shrub, except to increase its reproductive output for the following season, which is a common plant response to environmental stress.

The possession of an underground meristem naturally suggests that the plant may be capable of clonal growth: walls and bare rock faces are hardly suitable for this, but in some quarry sites where there are dense thickets of Buddleia growing on old workings several apparently separate plants can all be traced to one rootstock. This may be due to root grafting in some instances or to suckering; in either case it presents further difficulties for carrying out population studies, an area which is already fraught for workers on shrubby species. The lack of an aerial meristem or main trunk makes it difficult to age a plant accurately; dating a stem from the growth rings does not necessarily reflect the age of the whole plant.

1:2 The history of the name Buddleia.

The first *Buddleia* to be described came from South America, collected by Sir William Houstoun in 1730-33. He suggested in a manuscript that the genus was named after the Reverend Adam Buddle. Buddle was sometime Rector of Farnbridge in Essex and a prominent

English botanist and contemporary of John Ray. He specialised in collecting mosses and grasses : his herbarium was reputed to be the finest in the country and was constantly referred to by other great botanists including Ray and Dillenius. He bequeathed the collection to Sir Hans Sloane and it now forms part of the Sloane herbarium. Buddle also wrote a 12-volume English flora which was a more extensive and reliable source than the Ray Synopsis of British Plants which had recently appeared, but he never published the flora, probably because after the universal acclaim of Ray's works he felt that the improvements in his would not be noticed.

Although Buddle did not publish anything his contibution to botany was widely acknowledged and Houstoun suggested naming Buddleia in his honour. The fact that he wrote the generic name in a manuscript subsequently created a lot of problems in spelling it. Linnaeus, who first published the name in his Species Plantarum (1753) and Genera Plantarum (1754), spelt it Buddleja. However, Houstoun may have been using the tailed "i" then prevalent when wishing to produce a consonant sound, in later years succeeded by a "y" (R. Elliot 1983, pers. comm.). Sprague (1928) notes that since Linnaeus was the first to describe and publish the name,by the international rules of nomenclature his spelling must be adhered to even though Buddleia or Buddlea would be a better spelling. While this is true it does not take account of the original Houstoun manuscript: if that included a proper description of the genus it may constitute the first publication and then his spelling should be the one to be followed. Clearly, there is a need to inspect this manuscript and to refer it to an expert on 18th century calligraphy before the issue can be finally settled.

1:3 The classification and evolutionary history of the genus Buddleia.

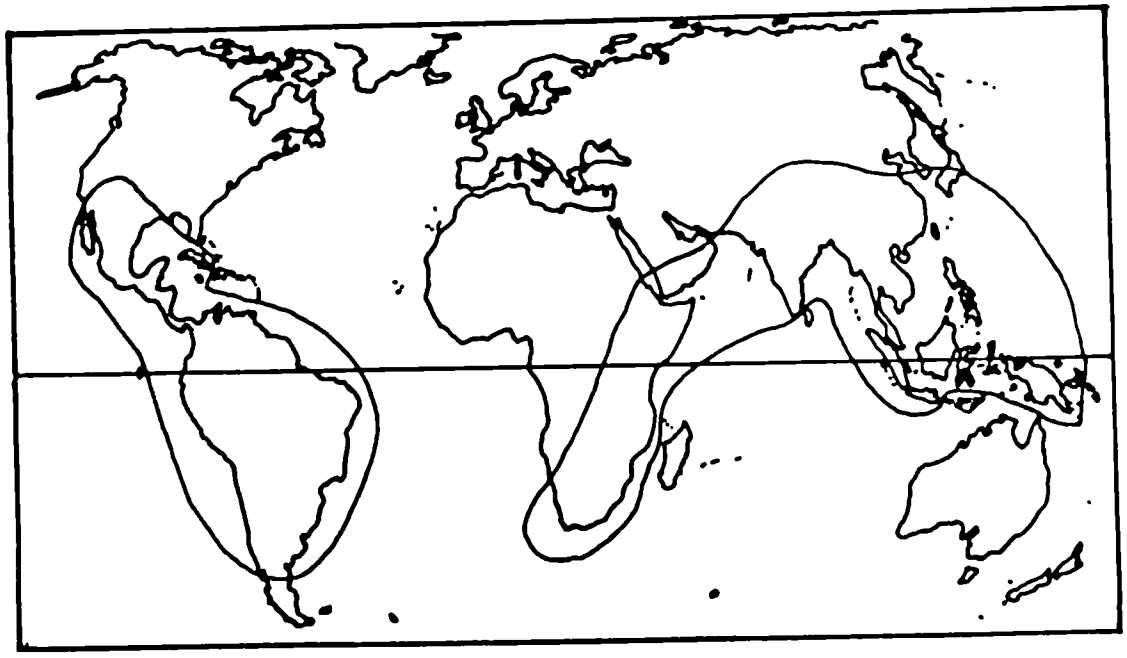
The genus Buddleia is listed in the Index Kewensis as comprising about 200 species but many of these are synonyms: a more accurate estimate, according to Moore (1947), is between 130 and 150 species. All of them are small trees or shrubs and they are widely distributed in tropical and temperate regions of Africa, Asia and the Americas, between the latitudes of 40o N. and 40o S. as illustrated in Figure 1.8 which is taken from Hutchinson (1959). Moore (1947) estimates that of the described species about 45% occur in South America, 23% in North America, 26% in Asia and only 6% in Africa.

Once again scientific debate has surrounded Buddleia, this time concerning the classification of the genus. It was originally ascribed to the family Scrophulariaceae by Don (1838) and Lindley (1846), but in 1857 Bentham placed it in the Loganiaceae while admitting its affinities to the former family. Embryological studies by Dop (1913) and Souèges (1940) indicates that it is very close to the Scrophulariaceae, although Solereder (1895) points out that the latter group does not possess stipules while Buddleia does. The family Loganiaceae is variously ascribed to the orders Loganales (Hutchinson 1959) and Contortae (Clapham, Tutin and Warburg 1965), but as Good (1974) comments "the order is too large a category to be of much practical importance", so that the family becomes the critical unit.

The Loganiaceae has 32 constituent genera mainly woody shrubs and vines from warm temperate and tropical regions in both

Figure 1.8 The origins of the Buddleiaceae: world distribution.

(after Hutchinson 1959)



hemispheres.It is widely regarded as an artificial group made up of genera related to the Scrophulariaceae, Apocynaceae, Gentianaceae and Rubiaceae. (Moore 1949). Solereder (1895 and 1897) proposes that it should be divided into two sub-families: the Buddleioideae and the Loganioideae. The former consists of 9 genera: Adenoplusia, Buddleia, Chilianthus, Emorya, Gomphostigma, Nicodemia, Nuxia, Peltanthera and Polypremum.

Wettstein (1935) raises the sub-families to family rank as Loganiaceae and Buddleiaceae. Hutchinson (1959) follows Wettstein in classing Buddleiaceae as a separate family, but he lists it as possessing only 8 genera. Polypremum and Peltanthera have been ascribed to two other families and Adenoplea has been described and added to the family since 1935. Of these members Nicodemia and Chilanthus are more closely related to Buddleia than are any of the others. Nicodemia is only separated by having a berry instead of a capsule as its fruit and Chilianthus has long, exerted stamens which Buddleia does not have. The origin of each genus as proposed by Hutchinson (1959) is summarised in Table 1.1.

Comparing this with the distribution map of Buddleia in Figure 1.8, it can be seen that all genera, except Emorya, are found in the Old World. More notably, Buddleia is found in all areas where the other members occurr, except in the Mascarenes. It is apparently replaced there by Nicodemia, which used to be known as Buddleia madagascariensis (Helmsley 1889).

The pantropical distribution of Buddleia and the limited extent of the other groups in the family indicate that Buddleia originated in Africa and the other genera evolved from it. As it spread from Africa many new species appeared in the new areas which it

Table 1.1 Origins of the genera in the family Buddleiaceae (excluding Buddleia)

Genus	Places of origin
Adenoplusia	Madagascar
Adenoplea	Madagascar
Chilianthus	South Africa
Emorya	Texas
Gomphostigma	Tropical and South Africa
Nicodemia	Mascarene Islands
Nuxia	Mascarene Islands and Africa

invaded. Since South America and Africa drifted apart about 100 million years ago, half way through the Cretaceous period, it appears that although Buddleia had evolved by then, speciation and the evolution of other genera occurred later. This speciation also suggests that it is a recently evolved family: a conclusion which supports the classification of the Loganales as one of the most advanced orders of the Lignosae (Hutchinson 1959). The floral morphology is advanced and it is adapted for pollination by Hymenoptera and Lepidoptera, the most specialised insects, which implies that the genus may have arisen in the early Tertiary when climatic segregation of floras was beginning so that seasonal variations in day lengths started to occur.

The photoperiodism and the polyploidy of the genus give further evidence of the age of the group. Buddleia can be either a short-day or summer-flowering plant, the majority of the short-day plants being diploid while the summer-flowerers are mainly polyploid. While polyploidy is regarded as an advanced characteristic, diploids may be primitive or advanced species. The photoperiodic habit is not directly altered by the level of ploidy but a change from the primitive short day habit to summer-flowering accompanies evolutionary advance. From this it is concluded that the original African stock was diploid and a short-day plant and this state, which is hardier than the polyploids, persists in those species which inhabit colder, more northerly latitudes (Moore 1961). The taxonomic position of buddleia was summed up a century ago in this way: "Throughout its whole area this genus represents such uniformity in structure that it has been designated one of the most sharply defined genera in the vegetable kingdom. Indeed, it is so distinct that it is not easy to determine its closest

relationship to other genera, and therefore there are different opinions as to its exact place in the natural system." (Helmsley 1889).

Cytological and morphological studies have provided further information for classification. Buddleia possesses inter- but not intra-xylary phloem, while the other members of the Loganiaceae have both; the latter group also have a nuclear endosperm but Buddleia has a cellular endosperm (Bendre 1975). Chromosomal analyses show that both Buddleia and Nicodemia have a haploid complement of 19 chromosomes and exhibit polyploidy. As a result of speciation there are 6x, 12x and 16x ploids as well as the most common diploid and tetraploid states. B. davidii is a tetraploid plant (Moore 1949).

One final area which is important in the context of taxonomy as well as in herbivory is the chemical composition of the plant and its secondary compounds. Buddleia possesses an iridoid, aucubin, which is also found in the Scrophulariaceae but not in the Loganiaceae (Paris 1963). This final piece of evidence appears to confirm the separate classifiction from the Loganiaceae and places the Buddleiaceae between these two larger groups.

1.4 The introduction of Buddleia in Europe.

The introduction of Buddleia to Europe and Britain is well documented. Buddleia globosa was the first member of the genus to arrive in Britain, sent from Chile in 1774 (Coats 1963). But it was not until the next century that most species were collected and cultivated in Europe. With the "invasion" of China by French missionaries and, in their wake, by Victorian traveller-naturalists, a

host of plants and animals which were new to Western science was collected and sent back to European centres for identification and description. Père David was responsible for introducing many of these species, sending back the plant specimens to Adrien René Franchet in Paris. In 1884 Franchet published his 2 volume Plantae Davidianae, including in it the Buddleia which he had recieved in 1869 and subsequently gave the specific name davidii, in honour of its collector. Helmsley, in England, was sent specimens of the same species from I-ch'ang by Dr. Augustus Henry, in 1887 (Bean 1970). Unaware of Franchet's description he called it B. variabilis; it was another 25 years before the prior claim of Franchet was recognised. The plant is still frequently referred to by Helmsley's name.

Buddleia seed (as opposed to specimens of the plant) was first introduced to Europe via Russia, collected by Russian explorers. The plants cultivated from these were weak , with poor flower colour and a semi-prostrate habit (Bean 1970). The early collections of seed are summarised in Figure 1.9. Helmsley (1889) reported that good seed was sent to England around this time, possibly by the Rev. E. Faber from Pa-tung, but that the plants thus produced had not yet flowered.This is probably the variety of B. davidii which Bean (1970) quotes as first cultivated in 1890 in Britain. In 1893, M. l'Abbé Jean André Soulie sent seed from Ta-tsien-lou to M.L.De Vilmorin in Paris. This produced plants that flowered in 1894 and were probably the source of the seed that De Vilmorin sent to Kew Gardens to produce the first flowering specimen in this country in 1896. Further collections of seed were sent from Mt. O'mei Shan in 1896 by Farges and in the following year from I-ch'ang, by Henry. The buddleia

Figure 1.9 The early collections of buddleia seed from China.

1. K'ang ting: Soulié 1893
2. Mt. O'mei Shan: Farges (record of de Vilmorin 1898)
3. Pa tung: (record of Helmsley 1889)
4. I ch'ang: Henry 1887 and Wilson early 1900's



seen in gardens today is probably derived from seed collected by E.H. Wilson between 1900 and 1908, from Hupeh and Szechwan in central China (Bean 1970). However, some of the wild plants may originate from the earlier seed collections or from hybrids with the original stocks.

The rise of the middle classes following the Industrial Revolution in Britain led to a large number of gardens being created in the middle of the 19th Century. Previously gardens had been the preserve of the landed gentry and aristocracy, usually involving large acreages of parkland and formal display gardens. Now smaller areas were enclosed in and around towns and filled with many ornamental plants. The exploratory and missionary activities that accompanied these social changes provided a convenient and extensive source of novel material for the new gardeners. As with all new fashions there was a strong element of competition in cultivating desirable and exotic plants in their gardens. Many new species were suitable for cultivation in the British climate, particularly those from the higher latitudes which required relatively little tending or shelter to persist where planted. They have since become standard features of the suburban garden, but remarkably few of these introductions from China and Japan have been able to persist in the wild in Britain. Salisbury (1942) lists only five: Lycium chinense (Tea plant), Petasites japonicus (Japanese Butterbur), 2 species of Polygonum and Buddleia davidii. Another shrub which originated further west in the Himalayas is Rhododendron, which has also escaped from gardens and become a weed in some parts of Britain, especially in forestry plantations.

1:5 The spread of naturalised buddleia through Britain.

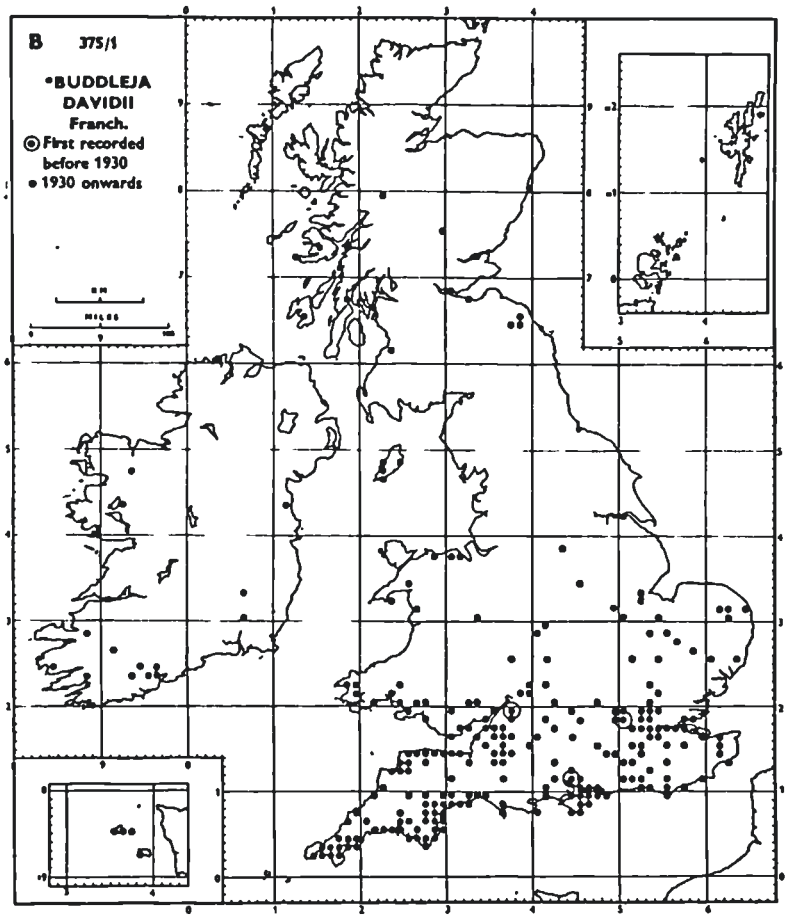
Although buddleia was planted in various parts of the British Isles at the beginning of the 20th century it was not until 1927 that there were any documented reports of the plant establishing itself outside gardens. The first record was from Middlesex, made by R. Melville and R.L. Smith (Kent 1975). Two other records were made pre-1930, one from Southampton and one from Gloucestershire where it was found in the Avon Gorge at West Nibley.

However it is difficult to assess whether these are genuinely the first occurrences of buddleia in the wild or if it was classed as a casual escape and hence overlooked in earlier reports. There is some evidence of the latter from the Flora of Cornwall in which it is stated that buddleia first appeared after the 1939-45 war, but Thurston (1930) recorded that it seeded freely in many Cornish gardens, which implies that it was regenerating naturally before 1930, though whether in the garden soil or on the walls or stony areas is not clear. Many of the county floras state that buddleia is under-recorded in their region, amateur botanists often excluding it from the check-list they submit to the Institute of Terrestrial Ecology recording scheme at Monks Wood. The scheme aims to produce national distribution maps for all indigenous and naturalised plant species by superimposing 10 x 10 km squares on the national grid and recording the occurrence of a species in each square. The resultant map for Buddleia davidii is shown in Figure 1.10.

Looking at this map the distribution appears to be concentrated over the south of England and in coastal regions. This may be partly a result of poor recording in other areas: new records have been made

Figure 1.10 The I.T.E. distribution map for Buddleia davidii in Britain.

(reproduced by permission of the I.T.E., Monkswood.)



since the map was drawn, so it probably occurs in more squares than shown, but the evidence of the county floras supports the overall appearance of the map. The frequency of wild buddleia is certainly highest in the south of the country.

A review of the county floras (listed in Appendix 1) shows that buddleia was reported as naturalised in Surrey, Dorset, Norfolk, Middlesex, and Angus in Scotland before the 1939-45 War. All the sources agree that it was only after this the population of buddleia underwent an explosion. This was a direct result of the bombing: newly created piles of rubble and crumbling masonry are ideal sites for this plant to colonise. Reports of it in Hertfordshire, Warwickshire, Bedfordshire, Cambridge, Oxford and Birmingham suddenly appeared and the numbers found in London, Bristol and the big southern cities increased markedly. However, in rural areas away from the coast it was much slower to invade. The first records for Nottinghamshire and Lincolnshire were in 1957, 1958 in Wiltshire, 1959 in Rutland, 1960 in Berkshire and not until 1969 in Derbyshire. There are still no reports of naturalised buddleia in Cheshire and Herefordshire and it is only rarely found in Monmouthshire. Interestingly, it has been wild in Scotland, which is near the limits of its growth, for many years. Apart from the Angus record of 1932, it is now recorded in E. Rosshire (1966), Kinross (1977), Moray and Inverness (1973). It is also recorded on several islands of the Inner Hebrides including Mull and Iona (1955).

Other interesting information to emerge from this review of the Floras concerns the types of substrate on which buddleia grows. It is most frequently reported to be growing in old walls, waste ground in urban areas, old quarries and gravel pits and railway banks.

Occasionally it is listed as growing in woodland, roadsides, river shingle and even on sand dunes in the Culbin Forest in Scotland. This diversity of situations suggests that the plant is an opportunist able to tolerate a wide range of physical conditions.

Buddleia appears to have spread originally along the railways: the trackside often provided a suitable medium for colonisation and the seeds were carried long distances by trains, rather than simply having to rely on their wind-dispersal mechanisms. Buddleia was not the first plant to benefit from the railways: Senecio squalidus which is another introduced species, spread along the railway network in a spectacular manner. Similarly, after the bombing in World War II, Chamaenerion angustifolium underwent a population explosion as it spread over bomb sites, particularly on the sites of fires. Buddleia did not appear to require the flush of nutrients that the fires provide, and was slower to move into and grow on these derelict sites, but once established it became just as spectacular as the willowherb.

The clearance and rebuilding that the War necessitated was followed by a series of planning policies in most of the major towns and cities which involved massive demolition and redevelopement projects. So when the bomb sites had disappeared there was still plenty of newly derelict land in most urban areas and thus a constant supply of fresh sites for buddleia to invade. During the last 30 years redevelopement and building has progressed at a phenomenal rate, even in small towns, accompanied by the dereliction of many railway lines. Obviously both of these factors have provided a series of new sites for buddleia: the dereliction of railway land has left large empty areas which would previously have been in constant use and so unsuitable for colonisation.

A consequence of these planning policies has been the sudden demand for stone and gravel which has resulted in a tremendous increase in quarrying and gravel extraction. Both of these activities provide further areas in which buddleia can flourish, growing not only on the newly exposed banks and quarry walls but also on the spoil heaps and piles of stones awaiting removal. It is particularly associated with the bases of the piles of chippings and with the margins of the quarries; presumably these areas are rich in humus and fine particles of soil which enhance the moisture and nutrient levels so that seeds that lodge there germinate and grow rapidly. All of these sites are in addition to the walls, in whose crumbling mortar buddleia has always been able to grow, and to the periodically disturbed sites in woodland and roadside where it may occasionally establish itself.

The original sites of naturalisation also include the seashore. In Rosshire it grows on sandy banks and gravel beds near the sea (Duncan 1980), and in Dorset it flourishes on the cliffs: the Undercliff at Lyme Regis has several vigorously growing specimens amongst its lush vegetation. These sites are in areas which are much less stable than inland: landslides and slips lead to new areas being exposed on which buddleia can readily establish itself. It is apparently able to withstand the low moisture levels which these substrates provide and the desiccating and damaging effects of the salt-laden on-shore winds which prevent many species from establishing themselves by the sea. These winds are advantageous to the plant in promoting its long distance dispersal, increasing the area of the seed rain, and hence its potential for moving into fresh areas, which is probably the main reason for the rapid spread of buddleia

around the coastline of Britain compared to what has happened inland.

1:6 Buddleia as a component of the wall community.

In 1969 Segal published his "Ecological Notes on Wall Vegetation" in which he presented an extensive analysis of the mural communities of western Europe. He separated the south west from the south east of England on the basis of the vegetation units in each. However, Buddleia is present in both areas, though it appears to be more consistently represented in the west. Southern England and north west France have the best developed mural vegetation in Europe, of a type known as the Filici-Saginetum. This is the commonest wall community in Europe and the only one which really constitutes a distinct community. It is found chiefly in maritime areas and in drainage basins of continental Europe.

The Filici-Saginetum is dominated by several species of fern: Phyllitis scolopendrium (Hart's tongue fern), Dryopteris spp. and Asplenium spp. (spleenworts), Tortula murialis and Bryum spp. (both mosses) and Poa annua (a grass). Buddleia is one of several species which appear consistently in the Filici-saginetum in the eu-Atlantic regions (Normandy, Brittany, western England and Scotland), thus creating a slightly different community but not occurring with sufficient frequency to serve as a differentiating taxon. As with most wall dwelling plants the members of this community are mainly small and low-growing species: it is only when the wall is in a fairly advanced state of decomposition that the larger plants such as buddleia can move into what is basically a succession gradient. Relatively few species are found growing in their optimum

conditions when on walls: their size and repoductive output is generally lower than that of individuals growing in more hospitable habitats; buddleia is a good example of this.

The aspect of the wall has a considerable effect on its colonisation. South facing sides present a more fluctuating environment than those with a northern aspect; though they warm up more on a summer day they also lose more heat at night and are subject to greater desiccation. Consequently the flora of a north-facing wall is usually much richer and luxuriant. Many walls display an extensive cover of vegetation, which helps to stabilise the micro-environment, but it is notable that buddleia frequently appears on walls which are almost devoid of other plants. However, its regional distribution in drainage basins and maritime areas means that the walls are subject to the ameliorating effects of proximity to a water course.

Segal (1969) classes buddleia as a neophyte, by which he means a plant that has recently become a self-perpetuating component of the wild flora and not merely a casual escape. He notes that it is found occurring as a neophyte in Britain, Brittany, Normandy and Languedoc and in the Lake Como region of Italy where it grows along stream beds (Sutter, 1962). It also occurs along the base of walls around Lake Constance, in Southern Germany (pers. obs.), but Segal points out that it is only rarely found in the Netherlands and then it does not behave as a neophyte. Why this last should be so is not clear: perhaps there is a shortage of suitably decomposed walls and derelict land in this region or it may reflect a more fundamental unsuitability of the climate.

Segal also classes the plant as a mesophyte according to the Iversen system of classification (Iversen 1939), which means that it

is mesomorphic, i.e. it cannot withstand appreciable loss of water and soon wilts completely, due to its moderate to weakly developed root system. This seems an unlikely category in which to place buddleia since it has an extensive rooting system and appears to be tolerant of quite lengthy interuptions to the water supply. Other woody plants that occur in similar situations are Sambucus nigra, Acer pseudoplatanus and Fraxinus excelsior. In fact S. nigra is frequently found growing alongside buddleia, among rubble and on derelict land: Segal states that they are both nitrophilous species as well as calcicoles. This is at variance with the descriptions of the 2 species by Clapham, Tutin and Warburg (1965) who clearly state that S.nigra is common on basic and nitrogen- rich soils but make no such claims for buddleia. Although it frequently occurs on highly calcareous substrates there is no evidence that it needs an enhanced nitrogen supply.

The composition of the community in which buddleia features indicates that it is the vegetation of a fairly advanced successional stage, similar to the scrub found in ravine forests but with more nitrophilous species. Phyllitis scolopendrium is another major component of ravine forest flora and is also found in many non-mural situations that are colonised by buddleia. In southern England it grows on calcareous rocks and gravel and in hedgerows, roadsides and in rocky woods, though these are mainly in damper districts.

Sambucus nigra provides an interesting native parallel to buddleia in Britain. At first glance it appears that the two species may be in direct competition for sites to colonise: it may be that the transient nature of the sites that they invade helps to minimise this, or that the difference in mode of distribution has some effect.

Alternatively, there may be differences that are not immediately apparent, such as the nitrogen requirements of elder, as mentioned above, or perhaps a greater tolerance of high levels of calcium, which may create a competitive advantage for one species at any given site.

Clearly there is a need for a thorough investigation of the optimum conditions and tolerance ranges for germination, establishment and growth of buddleia before one can proceed beyond speculative questions.

1.7 Secondary compounds and herbivory in buddleia.

Research into buddleia so far has tended to centre around a number of secondary compounds which it possesses. The leaves have been used for centuries in China for fishing since they produce piscicidal effects. So far three glycosides: catalpol, methylcatalpol and aucubin have been isolated by Duff *et al.* (1965) and five novel toxic sesquiterpenes have been isolated from the root bark. These are currently being investigated further by teams in Japan and France (Yoshida *et al.* 1978a, 1978b and Susplugas *et al.* 1978). These compounds are named buddledin A, B, C, D and E. Many sesquiterpenes have cytotoxic effects but these are the first to be isolated with a caryophylllene skeleton. The same chemical structures have been isolated from *B. davidii* and *B. japonica* which implies a homogeneity in the secondary compounds.

The sesquiterpenes are thought to be the piscicidal agents and they may also have a deterrent effect on potential herbivores in this country, but so far a suprisingly diverse fauna of leaf-feeders has

been identified. The leaves are palatable to slugs, snails and other polyphagous insects such as the aphid Myzus persicae, Tetranychus urticae, the red spider mite, and Trialeuroides vaporariorum, the glass-house whitefly (personal observation), and to several more oligophagous species. One would not expect to find a large specialised fauna on an alien species that has been in the country for under a century and widespread for less than 50 years. Work by Owen and Whiteway (1981), and Southwood and Kennedy (1983) demonstrates that this is so for buddleia, yet there is at least one oligophagous herbivore that has extensively adopted buddleia as a new food resource and that is the larvae of Cucullia verbasci, the mullein moth, which is normally confined to Verbascum and Scrophularia spp. This may reflect some similarity in either the physical or the chemical composition of the leaves: mullein moth larvae are certainly used to feeding on hairy leaves.

A few other specialised feeders have been found feeding on buddleia, for example, the weevil Gymnaetron tetrum, Amaromyza verbasci which is a dipteran leaf miner and Campylomna verbasci, a leaf bug. All of these species are alternatively found on Verbascum spp. Many lepidopteran larvae feed on buddleia but they appear to be either polyphagous species or occasional records where the female has laid on the bush for want of another more suitable substrate (Owen and Whiteway 1981). It will be interesting to see whether more species adopt buddleia as a food plant. This will partly be a function of the extent of the spread of buddleia in Britain: if it has now colonised most of the areas in which it can grow then it will have approached its maximum potential as a food resource when

the density and size of individuals are at a peak, and any increase in associated faunal species will be a function of time. However, if there is a massive increase in the sites on which it currently occurs, or if it invades new and different areas, then a much larger supply will be available for potential herbivores which naturally means one would expect to find more species feeding on it.

1:8 Aims of these investigations.

In describing the life histories of plants most workers have been concerned with annual or clonal species herbacious species, not trees or shrubs. Grime (1979) has suggested that most woody plants can be catagorised as stress tolerant competitors, according to his classification of plant strategies, but he points out that this is only speculation and needs more life history data for these groups, to test this hypothesis. Therefore, one aim of the study is to provide such information, to classify buddleia according to its established and regenerative strategies.

A further aim is to look at the life history characteristics of a large and influential new colonist, in order to assess its potential impact in both man-made and natural environments. Urban ecology is a recently developed field of study, which is currently mainly concerned with recording and mapping the flora and fauna of habitats created by man (Schmid 1975). Since such habitats are constantly changing and expanding, and new species are increasingly introduced to urban areas, this must be a growth area for ecologists, and one which offers them an important interface with the planners. If this is to be realised then ecologists must be able to provide predictive

information about colonising species, including those which originally escaped from gardens, rather than simply listing what is present. Our present knowledge of the life histories of such plants is mostly confined to native species and annual or biennial weeds. Yet non-native colonists, particularly if they are long lived, cannot be ignored: they may have important effects on both the landscape and the existing flora and fauna. This is the first attempt to record the life history characteristics of such a plant, growing in an alien situation; it is hoped that it will be followed by many others.

The striking thing about the present distribution of buddleia is its localised nature. Despite its occurrence throughout Britain and its tolerance of a broad range of soil and climatic types when planted as mature individuals,there is no indication that it will invade other areas which appear to be hospitable, such as freshly disturbed agricultural or horticultural land, as other escaped species have done. Gardening books recommend a rich, loamy soil and a sunny position for maximum growth, yet when they are planted into such a situation there is little evidence of seedlings occurring naturally around the parent.

The seed is the mobile phase in the life history of a plant, and for a species which is largely self-fertilised, it also represents the stage at which new material is brought into the gene pool of a population. Consequently, it is arguably the most productive area for any study which seeks to account for the distribution of a species. It is the response of the seeds and seedlings of buddleia which will determine whether a given habitat can be colonised by the species.

Why is buddleia limited to marginal habitats? What are the adaptations that enable it to successfully colonise these localities

and yet fail to become established in more mesic environments which, from the evidence of transplants, would be expected to present better situations for the growth and reproductive output of the adult plants? Is it just a poor competitor, or are there innate features of its ecology that prevent it from colonising other habitats?

In order to answer these questions the following studies were carried out:

1.) An evaluation of soil conditions characterised from a wide range of sites where buddleia occurs naturally.

2.) Surveys of seed output and of the spatial and temporal patterns of seed release are carried out to assess the potential for distribution and dispersal of buddleia.

3.) The fate of seeds in the soil is followed to establish whether they can form persistent seed banks and to quantify losses due to predation, fungal attack and unsuccessful germination.

4.) To identify which phases of the life cycle experience the heaviest mortalities and to suggest the major causes of these mortalities, a field investigation is described, carried out over 18 months and focusing particularly on the crucial stages between seed dispersal and seedling development.

5.) Laboratory experiments on the tolerance ranges and optimal conditions for germination and seedling establishment are reported for a variety of environmental factors.

The final aim of these studies to define which factors limit the distribution of buddleia, and hence to suggest what potential it has for further colonisation in Britain.

CHAPTER 2: SOIL ANALYSES AND DEMOGRAPHIC SURVEYS.

2:1 Introduction.

The distribution of buddleia has so far been described in 3 ways: its geographical occurrence, some general comments on the nature of the sites where it is commonly found and, from the work of Segal (1969), a brief review of the species associated with it in a mural community.

In order to define the necessary soil conditions for natural regeneration samples of soil were collected from beneath established self-set buddleia in a variety of locations in southern England. Analyses were carried out to establish the pH, cation exchange capacity, potassium and calcium content and the textural class of each sample.

In the south of England, buddleia is not confined to small scattered groups colonising rubble and old walls; it also occurs as dense thickets on disused but highly disturbed land, such as old quarry workings. This provides an opportunity to investigate the population structure and the interactions between plants in a situation which is probably similar to its occurrence in China.

2:2 Soil analyses: materials and methods.

2:2:1 Sample sites.

The locations and brief descriptions of the sites from which soils were collected for analysis are listed in Table 2.1. Any litter which was on the surface was removed along with the top 4 cm soil where this

Table 2.1 Location of sample sites.

Soil Reference	Geographical Location	Grid Reference	Site Reference
A	Folly Bridge, Oxford	SP515055	Wall on a river bank
B	Folly Bridge, Oxford	SP515055	Crumbling masonry on a river bank
C	Folly Bridge, Oxford	SP515055	Crumbling masonry on a river bank
D	Folly Bridge, Oxford	SP515055	Masonry beneath a thicket of buddleia.
E	Wookey, Somerset	ST540468	Floor of a limestone quarry
F	Wookey, Somerset	ST540468	Floor of a limestone quarry
G	Wookey, Somerset	ST540468	Floor of a limestone quarry
H	Wells, Somerset	ST568443	Cliff face in a limestone quarry
J	Wells, Somerset	ST568443	Rinsings from chippings on floor of a limestone quarry
K	Wells, Somerset	ST568443	Cliff face in a limestone quarry
P	Puriton, Somerset	ST313413	Roadside embankment
Q	Puriton, Somerset	ST325413	Cliff face in disused blue lias quarry
R	Puriton, Somerset	ST313414	Railway embankment
S	Puriton, Somerset	ST325413	Cliff face in disused blue lias quarry
T	Dry Sandford, Oxon.	SU470998	Floor of a disused sandpit
V	Dry Sandford, Oxon.	SU470998	Floor of a disused sandpit
L	Brasenose Wood, Oxon.	SP560054	Burnt area in woodland
M	Brasenose Wood, Oxon.	SP560054	Burnt area in woodland

was possible, although in the case of plants growing in rock crevices or piles of chippings the fragmentary material was collected from around the roots, discarding visible pieces of the plant in order to minimise error in measuring the organic matter.

The reference letters listed in Table 2.1 are those used throughout the chapter.

2:2:2 Mechanical analysis of the soils using sedimentation techniques.

The soils were initially air dried and the following procedure repeated for each sample. Having gently removed all the aggregates with a pestle and mortar 30 g soil was weighed out and passed through a 2 mm sieve. The fraction that was retained in the sieve was weighed and recorded as gravel.

In order to carry out the process of sedimentation effectively it is necessary to first break down all the bonds between the primary particles. The sieved material was treated with 50 ml of 10% H_2O_2 to oxidise the organic matter, and stirred at regular intervals until the reaction in the cold had died down. The mixture was then heated to boiling on a sand bath until there was no further reaction and all visible organic matter had disappeared.

The calcium carbonate was removed by adding 25 ml 2M HCl to the suspension in each beaker and stirring the mixture frequently for 1 hour. The acid soil was then filtered through a Buchner funnel with Whatman no.50 filter paper and then washed until the soil was acid free. The soil was then carefully transferred from the filter paper into the cup of a high speed blender (M.S.E. model "atomix") with about

400 ml distilled water. The clay particles were then saturated with sodium ions by adding 100 ml 2% Calgon solution. The suspension thus formed was poured through a 0.2 mm sieve into a litre measuring cylinder and rinsed thoroughly with distilled water. The fraction retained on the sieve was weighed and recorded as the coarse sand fraction.

The remaining suspension was then made up to 1 litre and stirred vigorously to ensure that all the particles had been thoroughly dispersed. The sedimentation technique makes use of Stoke's Law, which is represented by the simplified formula:

$$V = Kr^2$$

where V= the rate of settling in cm sec^{-1}

K= a constant

r= the radius of the particle Thus the time taken for all particles of fine sand to have settled through 20 cm was calculated and an aliquot of suspension was withdrawn at 20 cm depth at the end of this time ensuring that the sample contained only clay and silt. Similarly the settling velocity of silt was calculated and an aliquot removed after this interval so that a sample containing only clay particles was removed. These samples were transferred to evaporating dishes, dried on a sand bath and then in an oven at 100°C for 24 hours. The weights of these samples were then used to determine the weights of clay and of clay and silt in each sample.

A repeated process of stirring, sedimentation and decantation based on the fine sand sedimentation rate was used to retrieve the fine sand fraction for drying and weighing.

The results were used to calculate the percentage of the original samples which were coarse sand, fine sand, silt and clay.

A determination of the proportion of organic matter in each soil was carried out by accurately weighing about 30 g oven dried soil into nickel crucibles. These were then placed in a muffle furnace for 24 hours at 300°C and a further 24 hours at 500°C. The cooled crucibles were then reweighed and the difference in the original and the burnt weights recorded as an estimate of total organic matter in each soil. Errors due to carbonate in these soils will be quite high, so there is likely to be an overestimate of the total organic content.

2:2:3 Soil pH.

The procedure used for each sample was to mix 20 g air dried soil with 40 ml distilled water and stir frequently for 30 minutes,then leave to stand for 1 hour. The pH readings were then obtained using a Pye Unicam Model 292 pH meter with a glass calomel electrode inserted in the supernatant and allowed to equilibrate for 30 seconds before recording the reading.

2:2:4 The measurement of exchangeable calcium and potassium ion content and the estimation of the cation exchange capacity.

All of the following procedures were carried out on 3 replicates of each soil sample. 5 g samples were weighed into centrifuge tubes to which were added 25 ml ammonium acetate, buffered at a pH of 9.1. After shaking for 10 minutes these were centrifuged and the supernatant was filtered into a flask. The extraction was repeated and the supernatant added to that in the flask. The filter paper was then washed with more ammonium acetate and the accumulated filtrate made up

to 100 ml.

Standard solutions of KCl and $CaCO_3$ were prepared to give 0, 5, 10 and 20 mg $K^+ l^{-1}$ and 0, 50, 100, 150 and 200 mg $Ca^{2+} l^{-1}$ to obtain calibration curves for potassium and calcium using an "EEL" flame photometer.The amount of exchangeable potassium and calcium in each soil was then calculated as milliequivalents per 100 g soil.

The soil from which the exchangeable cations had been extracted was then washed with 10 ml methanol, shaken and centrifuged, discarding the supernatant. The washing was repeated twice so that all the excess ammonium ions entrapped in the wet soil were removed. 25 ml of $BaCl_2$ solution was added to the centrifuge tubes which were then shaken for 10 minutes. After centrifuging the supernatant was filtered into a flask and the procedure repeated, accumulating the supernatant. The latter was then made up to 100 ml with more $BaCl_2$ solution. A 25 ml aliquot of this solution was then removed and using phenolphthalein as an indicator, 0.02M NaOH was added until the solution was just alkaline. To this, 5 ml 40% neutralised formalin was added and the solution was left to stand for 5 minutes before titrating against 0.02M NaOH.

The ammonium ions combine with the formalin to release hydrogen ions according to the equation:

$$4NH_4^+ + 6CH_2O = C_6H_{12}N_4 + 4H^+$$

The cation exchange capacity was calculated from the titration values and expressed in milliequivalents per 100 g soil.

2:3 Results and discussion of soil analysis.

The results of the mechanical analysis of the soil samples and

estimation of organic content are shown in Figure 2.1(a-c) and have been applied to a textural triangle in order to classify each soil according to the England and Wales Soil Survey (Hodgson, 1976). The classifications are shown in Table 2.2.

The values of pH, cation exchange capacity and specific ion content of the soils are summarised in Table 2.3.

Charts in Figure 2.1 show that all soils have a high sand component; in most samples sand makes up over 75% of the total weight of the soil and in those where it is not such a high proportion, silt tends to dominate. Notable exceptions to this are soils P and R in which clay is a major component and the other fractions are more evenly distributed: in Table 2.2 soil P is classified as a clay soil and soil R as a clay loam. Both soils come from embankments, P from the roadside and R from the edge of the railway, which are sites frequently invaded by buddleia.

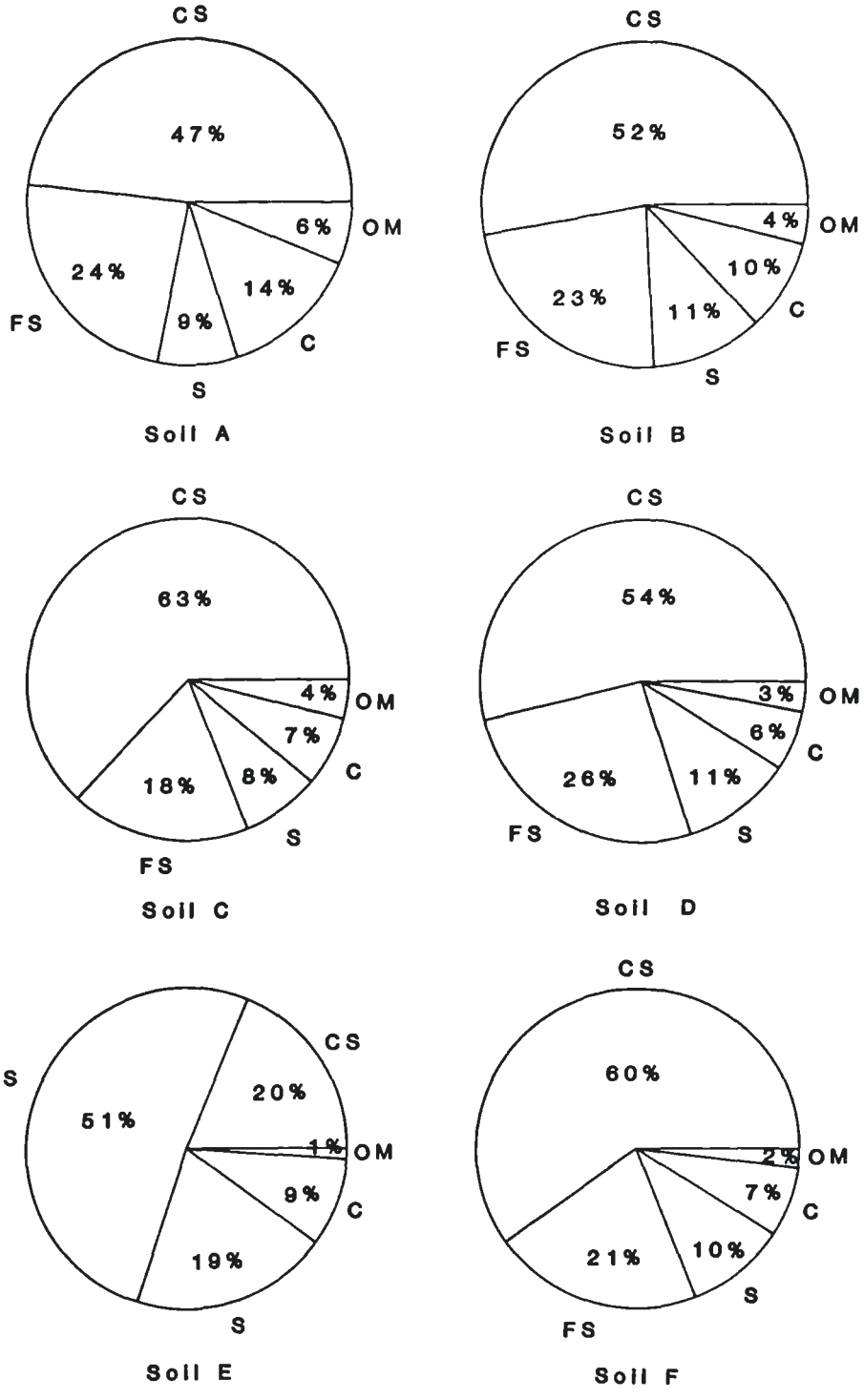
The rest of the samples have between 60% and 90% sand in their composition and are classified as sands, loamy sands or sandy loams. This indicates that the shrub is capable of establishing itself on highly porous and often poorly structured soils, but it is also capable of self-propagation on more balanced, loamy soils.

The organic content is fairly low in all the soils, but the sites had all been recently disturbed and it may be assumed that the higher organic horizons were removed at the same time. The limited colonisation of these immature soils has made little contribution to the organic content.

It is immediately apparent from Table 2.3 that buddleia is capable of colonising areas with a high pH; for example, soil K from Wells has a pH of 8.9. Increasing alkalinity in soils is accompanied by a

Figure 2.1 (a-c) The proportions of different textural classes in 16 soil samples from beneath self-established buddleia in southern Britain.

Figure 2.1(a) Textural composition of soils A, B, C, D, E & F.



CS - coarse sand
FS - fine sand
OM - organic matter
S - silt
C - clay
G - gravel

Figure 2.1(b) Textural composition of soils G, H, J, K, P & Q.

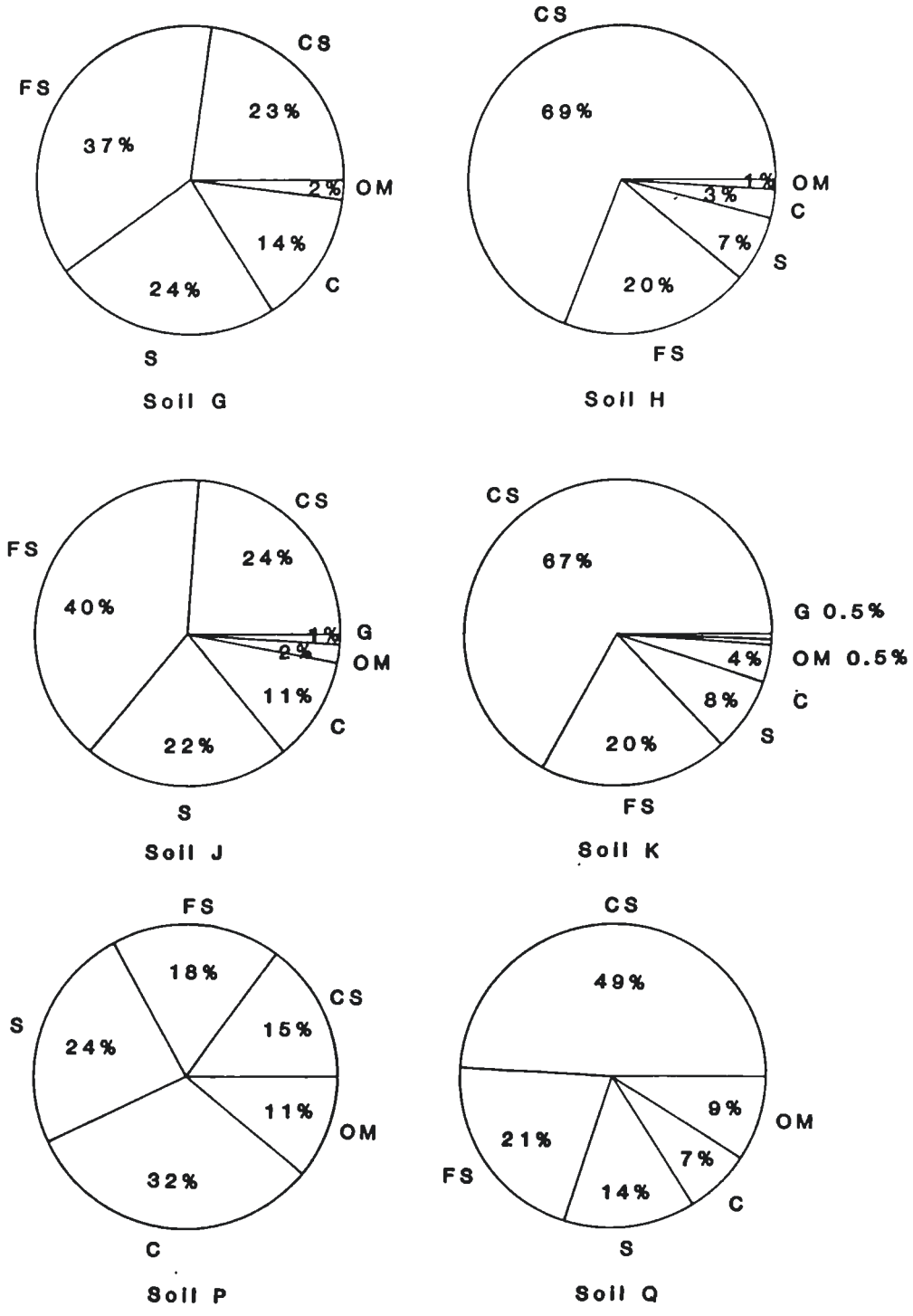


Figure 2.1(c) Textural composition of soils R, S, T & V.

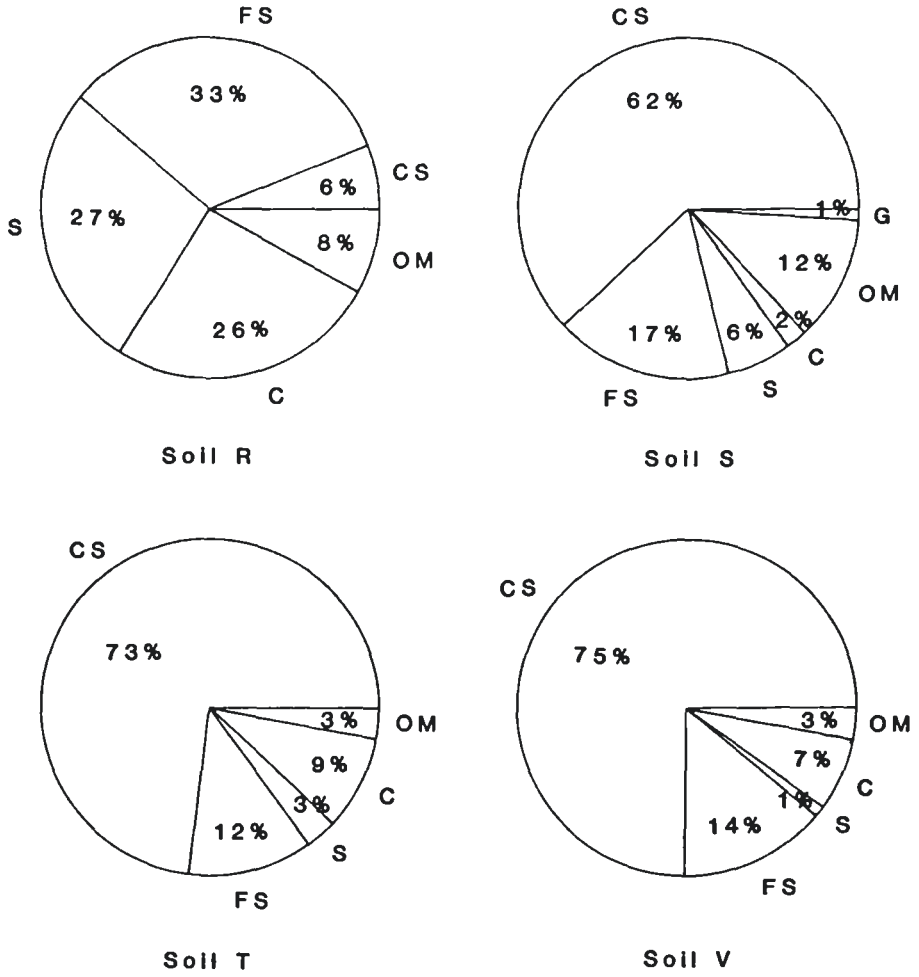


Table 2.2 Classification of soils from sample sites, according to the England and Wales Soil Survey.

Soil Sample Reference	Classification (from textural triangle)
A	Sandy loam
B	Sandy loam
C	Loamy sand
D	Loamy sand
E	Sandy loam
F	Loamy sand
G	Sandy loam
H	Sand
J	Sandy loam
K	Sand
P	Clay
Q	Sandy loam
R	Clay loam
S	Loamy sand
T	Loamy sand
V	Sand

Table 2.3 The pH, C.E.C., potassium and calcium ion content of soil samples.

Soil sample reference	pH	C.E.C. mmol/100g soil	K^+ mmol/100g soil	Ca^{++} mmol/100g soil
A	8.10	1.91	0.07	2.0
B	8.51	1.10	0.03	2.2
C	8.32	0.84	0.07	2.2
D	8.37	0.85	0.06	2.8
E	8.39	8.05	0.02	1.93
F	8.42	5.33	0.02	1.97
G	8.39	7.47	0.03	1.93
H	8.61	4.11	0.00	2.53
J	8.05	5.6	0.02	2.67
K	8.91	3.89	0.01	2.73
P	7.97	18.83	0.87	2.1
Q	7.57	13.39	0.34	2.8
R	8.21	14.83	0.74	1.8
S	7.67	11.41	0.26	7.8
L	5.90	-	-	-
M	6.00	-	-	-

decrease in the availability of certain mineral nutrients. Above a pH of 7 phosphorus and iron, and to a lesser extent manganese, zinc, copper and boron, are progressively withdrawn from solution and form insoluble complexes, often with calcium. Excess calcium may also hinder phosphorus absorption and utilisation in plants (Brady 1974). Plants growing in these soils often have visible symptoms of nutrient deficiency, in the form of scorching, chlorosis or abnormal pigmentation. However, there was no evidence at the study sites to suggest that the buddleia plants were suffering from deficiencies of these elements induced by high p.H.

Soils L and M, which come from a burnt area of oak woodland, show that it is also able to colonise soils with a pH around 6.0. It does not appear to require an alkaline environment to establish itself; there are no highly acidic soils represented, but since it is only a small sample it is unfair to place too much emphasis on this except to note that none of the reports and observations of naturalised buddleia collected over 4 years has come from areas with acid soils. A further investigation of the effect of pH on the germination of buddleia is reported in section 4:9.

The evaluation of the cation exchange capacity (C.E.C.) of each soil and of the proportions of that capacity taken up by the cations calcium and potassium was undertaken to provide some measure of the nutrient status of each soil. The cations adsorbed to the surfaces of colloids or micelles indicate what is available in the soil for plant uptake.

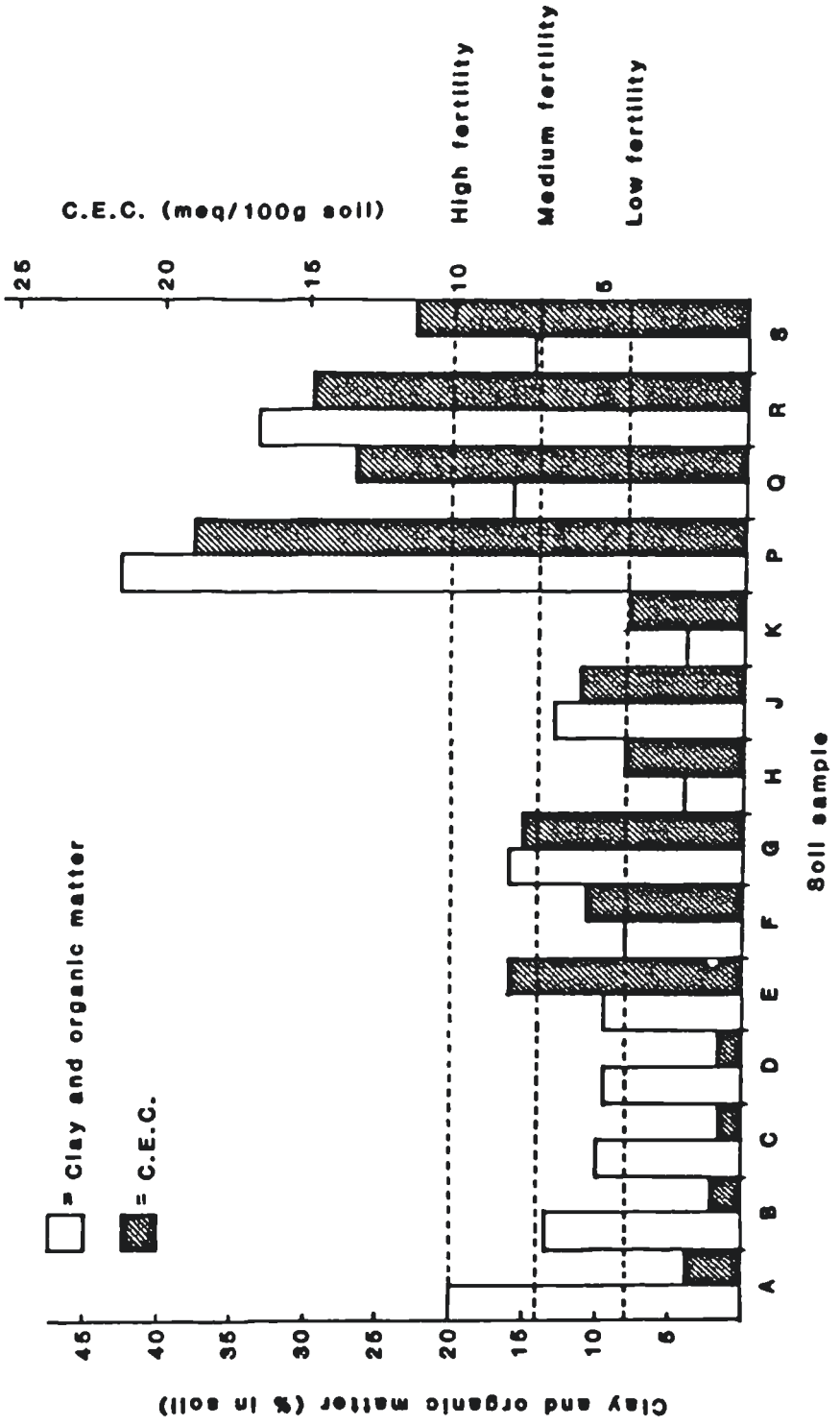
Since the C.E.C. reflects the availability of colloidal or micellular sites in the soil, there should be a close correlation between this figure and the humus and clay content of each soil

(Figure 2.2). It should be noted that the estimate of organic matter includes both the humus content and all of the undecayed plant material in the soil. In such young soils there may be little correlation between these 2 components, and since it is only humus which contributes to the soil structure and nutrient status, the total figure given may be a poor estimate of nutrient holding potential.

Somerset soils P-S have the highest C.E.C and correspondingly higher clay and organic contents than the other soils, and this trend is followed to a lesser extent in soils E-K. However, in soils A-D, which were taken from derelict masonry, the C.E.C. is much lower than expected from the proportion of clay and organic components. This reflects the high proportion of undecomposed organic material which is found in these soils, but it may also be connected with the type of clay. There are enormous differences in the availability of exchange sites which different types of clay possess. For example, kaolinite has a C.E.C. of only 8 meq per 100 g compared with 100 meq in montmorillonite (Brady 1974). It may be that the clay in soils A-D is mainly kaolinite, while soils E-K, which contain a similar amount of clay, have a higher proportion of the more adsorptive types.

An interesting comparison may be made between soils D and E, which both contain less than 10% combined clay and organic matter, with clay as the prominent component in each, as Figure 2.1 shows. However, the C.E.C. of soil D is almost a tenth of the figure for soil E. Similarly, soils H and K have a low organic content but show a high C.E.C. in relation to their clay composition. There is no reason to expect significant differences in the proportions of humus in the total organic content of soils from these various sites, so the results indicate that the clay in the Mendip sites has a good adsorptive

Figure 2.2 The relationship between the clay and humus content and the cation exchange capacity of the 16 soil samples.



capacity compared with that found in Oxford walls.

Soils P-S all have a similar organic component, but soils Q and S have less than a sixth of the clay content of P and R, yet the C.E.C. of all these soils is not so different as might be expected if clay was the most important source of exchange sites. In fact, comparing the C.E.C. of soils G, Q and S, which have similar total organic contents, the former has a much lower capacity, which reflects the higher proportion of clay to organic matter in this soil, compared with the ratio in the other 2 soils. In these it is organic matter that is the predominant component, and they have proportionately higher exchange capacities.

So the relative importance of the clay and the humus in providing exchange sites changes, depending on the types and composition of soils involved. The types of clay may be important when there is relatively little humus present, but this difference becomes less important as the humus levels increase.

A "typical" temperate soil composed of a balance of these clay types would have about 0.5 meq $100g^{-1}$ soil for every 1% clay in its composition and 2 meq $100g^{-1}$ soil for every 1% organic matter. So for a sandy soil with little clay or organic matter the average cation exchange capacity might be less than 5 meq $100g^{-1}$, but for a typical muck soil it would be about 200 meq $100g^{-1}$ (Brady 1974). Typical C.E.C. levels of high, medium and low fertility soils underlying stands of trees in North America, are shown on Figure 2:2, derived from Wilde (1958). From these it is clear that buddleia has no special requirements in terms of general nutrient status.

Turning to the figures for specific ions in Table 2.3, it also appears that calcium and potassium are not critical in its

distribution. Buddleia is found growing in soils in the range of 0.0 to 0.87 meq K^+ $100g^{-1}$ soil and 1.93 to 7.8 meq Ca^{2+} $100g^{-1}$ soil. The potassium figures are not particularly notable: the figures obtained from the Wells quarry site demonstrate only that the soil there, which came from crevices in the limestone, was very immature and releasing few K^+ ions. The values obtained for calcium seem remarkably low for a calcicole growing on limestone and sandstone sites. They are probably largely explained by the use of ammonium acetate at pH 9.1 as the extractant. This extractant is normally used at pH 7.0, but since this produced abnormally high values for calcium it was concluded that additional ions from $CaCO_3$ in the soils were being released. Therefore a more alkaline extractant was used to avoid this source of error. This means that the measurements obtained are not directly comparable with figures by other authors for calcium components of various soil types.

Comparing the figures for K^+ and Ca^{2+} ions and the overall C.E.C. there is an obvious disparity in soils A-D, which were derived from mortar in decaying walls. In soil D there were 3 times more calcium ions than there were available exchange sites. This indicates that much of the calcium in these samples was derived from the mortar and present in the soil solution rather than bound to the soil particles. The fact that this shows up despite the alkaline extraction implies that the sites were remarkably calcium-rich and that any plants growing there must be extreme calcicoles. The other sites do not show a similar pattern; in fact the low calcium figure leaves a considerable proportion of the total C.E.C. unaccounted for by the remaining cations. Hydrogen ions are likely to be present in relatively small amounts in such alkaline soils and there is no reason to suppose that

any of the other major cations such as sodium and magnesium will be found in large quantities in these soils. Presumably it is the low calcium values obtained by this extraction technique that are responsible for this disparity.

The indications of the analysis are that although buddleia is capable of growing in highly calcareous substrates it is not an obligate calcicole. Even allowing for the problems reviewed above, the figure for exchangeable calcium ions is lower than expected in sites such as the road and railway verges which were the sources of soils P and R.

2:4 Survey of Natural Populations: the Site.

The gravel pit owned by Amey Roadstone in Slindon, West Sussex (grid reference SU 978073), has been extensively colonised by buddleia. It was chosen as the site for a survey of self-established populations of the shrub because it provided extensive and relatively uniform tracts of disused land for colonisation, and the successive abandonment of old workings has produced a series of sites at different successional stages. Most of the buddleia seen in Britain occur as isolated individuals or in small groups and their dispersion patterns and relationships are often masked by the heterogeneity of their environment.

It was originally intended to establish the population age structure, as well as the patterns of distribution of the shrub, by surveying several sites in the gravel pit. However, it was immediately apparent that none of these sites possessed a complete age range of buddleia. Land that had recently become disused had only seedlings

present, but older areas had populations of mature buddleia of remarkably similar ages. In one undisturbed corner of the pit there was a dense single-species stand of mature buddleia, shown in Figure 2.3, but no plants under 5 years old.

An extensive tract of land which appeared to show a range of individuals from all age groups had been highly disturbed recently. Several mature plants had been left at the margins but the centre of the site had been cleared of surface vegetation 18 months previously. The top soil was massive clay, at least 40 cm deep, and most of this was devoid of vegetation, as can be seen from Figure 2.4. Buddleia and 2 or 3 other woody species were the only plants scattered sparsely over the site; they had presumably regenerated from undamaged roots. Digging into the clay revealed that the young buddleia plants on this site were actually new shoots arising from the buried roots of the original plants. Despite the open ground, the recent heavy disturbance and the abundant source of seeds provided by the surrounding adults, there were no seedlings present.

The clay presented a solid, unbroken surface except at the margins where trees and shrubs were growing healthily. One side was bounded by open birch and willow woodland in which the dominant understorey plant was buddleia, growing in the large fissures caused by the drying and cracking of the clay under the trees. This buddleia was not more than 0.5m high and appeared to be exclusively young plants less than 5 years old, although the records of the gravel pit and the size of the trees indicated that the woodland was at least 15 years old. These observations are consistent with reports of buddleia growing in nearby coppiced sweet chestnut woods, which are extensive on this part of the South Downs (A.G. Gaydon, Pers.Comm.).



Figure 2.3 The edge of a dense stand of mature buddleia at Slindon, Sussex.



Figure 2.4 The sparse regrowth of vegetation, mainly buddleia, on a clay-based site at Slindon, Sussex, cleared 18 months previously.

Having recorded these features for future reference there was no point in attempting to survey the age structure in the populations of buddleia at any of the sites. However, it is not only the distribution of individuals in time that invites investigation: their arrangement in space reflects both the availability of suitable microhabitats for the seedlings and the interactions between plants. Recording and analysing the dispersion patterns and densities of plants on the Slindon site, at three different stages of the life cycle, should reveal more about the relationships between individuals at each stage.

2:5 Materials and methods of measurement.

The 3 stages of the life cycle to be investigated were selected on the basis of ease of recognition. It was possible to ascribe an individual to one of the age catagories listed in Table 2.4, based on knowledge of growth rates. The criteria for age classification did not apply where aerial parts had regrown from rootstocks. Therefore, sites where this had occurred were excluded from the survey. The 3 categories will be referred to by the reference letters given in Table 2.4 throughout the text.

The techniques used were all based on plotless sampling methods, using point-to-plant and nearest neighbour distance measurements as advocated by Cottam and Curtis (1956).

Sites 1 and 2 were on clay, with some gravel, and had been left to colonise for several years. The point-centered quarter method (Cottam, Curtis and Hale 1953) was employed to sample the distribution of the type Y buddleia plants which were the dominant age group on both sites, as illustrated in Figure 2.5. A grid was laid out across the site and

Table 2.4 Age categories and descriptions of buddleia plants.

Age category	Reference Type	Distinguishing attributes
Seedling (Plants 2 years old or less)	S -type	Less than 10 cm tall. Have not yet flowered.
Young adult (Plants 3 - 5 years old)	Y -type	10 cm - 2 m tall. Basal stem < 2 cm diameter. Have flowered.
Mature adult	M -type	Plants over 5 years old. Usually several meters high. Stem diameter over 2 cm.



Figure 2.5 The open vegetation on sampling site 2 at Slindon, Sussex.

points placed at intervals determined by random numbers of paces between them. Four quadrants were orientated around each point, in a constant direction. The distance from the sampling point to the nearest type Y individual was then measured in each quadrant, together with the distance between this plant and its nearest type Y neighbour.

Site 3 was mainly on gravel, with some compacted material (clay and tarmac) in small patches. It was the most recently disturbed site and hence dominated by seedlings, not adult plants. The site was narrow so the sampling points were arranged at intervals along a linear transect, and both nearest neighbour and closest individual methods were employed. From each point the distance to the nearest individual of type S in any direction was recorded and the distance to its nearest type S neighbour.

Site 4 was on clay and gravel that had been undisturbed for at least 10 years, with the result that the population was composed exclusively of type M buddleia. The nearest neighbour technique was again used but in this case the entire population of 40 individuals was recorded rather than a sample, as on the other sites. The distances between each individual and its first, second and third nearest neighbour were recorded. In addition, the diameters of all living stems were recorded at 7cm above the ground for all the nearest neighbours in order to investigate the relationship between density and productivity in a mature stand. The diameter at this height was found to give a reasonable measure of the relative size of each plant: those with the greatest aerial biomass had thicker and more numerous stems at this point than the smaller plants. Since the number of individuals was known, by measuring the area in which they were growing it was possible to calculate the density directly.

2:6 Analysis and interpretation of the distribution data.

The data collected by the closest individual method, used for buddleia type S, were used to calculate the densities of these plants at site 3, according to the formula

$$\text{Density} = \text{Unit Area} / (2 \times \text{mean point-to-plant distance})^2$$

The data from the Point-Quarter method, employed at sites 1 and 2 for buddleia type Y, were used to calculate the densities at both sites, according to the formula

$$\text{Density} = \text{Unit Area} / (\text{mean point-to-plant distance})^2$$

The density of individuals of the particular age group at each site is recorded in Table 2.5.

Since all the density estimates were collected from different sites they are not directly comparable. However, as they were all situated in the same quarry and on similar substrates it seems reasonable to comment on their relative values. The progressive decrease in density with increasing age that appears in Table 2.5 is an expected result from general principles of plant competition, but the density of mature plants remains unexpectedly high.

The point-to plant distance and nearest neighbour data from sites 1, 2 and 3 were then analysed using the Coefficient of Aggregation, A, (Hopkins 1954). This is the ratio of the mean of the square of all distances between a point and an individual (P) to the mean of the square of the distances between an individual and its nearest neighbour (I), summarised by the formula

$$A = \Sigma(P^2) / \Sigma(I^2)$$

With random distributions of individuals this coefficient is unity, it is less than 1 with regular distributions and it exceeds

Table 2.5 Density of individuals of specified age group on sampling plots.

Site reference	Age category	Density m^{-2}
3	Seedlings	4.3
2	Young adults	3.28
1	Young adults	1.82
4	Mature adults	1.93

1 when the pattern is contagious. To test the significance of the deviation of A from unity the parameter x = A/(A+1) is calculated, having a value of 0.5 for a random distribution, with a variance of 1/(4(2n+1)). As the number of pairs of observations (n) increases, the distribution of x tends to normality. For $n < 50$ the value of x must be related to tables of the incomplete beta function (Pearson 1934). Hopkins (1954) has produced a diagram from these tables from which the 5%, 1% and 0.1% probabilities of x deviating significantly from randomness can be easily read, for any n value between 20 and 50. Table 2.6 shows the values of A, x, their levels of significance and the pattern that they indicate, for these plants.

The distance data collected from site 4, the mature plants, were analysed using the statistic developed by Thompson (1956). This makes it possible to compare the mean nearest neighbour distance with that expected from the density of the plants, to show whether the distribution is normal i.e. random, or skewed, indicating aggregation or regularity depending on the direction of the skew. This can be done for the nth nearest neighbour, to see if there is a larger scale pattern of dispersion which is not apparent from the single nearest neighbour techniques. The statistic used is

$$\Sigma 2\pi D r_n^2$$

where D = density

and r_n = distance to the nth nearest neighbour

This statistic is distributed as a Chi-square with $2N_n$ degrees of freedom, so the significance of the results for each order of nearest neighbour can be checked from Chi-square tables.

The results of these calculations, their significance and the patterns that they indicate are recorded in Table 2.7.

Table 2.6 Coefficient of aggregation applied to stands of buddleia.

$$(A = \Sigma (P^2) / \Sigma (I^2))$$

Age category:	Seedlings	Young adults	Young adult
Site reference:	3	2	1
(P^2)	1242.29	4343.06	8468.86
(I^2)	639.66	1754.25	1167.07
A	1.94	2.84	7.26
x(A/A+1)	0.66	0.71	0.88
n	35	32	28
probability	$0.001<p<0.01$	$p<0.001$	$p<0.001$
pattern	contagious	contagious	contagious

Table 2.7 Application of the Thompson statistic ($\Sigma 2\pi Dr_n^2$) to a stand of mature buddleia

	First nearest neighbour	Second nearest neighbour	Third nearest neighbout
Density m^{-2}	1.93	1.93	1.93
$2\ Dr_n^2$	119.94	219.51	341.03
n	40	40	40
d.f.($2N_n$)	80	160	240
probability	$0.001<p<0.005$	$p<0.001$	$p<0.001$
pattern	regular	regular	regular

From Table 2.5 it is clear that the seedlings and young adult plants were highly contagious in their distribution. The heterogeneity of the sites on which they occurred may account for some of this, particularly at site 3 which was a mixture of substrates. However, the established plants at sites 1 and 2 were on a more even, soil-like substrate but they show an even more marked degree of aggregation. The densities of the young adults on site 2 were shown to be less than half that observed in the seedlings so it appears that although the numbers of survivors decrease with age the arrangement in clumps is still as marked. This implies that the degree of competition among these groups of plants is not severe, or a decrease in the aggregation as well as in density would be expected.

The results in Table 2.7 show that there is a significantly regular distribution of the mature shrubs at the most local level, with a probability value of 99.5%. The second and third nearest neighbours show an even greater regularity, the probabilities exceeding 99.9%. Such a marked pattern generally indicates that there is intense competition between the individuals for some limiting resource such as water, light or nutrients. The other explanation for this phenomenon, which is comparatively rare in nature, is that the plants are exerting some direct effect on each other by chemical exudations, which limit the spread of individuals and prevent seedlings from colonising the area. The absence of other vegetation, including buddleia seedlings, from the area populated by these shrubs does suggest that allelopathy may be involved. Alternatively, the degree of competition for limiting factors or the denseness of the canopy of the shrubs may be sufficient explanation for the regularity of the pattern and the inability of new and different plants to grow beneath the mature plants.

The change from the highly aggregated pattern of the seedlings and young adult plants to the highly regular arrangement of the mature plants indicates that buddleia, which initially shows little intraspecific competition, becomes increasingly competitive as the established plants mature. The average height of the the young plants was about 1m but the mature plants that were surveyed were between 3 and 5m tall, so the intensity of the competition would be expected to increase considerably.

However, referring to the density data in Table 2.6, it is apparent that despite changes in the degree of competitive interaction, the densities remain remarkably similar as the plants age. This may be anomalous, a consequence of differences in the sites studied for each age group; this possibility is minimised by looking in detail at the densities in the single stand of mature buddleia. Disregarding, for the moment, the possibility of allelopathy, it might be expected that as the plants grow and compete more vigorously, the density would decline as self-thinning occurred. Since there is no evidence that this occurs, it may be useful to consider the extent of self-thinning in these plants. This can be done by analysing the data further to see if they fit the self-thinning rule proposed by Yoda et al.(1963).

This rule, also known as the -3/2 power law, is the most general principle of plant demography yet discovered, according to Westoby and Howell (1982). It expresses the relationship between density and total biomass of individuals in a population as follows:

$$W = C\ p^{-3/2} \quad \text{i.e. } \log W = \log C - 1.5 \log p$$

where W is the dry weight of the surviving plants, p is the density of the survivors and C is a constant that relates to the growth characteristics of the particular species involved. The measurements

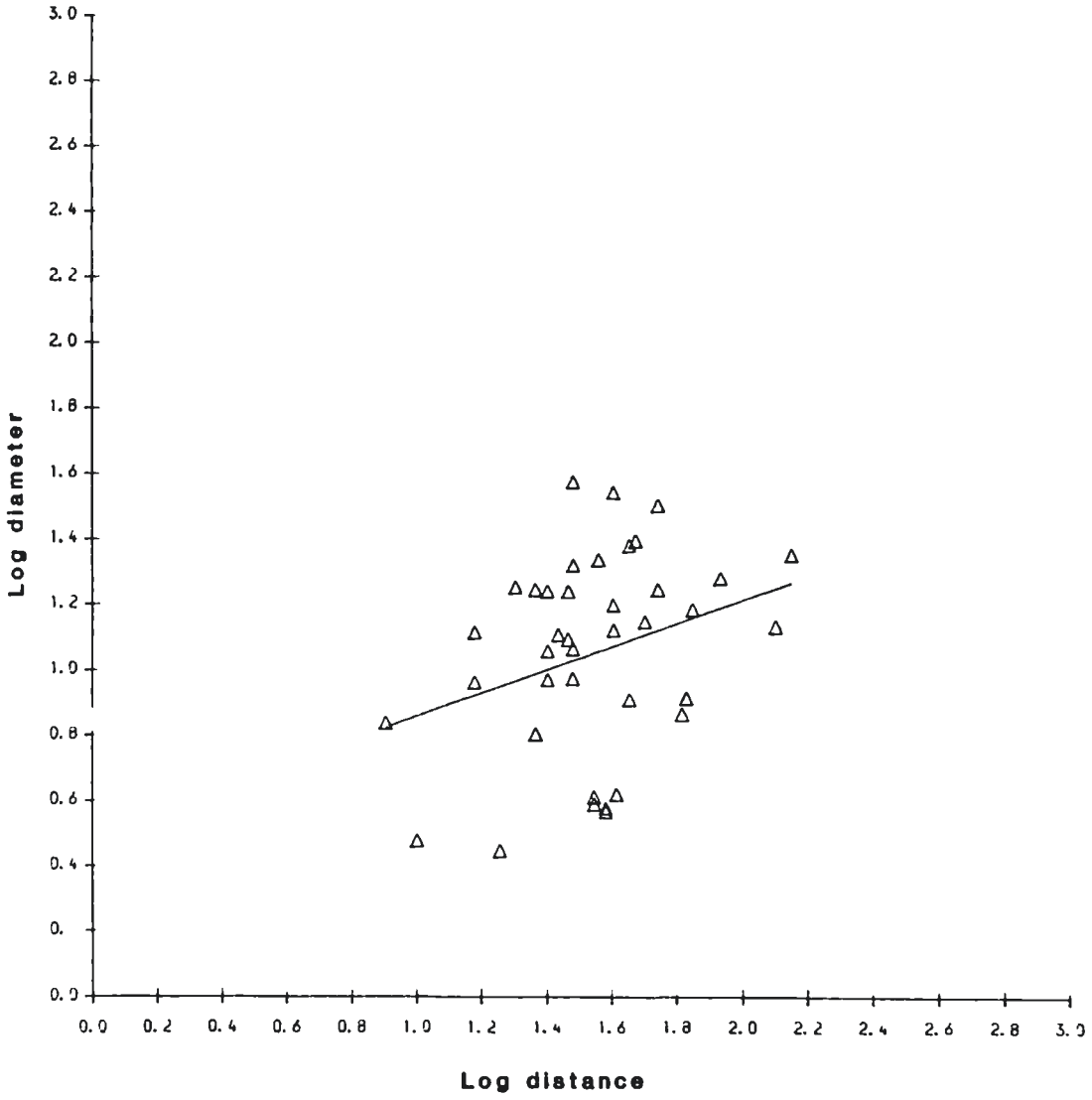
recorded for each pair of nearest neighbours in the population of mature buddleia included the diameters of both individuals. The diameter can be used as an estimate of biomass and the square of the distance between 2 plants is the reciprocal of the density. So by plotting the log of the combined diameters of each pair against their log density, these data can be used to examine the relationship between growth and density in terms of intraspecific competition. Figure 2.6 shows the resulting scatter of points with a regression line fitted; the -1.5 gradient line is shown for reference.

The correlation coefficient (r) for the points in Figure 2.6 is -0.3565, a significant value at the 95% probability level, which indicates that density and biomass of the mature plants are inversely related, but it is not a strong relationship, as the scatter of points shows. The regression line fitted to them has a gradient of -0.2118 which is clearly much lower than the expected gradient of -1.5. The points do fit the latter gradient line, but with considerably more "noise". It appears from Figure 2.6 that there is a poor correlation mainly due to the limited variation in diameters of shrubs compared to the wide range of colony densities.

The results of this analysis do not reveal a high level of intraspecific competition as expected from the pattern analysis. However, there are several factors that may account for the anomalies.

First, the use of stem diameter at this height may not be sufficiently sensitive as a measure of biomass to show the relationship with changing densities clearly. It might also be argued that no account was taken of the biomass below ground, which for shrub species is usually considerable. However, it has also been proposed (White 1981, Lonsdale and Watkinson 1983) that the relationship between volume

Figure 2.6 The relationship between the log diameter of the stems and the log density of individuals, in a stand of mature buddleia on a gravel pit in Sussex.



and density is a better application of the power law than biomass and density, where volume is the volume of the canopy not of the whole plant. So the measure of the stem diameters and hence, by assumption, of aerial biomass should give a better fit than a figure incorporating root mass.

Secondly, since the actual age of each individual was unknown, the population may not have been at the asymptotic level where self-thinning was complete. Instead there may have been younger individuals scattered through the stand which will ultimately be removed by self-thinning but that are presently causing an increase in density without a proportionately large decrease in total biomass.

Thirdly, the distribution of individuals, although regular, may also reflect local heterogeneity of the plant environment, so that in some areas of the stand it may have been possible for larger individuals to grow at higher densities than in others.

Despite all these mitigating factors the gradient of the slope in Figure 2.6 remains unexpectedly low. The -1.5 slope indicates that a reduction in density causes such enhanced growth of the survivors that the total productivity in an area is increased by thinning. A gradient of -1.0 would indicate that the plants were at least growing enough to compensate for the thinning by producing an equivalent total biomass to an unthinned plot. The slope of -0.2118 is so far below this as to imply that the although the plants do increase in size with declining density, they do it so slowly that they do not even compensate for the removed plants, or conversely, increasing the density does not lead to such a rapid decrease in biomass as the power law predicts. Figure 2.6 shows that at high and low densities there are not corresponding extremes in size: this remains remarkably constant.

Gorham (1979) points out that this single simple rule relating shoot weight and standing crop to density is remarkable in its generality: it applies to mosses, ferns, gymnosperms, mono- and di-cotyledonous species, but only in the situation where there is competition for light. Several authors, including Westoby (1977) and Lonsdale and Watkinson (1982) have pointed out that light is the critical factor determining the -3/2 response curve. In natural situations where the canopy is not closed, so that there is no competition for this resource, the line approaches a gradient of -1.0 (Gorham 1979).

So it appears that despite the regularity of its pattern of occurrence, the data for the mature buddleia stand demonstrate that it is density independent: the competition is not for light, as it usually is in the case of monospecific stands. On the contrary, it appears that the pattern is determined by some other limiting factor, possibly of root competition for water, as is often the case with shrubs in arid areas (Woodell et al. 1969). Furthermore, even though the regularity implies that the competition is intense for some other limiting factor, the plants growing at particular densities are not evenly sized. Individuals with slender single stems were observed growing between large plants with many stems, thus creating a high density figure, but although these plants were obviously the poorer competitors they appeared to be capable of persisting for prolonged periods. They will presumably eventually die, but not necessarily as rapidly as predicted by Yoda's Power Law.

It is a feature of stress-tolerators (Grime 1979) that they are capable of persisting for prolonged periods with limited resources, which is generally achieved by limiting their growth to minimise their

resource requirements. Buddleia undoubtedly does this in the early stages of its life cycle, so if it can behave as a stress-tolerant competitor (sensu Grime, 1979) as a mature plant, switching between the 2 strategies as the circumstances demand, this may explain the scatter of points about the regression line in Figure 2.6.

It may be concluded from this survey of natural populations of buddleia, that the plant is originally dispersed in a contagious pattern, which probably reflects the heterogeneity of the seedling environment. Where there is a suitable site for germination in an area with a plentiful seed supply a number of seedlings colonise it. The seedlings are able to grow in high densities within small groups and are not apparently subject to appreciable self-thinning until a comparatively late stage of development. This is shown by the contagious pattern of dispersion in the young adults and the regular pattern of mature shrubs. Even then they are capable of persisting in relatively dense stands under conditions of intraspecific competition, which indicates that they are stress-tolerant competitors. The possibility that these plants exhibit some form of allelopathy to increase their competitive advantages and prevent the growth of new seedlings has also been investigated and is reported in section 4:11.

CHAPTER 3: SEED PRODUCTION AND DISPERSAL.

3:1 Introduction.

The survey of wild stands of buddleia in Sussex revealed interesting and unexpected features of its biology.

Firstly, the young plants are capable of persisting in close proximity to other individuals without much evidence of competition.

Secondly, the well-established plants are regularly spaced, indicating that competition is intense by this stage.

Thirdly, all of the stands are remarkably even-aged, with no new seedlings growing beneath the mature adult plants. Even after removing all the aerial growth from an area there is no sudden influx of new seedlings on the bare ground between the plants, which may either indicate persistent competition by the roots or that the surface of the ground has been modified in such a way that germination can no longer occur there.

The absence of a normal distribution of ages suggests the populations are not constantly recruiting new individuals. Two theories can be advanced to account for this. First, there may not be a sufficient supply of viable seed every year. Second, there may be a certain set of environmental requirements for the successful establishment of seedlings, which are not met every year at any one site. To investigate both of these possibilities and to explain the distribution patterns observed, the logical starting point is to look at the production of offspring by the adult plant, and to follow their fate.

The plants used were found on 3 sites, all within a 15 km radius

of Oxford. Site A, Folly Bridge, is a derelict city building site on the banks of the River Cherwell, in the City; buddleia plants of all ages are established here. Site B, at Dry Sandford, is a disused sandstone quarry, now a nature reserve and owned by the county Naturalists' Trust. In addition to grassland and woodland, the site incorporates areas of wetland and exposed cliff faces on which buddleia has been established for several decades. Most of the plants are mature individuals: occasional seedlings are found only on the cliff faces. Site C is a disused coal yard on land owned by and adjacent to the railway station in Oxford. It has a single mature plant growing in the middle of an extensive tract of derelict land. This land has a sparse cover of vegetation, chiefly bryophytes and lichens, since it is composed mainly of cinders and coal dust, criss-crossed with old railway lines and concrete tracks.

These plants were used to investigate the capacity of buddleia for self-pollination, the production and viability of seed, and the way seed is dispersed in space and in time.

3:2 Self-pollination.

Twenty-five bags measuring 25x30 cm. were made from medium-grade dressmaker's interfacing, a material with very fine pores which will keep out insects, but which is gas permeable and has good drying properties The sides were held together by "Vilene", a heat-sealed bonding which does not leave gaps or holes through which insects might enter. The bags were placed over inflorescences which had not yet flowered, and a piece of plastic sponge wrapped round the base of each inflorescence. The bag was tied tightly around the sponge to

ensure that no insects could crawl up the stem and into it.

The bags were left on the bushes for the duration of the flowering season, and then removed together with an equivalent number of unbagged inflorescences from the same bushes. They were subsequently examined in the laboratory where the intention was to record the numbers of seeds and capsules formed on each stem, and compare the results from the 2 treatments. However, this proved impossible since no capsules were formed on the inflorescences from which insects had been excluded, although large numbers of capsules, tightly packed with seeds, had been produced on those that not been enclosed and thus received visits from pollinators. The enclosed inflorescences had dried out but had retained their structure, indicating that they had not become water-logged. This result demonstrates conclusively that buddleia has no self-pollination mechanism.

Insect pollination has advantages as a method of enhancing genetic diversity in a population, provided there are sufficient insects about in the flowering season to act as pollinators. Many plants have evolved features to attract insects, but they often have alternative mechanisms to ensure that there is some form of reproduction in the event of failure by the pollinators. Self-pollination guarantees there will be offspring, even though they have identical genes to the parent; as such it is often exhibited by pioneer species which need to maintain seed production in areas where insects are likely to be scarce. The term self-pollination is used to mean the transfer of pollen from the anther to the stigma of the same flower without the aid of insects or other vectors.

Field observations of the insect visitors to buddleia indicate

that once an insect locates and lands on the inflorescence it spends prolonged periods feeding there and if it does move it is usually to another flowering stem on the same plant. It may therefore be assumed that the plant is not self-sterile, since many of the insect visitors are unlikely to move sufficiently frequently between different plants to transfer different pollen to each flower.

The absence of self-pollination explains the heavy allocation of resources to insect attractants and rewards in this species; the flowers are highly conspicuous because of their size and colour, and have a pungent scent with an abundant supply of nectar (Percival 1963). So it is bound to attract a high proportion of the nectar feeders which visit its locality, which leads to speculation about the impact that buddleia has on the pollination and seed production of the less attractive species in the same area.

Production of seed in buddleia reflects the activity of insects during the flowering season; a wet or cool summer produces proportionately fewer seeds than a warm, dry one. Nevertheless, for the reasons outlined above, buddleia probably does not suffer a marked reduction in output: this should become more apparent from the results of section 3:4.

3:3 Seed Production.

Having acknowledged that there is considerable annual fluctuation in the output of seed, it is important to have an estimate of the numbers of seeds produced by a self-established adult shrub in order to look at its life history characteristics.

A representative sample of infructescences from sites B and C

was collected and the number of seeds per capsule and of capsules per infructescence recorded for a sample of 50 capsules from 20 seed heads. The numbers of infructescences were counted on 15 plants, all between 1 and 3 m high and 1-1.5 m^2 in diameter of canopy. The figures are shown in Table 3.1. It should be stressed that this is only a guide to the magnitude of seed production: as the standard deviations in Table 3.1 show, there is marked variability in numbers of seeds produced per capsule (between 6 and 79) and in capsules per infructescence (240 to 1,650, in this instance), which is repeated in the numbers of infructescences per plant. As noted in the introduction, the size of the shrub and the panicles may also vary enormously. However, the figures in Table 3.1 may be used to estimate the seed production of a "typical" adult bush, about 2 m high with a canopy area of about 1 m^2, suggesting an output of 2-3 million seeds. This presumably means that the supply of seed is not normally a limiting factor in the colonisation of sites by buddleia.

3:4 Seed viability and innate dormancy.

Factors which have not so far been considered are the viability of the seed and possible dormancy mechanisms. A seed is described as dormant if it does not germinate despite an adequate supply of air, moisture and warmth, due to some physical or chemical blockage in the embryo or seed coat; overcoming that blockage constitutes the end of dormancy. Breaking dormancy is physiologically distinct from germination: the factor reponsible for ending dormancy is often not required for successful germination. Dormancy is usually broken by a

Table 3.1 The mean reproductive output from a sample of mature buddleia plants, growing on sites B and C.

	No. of seeds per capsule	No. of capsules per infructescence	No. of infructescences per plant.
Sample size	50	20	15
Maximum number	79	1,650	280
Minimum number	6	240	80
Mean	35.5	506.6	154
Standard deviation	20.1	373.9	77.4

discontinuity in the environmental conditions but once it has been broken then germination may proceed under the original conditions. For example, dormancy may be broken by exposing the seed to fluctuating temperatures or a flash of light but germination will then readily proceed at constant temperatures or in the dark.

Several forms of dormancy have been recognised and they are referred to by different names by different authors. Bewley and Black (1982) refer to relative dormancy as a condition which is only manifest under a certain range of environmental conditions which may change with time. Harper (1957) calls the same phenomenon enforced dormancy. Complete dormancy, on the other hand, is when the seeds are dormant regardless of the surrounding conditions. Innate dormancy (Harper 1957) is inherited from the mother plant and present when the seed is shed; it is called primary dormancy by Bewley and Black (1982) but this may lead to confusion since other authors (Crocker 1916 and Koller et al. 1962) have used it to refer collectively to innate and relative dormancy. The third major catagory is called secondary dormancy by Bewley and Black (1982) and by Mayer and Poljakoff-Mayber (1982) or induced dormancy by Harper (1957) and Barton (1965). Both names describe that state which arises when a mature imbibed seed is left under conditions which are unfavourable to germination. Subsequent modification of those conditions to a level which would originally have supported germination will no longer do so. The seeds have developed some form of dormancy which will only be broken by further environmental changes, such as chilling or scarification.

The one feature that all of these dormancy mechanisms have in common is that they are broken when the seed is subjected to

environental discontinuities.

The effects of pre-maturation experiences on the seed are well known in the literature on seed germination (Bewley and Black 1982). It is possible that there are considerable differences in the viability of seed from one year to the next as a result of environmental conditions prevailing at the time of seed formation and development.

To find evidence of innate dormancy in buddleia and to assess the changes in viability from year to year and with prolonged storage, seeds were collected from sites A and B in 1979, 1980, 1981 and 1982. They were stored for 6 months before being tested and then stored for periods up to 42 months, with a check on viability at annual intervals. The seeds were set up in a standard procedure (Proceedings of the International Seed Testing Association 1966), which was used in all the germination tests.

100 seeds were arranged regularly on 2 sheets of Whatman No.1 filter paper in a plastic petri dish and moistened with 4 ml distilled water. Four replicates were set up, the dishes sealed around the rim with Parafilm to prevent desiccation and then placed on a shelf in a Fisons Growth Cabinet, series III Model 140G2. The cabinet was illuminated with 16-18,000 Lux for a 16 hr. day at $24^{o}\pm2^{o}C$, and a period of 8 hr. darkness at $20^{o}\pm2^{o}C$. The dishes were left in the cabinet for four weeks when it was assumed that all of the viable seed had germinated. Daily inspections were made and the numbers of seeds that had germinated were recorded. Throughout, germination is recorded when there is a protrusion of at least 1 mm of the radicle through a break in the testa.

The results of the dormancy and viability tests on buddleia seed

collected in the 4 successive years are shown in Table 3:2, and the effects of storage are shown in Table 3.3. There is no evidence from these that there is any form of primary dormancy in buddleia seeds. As far as is known any factor which inhibits germination of a seed may also induce secondary dormancy. It appears from Table 3.3 that the storage of buddleia seeds has not had any such effect but there is no evidence to indicate that under field conditions the seeds do not enter a period of secondary dormancy.

Examination of Table 3:2 with respect to the viability of the seeds, the analysis of variance produces $p<0.01$, which indicates that there is a significant difference in viability over the 4 years. However, the viability is high, even in the poor year of 1981, and the changes probably reflect differences in the weather and other external conditions that influence the parent plant at the time of seed formation and maturation (Bewley and Black 1978).

Table 3.3 shows that the viability remains remarkably high during the first 2 years of storage but there is a marked decline between 30 and 42 months: by the end of this period none of the seeds tested were still viable. Moreover, since the seeds were stored in an air tight plastic box in the laboratory, this may be an over-estimate of their persistance under field conditions. Viability declines more rapidly under moist and warm conditions than in cool, dry ones (Bewley and Black 1982), so it might be expected that seeds deteriorate more rapidly in the field. It is unlikely that there is a total loss of viability within one year, but from the above evidence it is not expected that any seeds in the soil would germinate after 3 years and probably few would persist after 2 years.

Seed was also tested from 2 garden sources in the Oxford area to

Table 3.2 Differences in seed viability in 4 successive years.

Year of seed production	Mean percentage germination
1979	99.0
1980	89.75
1981	85.5
1982	97.5

$p<0.01$, a significant difference between years.

Table 3.3 Changes in seed viability over a 42 month storage period.

Length of time stored	1979 Seed	1980 Seed
6 months	99.0	89.75
18 months	95.75	90.5
30 months	n.a.	79.25
42 months	0	-

see if there was any significant difference in the germination potential of seed from cultivated and naturalised buddleia. The results of this are presented in Table 3:4 and the results of an analysis of variance test carried out on the data indicate that there is no significant difference in the viability from either source. It must be noted that none of the seeds was tested in conditions other than the mesic ones described above; it is possible that variation between plants or between years may not be apparent under such conditions, but that in the typical marginal habitat of buddleia these variations may be more obvious.

The germination in response to various environmental conditons, including temperature, light, oxygen and water supply, is described in detail in Chapter 4.

3:5 Seed dispersal: the timing of release.

Although it is known that buddleia seeds are retained on the shrub over winter and released in spring, the time period over which this release occurs has not been previously recorded. The following field investigations were designed to monitor the timing of seed release.

Seed heads were harvested in the third week of November 1981, when the capsules had swollen and turned brown. After air drying in the laboratory for 3-4 hrs. the capsules had opened sufficiently to remove the seeds. The viability of these seeds was assessed using standard germination tests described in section 3:4. The resulting figures for viability are shown in Table 3.5. Comparing these with the figures for seed viability in Table 3.2, it is clear that seed was

Table 3.4 Differences in seed viability from cultivated and self-established buddleia sources.

Seed source:	Mean Percentage Germination
Oxford garden	99.5
Dorset garden	99.25
Kidlington garden	98.0
Westgate, Oxford (self-established)	98.75

$p>0.05$, indicating a non-significant difference.

Table 3.5 Viability of 1981 seed from site B, harvested and sown on 21.11.81.

Replicates	Percentage germination
1	86
2	87
3	90
4	83
Mean	86.5

mature by November and was retained on the parent after ripening.

Twenty-four representative infructescences were then selected from 12 shrubs growing on site B, and each was enclosed in a cloth bag to collect seed as it was released from the capsules, as shown in Figure 3.1. The bags were made of a tough terylene/cotton fabric which dries out rapidly and prevents water-logging - which might have affected the results by keeping the seed heads moist. Each bag measured 17 x 25 cm and was attached to the plant by tying securely round the base of the infructescence with wire, preventing seeds from escaping even in high winds. Bags were replaced at two-week intervals from the third week in January, when the first seeds had been released, until May, when seed heads had degenerated, so that the only remaining seeds were those which were caught up in fragments of capsules that still clung to the stem. Numbers of seeds were recorded from each bag. Where the numbers were particularly high the following sub-sampling technique was employed. All the seeds in the bag were shaken-up with 1 litre of water. As they are so small they were evenly distributed through the column of water, so by removing a sample of 50ml water and counting all the seeds in it a number was obtained which could be multiplied up to give a total.

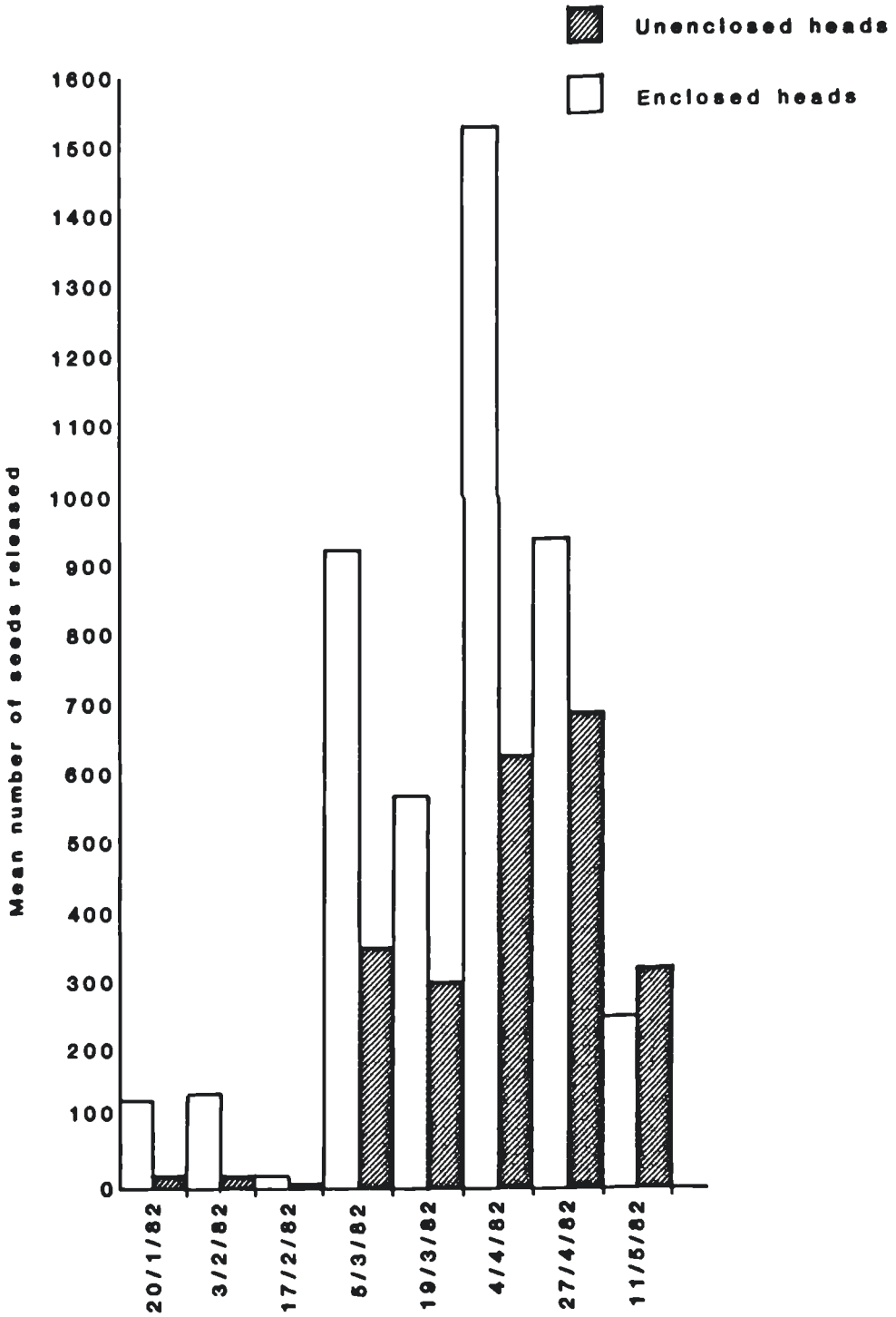
Two different experiments were devised with these bags. The first involved enclosing the same 12 infructescences throughout the season, while the second used 12 different ones each time the bags were changed. This was designed to show whether the bags had any long term effects on the retention of seeds in the capsules or if the pattern of release was constant.

The results of the 2 experiments are shown in Figure 3.2. Most of the seed is released between the middle of February and the end of



Figure 3.1 Buddleia infructescence enclosed in a cloth bag to collect seeds as they are released.

Figure 3.2 The pattern of seed release over a 5 month period in 1982: seeds from continuously enclosed and previously unenclosed seed heads.



April (over 88% in this instance), with a peak at the end of March and the beginning of April. This distribution reflects the weather at this time of year; with a typical British spring alternating between gusts of wind and squalls of rain, the season for release is protracted but has peaks coinciding with the dry spells.

The seeds which were collected from different infructescences each time show the same overall pattern as those released from the continuously enclosed heads. The actual numbers are lower, but this is probably because without the impedence of the material many more seeds are lost during the period before the seed head is enclosed, leaving fewer to be collected during the 2 week period in the bags.

The timing of seed dispersal and the subsequent patterns of dispersion in space often determine the fate of the seed. In many species a marked pattern of release reflects a specifically evolved response to environmental triggers. The seeds may be shed immediately after ripening or retained on the parent for a specific time interval after which they may be released synchronously or gradually, over a period of weeks or months. These different stategies enable plants to exploit their natural environment, for example, by delaying the release of seed until conditions are favourable for germination, thus protecting the seed from the risks of prolonged residence in the soil.

Buddleia retains its seeds on the plant throughout the winter, releasing them from early spring onwards. It employs a simple physical device to prevent the release of seeds in unfavourble dispersal conditions. The seed capsules remain closed under wet conditions, but when dry they open, the sides of the capsules curling outwards, enabling the seeds to drop out if there is sufficient air movement to shake them loose. When the atmosphere becomes humid again the capsules

close; this takes less than 5 minutes and dispersal ceases until drier weather ensues.

This seed retentive mechanism is a great advantage for a wind-dispersed opportunist: it ensures that seeds are only released when they can become airborne, thus increasing the area of dispersion.

If buddleia has no primary dormancy mechanisms, the seed presumably germinates once it reaches a safe site on the ground, under appropriate environmental conditions. Retention of the seed on the parent prevents autumn germination with the subsequent problems of over-wintering that newly emerged seedlings would encounter. The rapid closure of capsules also prevents seeds within imbibing water and then germinating before they have dispersed. The spring weather may provide good conditions for seed release and transport over prolonged distances, together with moist conditions for subsequent germination. Consequently, there is a good chance that the seed will germinate soon after dispersal, provided that it can lodge in a suitable "safe site" (Harper 1977).

3:6 Seed dispersal patterns in space.

Since buddleia is a pioneer species, the small, winged seeds are typically adapted for mobility, capable of remaining airborne for prolonged periods once they are caught by the wind. What proportion of seeds is actually carried away from the parent plant and what proportion drop to the ground below and thus remain within the sphere of maternal influence is not known. So the following seed trapping experiment was also run through the spring of 1982 to investigate the dispersion of seed originating from a single source, the isolated

buddleia plant which grew on site C, the disused coal yard.

Five concentric rings of traps were placed at 16 compass points around the plant, at distances of 0.5 m, 1 m, 2.5 m, 5 m, and 10 m from the base. Figure 3.3 shows a row of the traps radiating from the experimental plant. The traps were made of an 11 cm square of hardboard, supported on a 15 cm nail which was pushed into the ground until the top of the trap was only 2 cm above the surface. A square of thick polythene was stuck to the top of the trap using a waterproof silicon compound, so that it could stand up to heavy rain. A disc of greaseproof paper was stuck to the polythene with "Copydex" and this paper was covered with a thin film of fruit tree banding grease, produced by I.C.I. Plant Protection Division. Figure 3.4 shows a finished trap: the area of the actual sticky trap was 62.5 cm^2.

Previous tests showed that once a seed lands on the trap it is unlikely to blow off again, since the grease remains sticky even after several weeks of exposure to rain and sun. The grease is almost colourless and on wetting turns opaque, but buddleia seeds which land on it become a distinctive orange colour, making them easy to distinguish from all the other seeds caught. In Figure 3.5 two buddleia seeds trapped on the grease can be easily identified. The numbers on each trap were recorded at 2-week intervals, and the plastic and sticky parts replaced, so that the traps remained effective.

Figure 3.6 is a polargraph which indicates not only the direction and distance that the seeds had travelled before they landed, but also the densities landing on each trap. This clearly shows that the highest density of seed rain is directly beneath the parent shrub. However, the traps obviously sample a much greater



Figure 3.3 A row of sticky traps, used to monitor seed dispersion patterns, extending away from an isolated mature buddleia at site C, in Oxford.

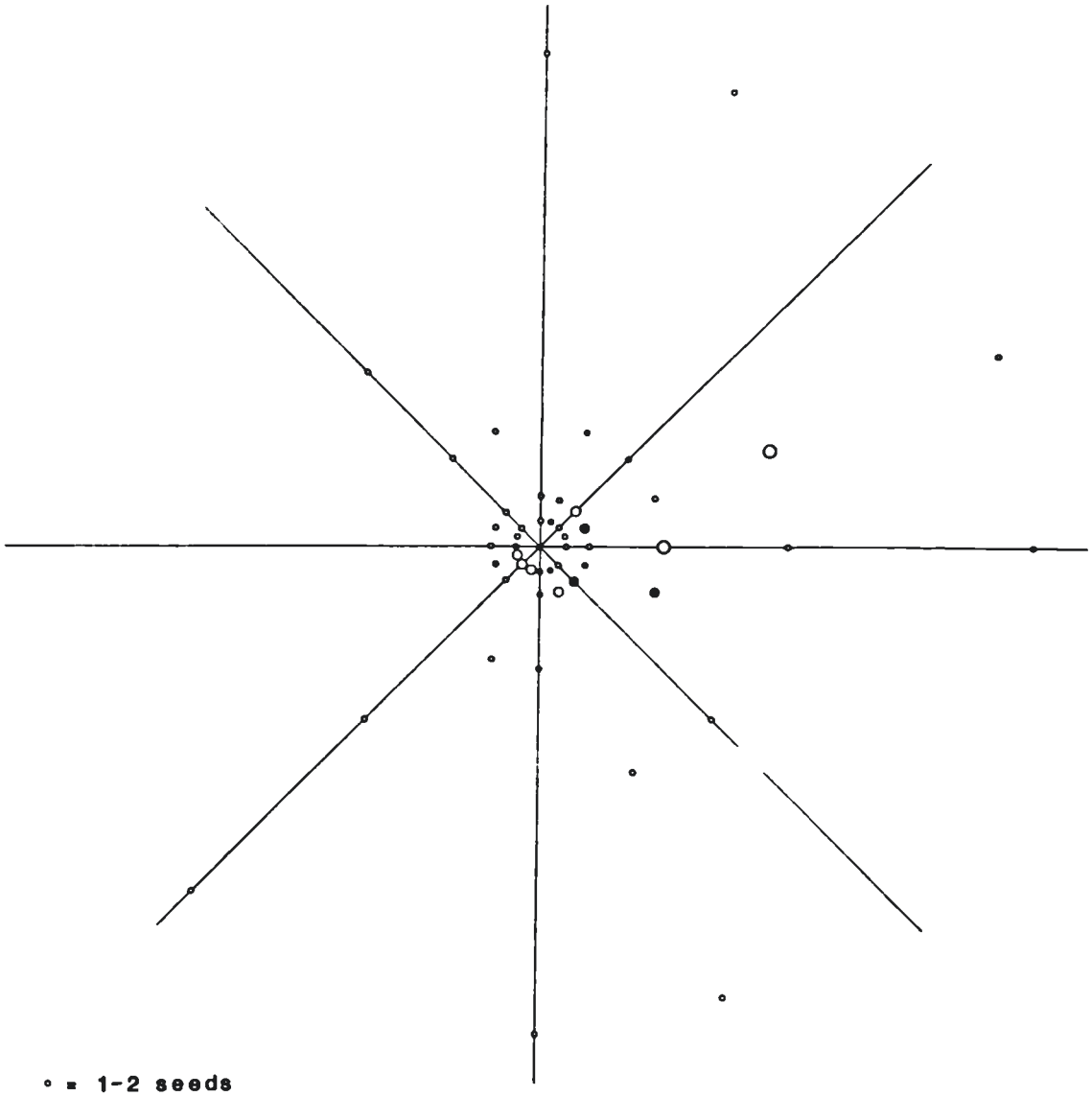


Figure 3.4 A sticky trap in use on site C, catching seeds and the odd insect.



Figure 3.5 The surface of a sticky trap, with the buddleia seeds in the centre of the picture clearly distinct from the other material.

Figure 3.6 The distance, direction and density of seed dispersion from an isolated mature buddleia plant, over a 4 month period.



° = 1-2 seeds

• = 3-4 seeds

o = 5-6 seeds

● = 7-8 seeds

O = 16-18 seeds

proportion of the area directly around the bush than of the area some some distance away. To overcome this discrepancy, numbers of seeds falling over the entire area of a concentric ring 1m wide, at each trapping distance from the plant, have been calculated and are presented in Table 3.6. From these figures it is apparent that only a small proportion of the total seed output falls beneath the parent plant; as the distance from the parent increases towards 10 m from the plant, so does the deposition of seed. Unfortunately, it was not possible to extend the traps beyond 10 m, so the point where it starts to decrease (which marks the limit to which the majority of seed travels) remains unknown.

Using the data from Table 3.6, it is possible to calculate the expected numbers of seeds falling over concentric rings 1 m wide, at 1 m intervals from the shrub, up to a distance of 10 m. The total number indicates the seed rain over the entire area within 10 m of the plant, as shown in Table 3.7. The output of seed from a mature, healthy buddleia of this size was estimated in section 3:2 as about 3 million seeds, and since Table 3.7 shows that only about 150 thousand are falling within 10 m of the shrub, it appears that over 95% of the seed are deposited on sites over 10 m from the parent. This may seem to involve an unacceptable level of approximation, but even with a conservative seed production figure of 1 million, the proportion of seeds falling within 10 m of the parent is still only 15%.

This high level of dispersal of seed which takes place over a prolonged period is advantageous to a colonising species. It also has benefits for a mass-flowering species in which outcrossing is likely to be infrequent. This makes it important that new individuals appearing near an established shrub should be of different genetic

Table 3.6 Changes in seed rain with increased distance from the source, expressed as density per m^2 and as numbers deposited within a 1 m diameter ring at each distance from the source plant.

Distance m	Area of ring m^2	Density per m^2	No. deposited within each ring
(D)	($4\pi D$)	(n_m)	($4\pi Dn_m$)
0.5	6.283	561.91	3,530.5
1.0	12.566	423.90	5,326.7
2.5	31.416	414.04	13,007.5
5.0	62.832	207.02	13,007.5
10.0	125.664	167.59	21,060.0

Table 3.7 Changes in seed rain with increased distance from the source, expressed as density per m^2 and as numbers deposited within a series of 1 m diameter rings, at 1 m intervals from the source plant, calculated from the observed values.

Distance m	Density per m^2	No. deposited within each ring
(D)	(n_m)	($4\pi Dn_m$)
1.0	424	5,327
2.0	416	10,455
3.0	373	14,062
4.0	290	14,577
5.0	207	13,006
6.0	199	15,004
7.0	191	16,801
8.0	184	18,498
9.0	176	19,905
10.0	168	21,112
		148,747

composition so that in the event of cross pollination occurring, the gene pool will be increased. However, this dispersal is unlikely to account for the absence of seedlings beneath the parent. The density of seed rain is still heavier here than in any area further from the plant, so it cannot be simply accounted for by the lack of an adequate seed supply.

The explanation may lie in the response of the seeds to factors influencing germination. Before making any further observations of the seeds and seedlings in field situations, it is necessary to carry out controlled laboratory studies, to determine the range of environmental conditions which produce successful germination and seedling emergence. Equipped with this information, it is possible to move beyond speculation in attempting to explain the distribution of buddleia and the patterns in which it naturally occurs.

CHAPTER 4: LABORATORY SEED TESTING.

4:1 Introduction.

The seed is the mobile phase of the life cycle of most plants and as such of particular interest in the study of a colonising species. This is the point at which the plant can move to a new site, but it will only germinate if that site fulfills a specific set of requirements. Similarly, the seedlings can only survive within a certain range of environmental conditions, but as the plants develop, they become tolerant of a variety of stresses that would be lethal at earlier stages. It is these early phases of plant development that determine which sites are colonised and which are not, depending on site conditions and the extent to which a particular species can tolerate them. Consequently, it is important to establish which environmental conditions will support or limit germination and seedling establishment of buddleia, in seeking to account for the distribution of this plant.

The combination of factors which cue the onset of germination reflect the conditions to which the seed is exposed in its native habitat. Modifications such as dormancy mechanisms and specialised germination requirements are often responses to periodic environmental stress, enabling the plant to remain in its most resistant state, usually the seed, until there is a change in environmental conditions to those which favour seedling establishment.There is considerable interspecific variation in both the optimum and the range of such conditions. For any particular species this is further complicated by

interaction between the factors: for example, under low light intensities many seeds become more temperature sensitive and vice versa.

Consequently, in order to understand and interpret field data on the fates of the seeds, a series of laboratory investigations was carried out to determine seed responses to a range of environmental factors under controlled conditions. The responses were generally recorded in terms of percentage germination, and in some cases the maintenance of viability. From this information it will also be possible to establish whether there is any mechanism for dormancy.

The factors initially considered were the dominant environmental variables: temperature and light (and the interactions of these two) and oxygen supply. The less obvious but important influences of seed storage on the bush, substrate pH and autotoxicity were also considered individually.

4:2 The process of germination.

It is useful at this point to digress and restate the meaning of several reference terms. Dormancy is the arrest of development resulting from structural or compositional factors within the seed, as opposed to quiescence, the term used to describe arrested development imposed by unfavourable environmental conditions (Villiers 1972). Germination is a separate process, under different physiological controls from either the dormancy which may precede it or the growth of the seedling which occurs subsequently. Essentially,it is the transformation of an embryo into a seedling by

the resumption of metabolic pathways typical of growth or development which had earlier been suppressed or suspended (Jann and Amen 1977).

The actual process of germination is triphasic (Bewley and Black 1978). Phase I is the uptake of water, or imbibition, which occurs in both living and dead seeds. Only living hydrolysed seeds then enter Phase II which is a period of activation of the arrested metabolic processes preparatory to elongation and growth of the embryo, and occurs in both dormant and non-dormant seeds, although it only leads on to Phase III in the latter type. This is the start of visible germination, where the embryo elongates and stored reserves are mobilised. It may involve cell expansion alone or in conjunction with cell division. The expansion and the division of cells are under separate control mechanisms during germination, rootlet protrusion resulting from cell elongation with little or no contibution from cell division (Haber and Luippold 1960). Germination and seedling development can occur without any cell division in some species, e.g. wheat (Haber and Foard 1964), implying that expansion precedes division in seed cells. However, in other genera such as Prunus the cell expansion occurs simultaneously with cell division or even after proliferation of cells in the embryo (Pollock and Olney 1959).

So, it may be concluded that cell expansion is responsible for the third phase of germination, with cell division involved sometimes, to a lesser degree, but once the cells start to proliferate and cause the root to protrude beyond the outer coat of the seed, then germination is complete and the growth stage has begun.

A brief mention should be made of the problems of defining when a seed is no longer capable of germination. Few workers refer to the

"death" of seeds, but instead use phrases such as "failure to germinate" and "loss of viablity". This is because it is often difficult to determine when the seed is dead, and not particularly relevant to tests of viability. The seed may be incapable of producing a normal seedling and so defined as non-viable and certainly dead from the evolutionary point of view, without all the seed tissue being dead. Conversely, some tissue damage does not necessarily result in failure to germinate, so reference to damaged and undamaged seeds does not indicate which are viable. The only way to be sure that an ungerminated seed is dead, is to test it with tetrazolium solution, which stains the embryo and endosperm of living seeds. Throughout these investigations any seeds which had not germinated within 2 weeks of the majority were recorded as non-viable.

4:3 Germination and development of buddleia seeds.

The following description of the process of germination and development of seeds is the result of observations of more than 5000 seeds in laboratory germination tests, as described in section 3:4, in a growth cabinet with 16 hr. photoperiod of 16-18,000 Lux, at 25°C and 8 hr. in darkness at 18°C. The cabinets used throughout the laboratory studies were Fisons controlled environment cabinets, unless otherwise stated. Similarly, the lighting and temperature regime was always as described above, except where stated to the contrary.

The initial phase of imbibition in buddleia is followed by the leaching of a yellow pigment from the seed within 24 hours of hydration. Neither the composition nor the function of this leachate

are known, but it is possibly a flavone or chalcone, acting as a germination inhibitor (Bewley and Black 1978). The membranous outer coat, which is drawn into 2 wings for dispersal, swells during imbibition to form a sheath within which the embryo expands and the radicle elongates until it finally ruptures the seed coat at a point about half-way along the length of a wing.

Immediately before it ruptures, a circular ring of fine hairs, originating from the junction between the hypocotyl and the epicotyl, extend through the seed coat, a phenomenon which has also been reported in germinating seeds of Populus tremuloides (Fechner et al. 1981). They function as initial absorptive organs, taking up sufficient water to support the rapid expansion and proliferation of cells in the subsequent stages of development. When the radicle has emerged the gap in the seed coat is widened by the growth of the hypocotyl until the cotyledons are drawn out behind it. The seedling is often completely free of the seed coat at this stage, but it sometimes remains attached to the cotyledons for several days. When germinated in the dark, the cotyledons remained folded in the seed case, implying that it is removed as a result of the cotyledons drawing apart.

4:4 Temperature requirements for germination.

Seeds collected at the end of 1979 from site B were used in the germination tests. Three similar Fisons environmental cabinets were set at constant temperatures of 15°C, 24°C and 29°C and 4

replicate dishes of seeds were placed in each. A fourth set was placed in an improvised* environmental cabinet at 9°C, which previous tests had shown remained constant to within 1°C and so comparable in constency to the Fisons growth cabinets. The lighting intensity in these cabinets was lower at 6-8,000 Lux. A fifth set of seeds was placed in an unlit incubator at 29°C. The number of seeds which had germinated was recorded on 6 occasions over the following 2 weeks after which it appeared that all the viable seeds had germinated, so that the rate as well as the final percentage germination could be compared.

The results shown in Figure 4.1 indicate that buddleia seed can germinate over a range of at least 14°C. It is notable that seed at 9°C is apparently too cold to become metabolically active. The rate is faster at higher temperatures, which reflects the thermal control of metabolic rates. The optimum temperature from this limited range appeared to be around 24°C, which is rather higher than the plant is likely to experience in a British spring.

A second series of tests was carried out using stored seed from 1981 and fresh seed formed in 1982. The 2 seed batches were subjected to alternating temperatures, linked to the 16 hour photoperiod, to represent the nocturnal drop in temperature that would be experienced naturally. This was carried out at both high and low pairs of temperatures, one set of seed from each year experiencing 25°C for 16 hours and 18°C for 8 hours, and the other set alternating between

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* The cabinet was made from a perspex box, to which was attached a small heating unit with a fan, to maintain a constant circulation of warm air. A thermostatic control ensured that the temperature remained constant, and the cabinet was kept in a cold room, at 4°C, to prevent overheating.

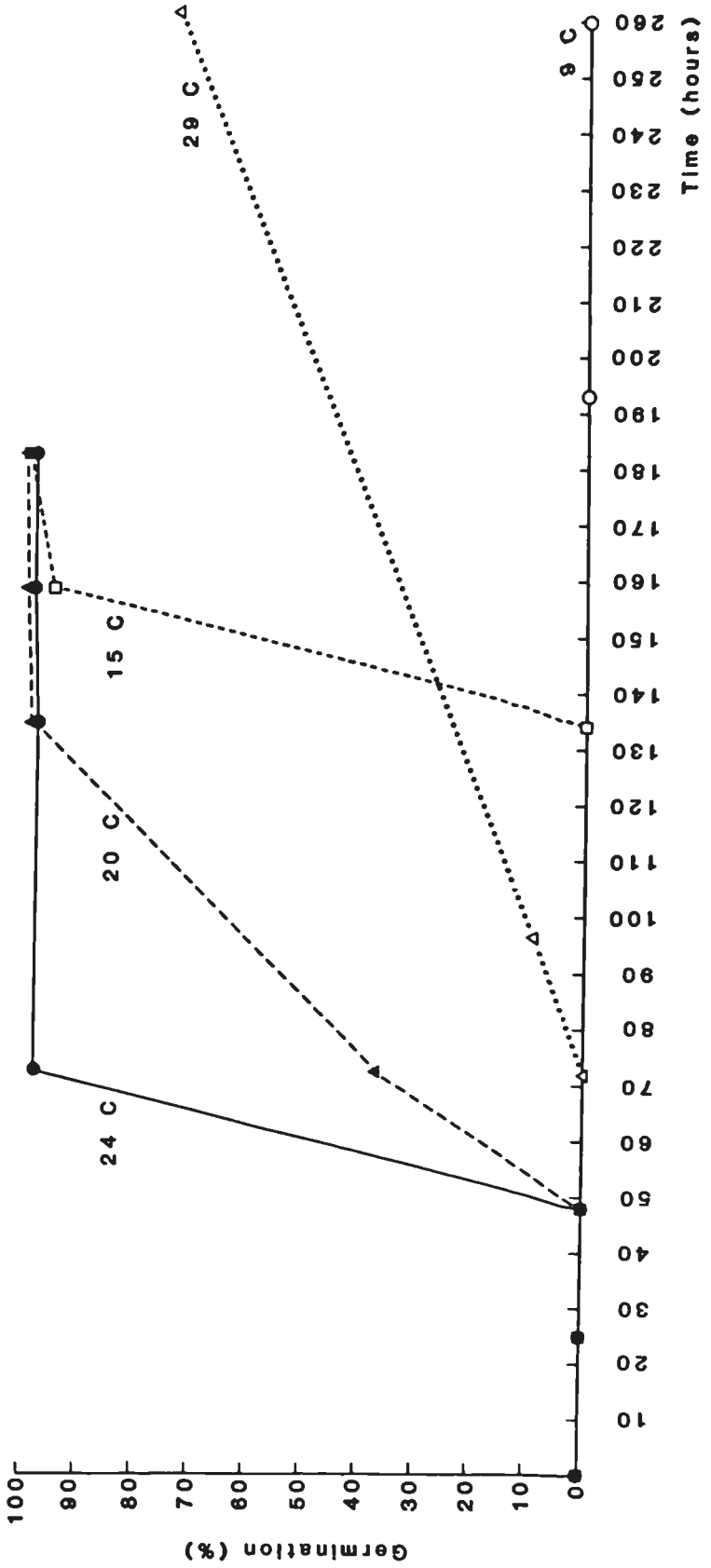


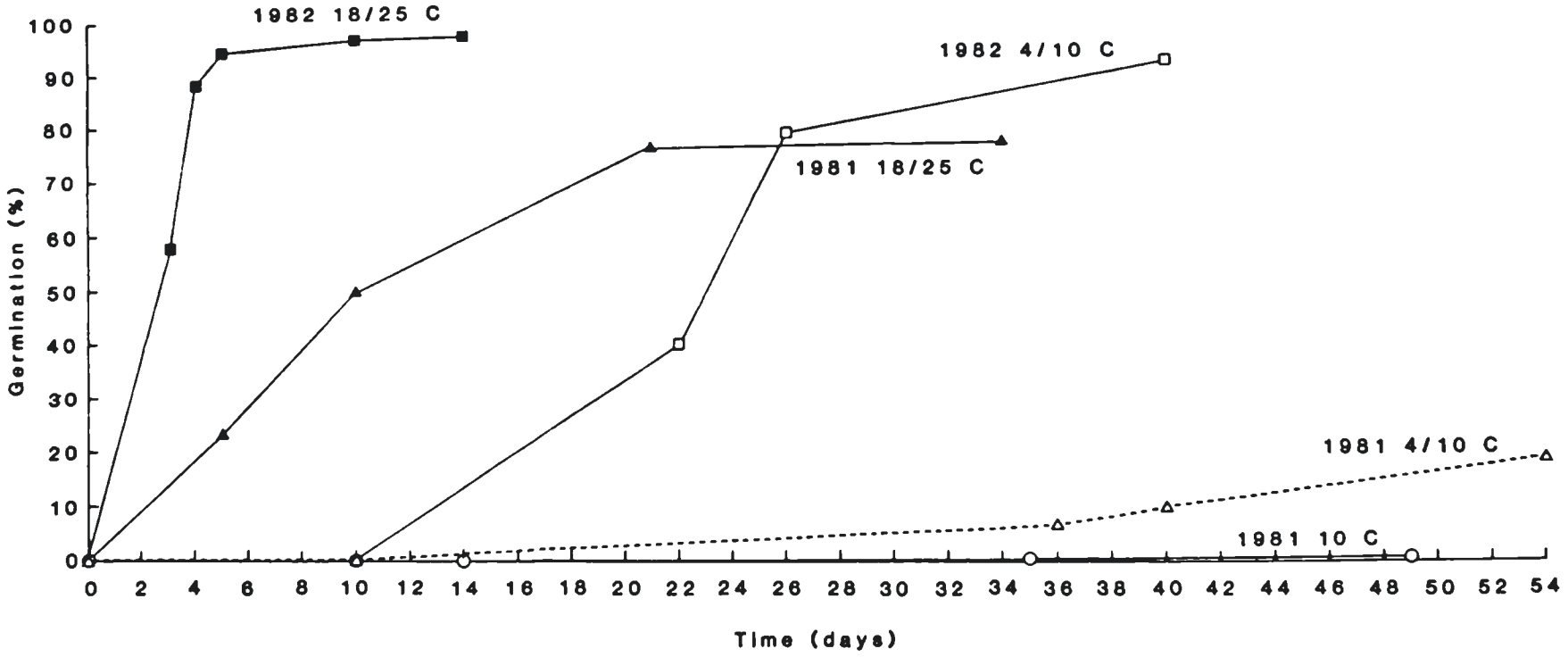
Figure 4.1 Rates of germination in response to a range of temperatures with a 16 hour photoperiod.

10°C and 4°C. For comparison a set of 1981 seeds was kept at 10°C constantly. There were 4 replicates of each treatment. Again the number that had germinated was recorded on 5 occasions over the following 7 weeks when it was decided all viable seed had germinated.

The results are presented in Figure 4.2, from which 2 interesting features emerge. Firstly, the seeds did not germinate at a constant 10°C, but when the temperature was dropped to a lower night-time level of 4°C the fluctuation resulted in some germination. At higher temperatures the germination was high anyway, so no change was evident, which suggests that it only becomes important when the seeds are in a more extreme environment. Secondly, the difference in the behaviour of the seeds from the 2 successive years was pronounced, particularly at the lower temperatures. The final percentage germination of 1982 seed was well over 90% in both high and low temperature treatments, although the rate was predictably slower in the cooler treatment. Seed from 1981 was not only slower to germinate at low temperatures, but also only achieved a fraction of that occurring at the high temperatures.

This implies that seed viability deteriorates rapidly, so that when subjected to conditions which are near the limits of its tolerance only a small proportion of seed can germinate. When seed batches were tested for viability at optimal temperatures (section 3:4) the deleterious effects of ageing are largely lost. However, at field temperatures the ageing factor may be of greater significance.

Figure 4.2 Germination of fresh seed (1982) and stored seed (1981) at high and low temperature regimes.



4:5 Light and temperature interactions.

There is no evidence for innate dormancy in buddleia, but since all previous tests were carried out with illumination it is possible that the seed is photosensitive, remaining dormant in total darkness. Light is of great ecological significance as a factor controlling dormancy in many species: the position in the soil where germination occurs may be determined by the seed response to light. Species which are buried or under dense canopies may be prevented from germinating in conditions that are poor for survival by some form of light contolled dormancy, which is what happens to Arabidopsis thaliana (Cruciferae), Digitalis purpurea (Scrophulariaceae) and Epilobium hirsutum (Onagraceae) (Grime 1979). On newly disturbed soils, light is responsible for much of the ensuing flush of germination.

The interaction of light and temperature produces the seasonal germination patterns observed in many species, for example, Poa trivialis (Gramineae), Rumex obtusifolius (Polygonaceae), Stellaria media (Caryophyllaceae) (Grime 1979). The modifying effect which temperature often has is readily understood since the phytochrome which is responsible for photodormancy is subject to thermal modifications (Bewley and Black 1982). The interactions may become apparent at low, moderate or alternating temperatures, depending on the mechanisms involved. They represent further adaptations which permit germination in the field only at times when establishment is most likely to succeed.

To establish whether there is photodormancy in buddleia, with

any form of temperature interaction, the following experiments were carried out.

Eight sets of 1981 seeds were used in the standard germination procedure, but with 4 of the sets the preparation was carried out without even the illumination of a green "safe" light once the water had been added to the petri dishes. These dishes were double-wrapped in aluminium foil so that no light could reach the seeds before they were placed in the growth cabinets, together with the control dishes for germination in the light. The seeds at 15°C, 25°C and alternating 25/18°C regimes were placed in Fisons cabinets and those at alternating 10/4°C were placed in the improvised cabinet.

The results are presented in Figure 4.3, and the results of t-tests between the means are in Table 4.1. Unfortunately it was not possible to record the rates of germination since this would have meant illuminating the dark sets of seeds. The variety of temperatures used was designed to show if there were any differences in the light reponse at either end of the thermal germination range. As Figure 4.3 shows, the dominant factor here is temperature: as in the previous experiment there is a marked decline in the performance of seeds at the lowest temperature and again it is only about a quarter of the germination achieved at the higher alternating temperatures. From Table 4.1 it is apparent that the effect of illumination on the germination was not significant except at 15°C ($p<0.01$). This may indicate that the seed shows some degree of photoinhibition at reduced temperatures, but the means from the 2 lighting conditions at 10/4°C were not significantly different ($t = 1.02$), which suggests that the

Figure 4.3 Total germination with and without light and under 4 different temperature regimes.

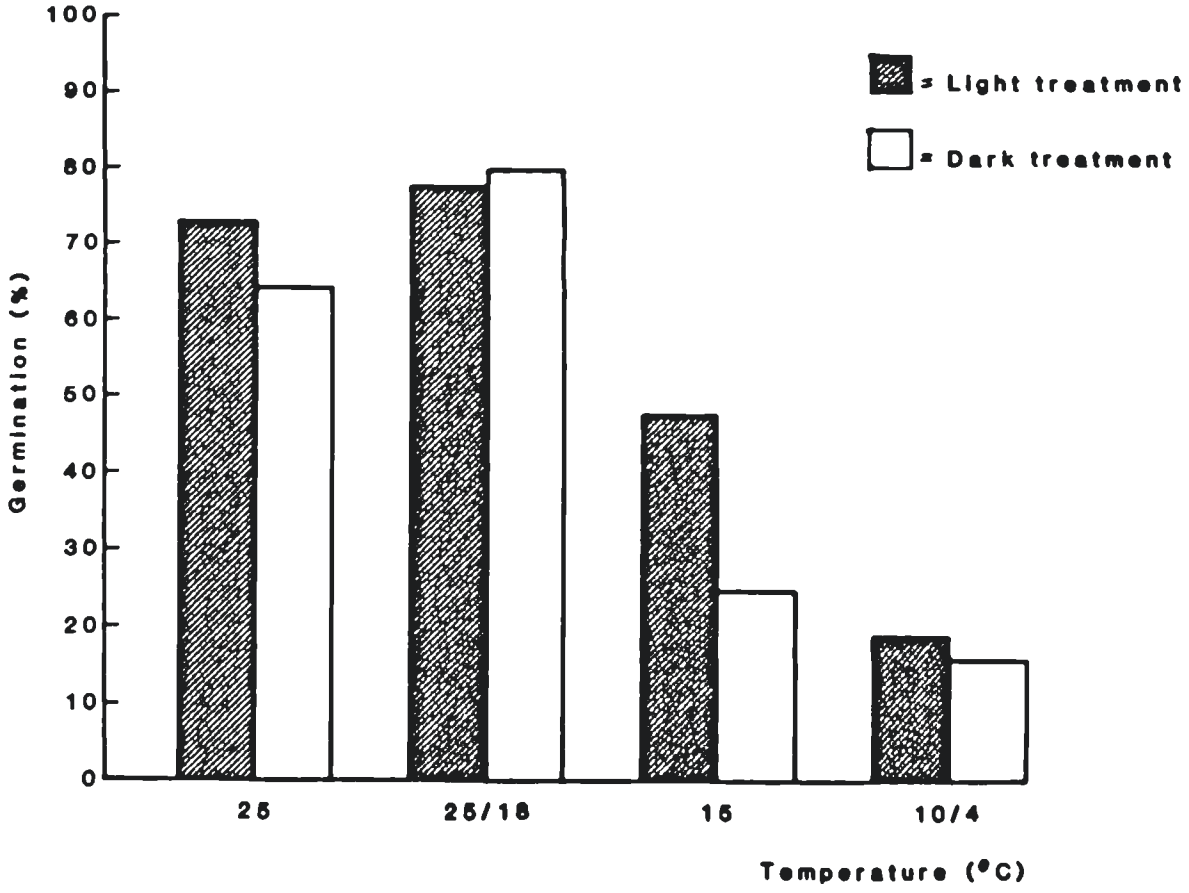


Table 4.1 The results of t-tests to compare the mean germination under light and dark conditions, at 4 different temperature regimes

Temperature treatment (°C)	Calculated t value	Significance
25	1.36	$p>0.1$
15	5.78	$p<0.01$
18/25	0.53	$p>0.1$
4/10	1.02	$p>0.1$

results at 15°C were anomalous.

So, it appears that light is unimportant in the germination of buddleia seeds, irrespective of the temperature at which they are tested. It is also clear that there is no innate dormancy in buddleia, an important fact when considering the fate of the seed in the field.

The seedlings which had germinated in total darkness had an average hypocotyl length of 15 mm and roots only 1-2 mm long, which is the reverse of the normal proportions. Since there was an adequate water supply the seedling could divert its resources away from root extension and into elongation of the hypocotyl. However, in field situations where water is limited, root extension will proceed at the expense of shoot development. The effect of this on the seedling emergence will be considered further in section 4:8, in relation to the depth of seed burial.

4:6 Low temperature effects on imbibed seeds: the impact on viability.

The seeds from the preceding experiment which had been kept in the dark for 10 weeks, at alternating temperatures of 4/10°C, on a moist substrate, had experienced similar conditions to those which prevail on the ground during winter, so it is interesting to note what effect this treatment has had on the viability of the seeds.

The dishes were transferred to a growth cabinet under the 18/25°C lighting and temperature regime, and left for 4 weeks, when germination appeared to be complete. The number of seeds which

germinated was recorded and compared with the number that germinated in the 18/25°C light treatment in the previous experiment.

The figures for both sets of seeds and the results of a t-test between the means are given in Table 4.2. These show that there has been a highly significant loss of viability in the seeds which were kept imbibed at low temperatures, although over 50% of the seeds were still capable of germinating after 10 weeks under these conditions.

There are 2 possible explanations for the loss of viability: firstly, it may be due to metabolic degradation of the seed, or secondly, it may be due to microbial action on the imbibed seeds. The degradation of seeds at high moisture levels and low temperatures has been the subject of exhaustive research using Phaseolus lunatus (Pollock 1969), which has demonstrated that the seed is most sensitive to chilling damage during the initial phases of imbibition. Severe injury may occur at 5°C if the seed moisture content is below 20%, due to the physical disruption of metabolic systems involved in this crucial early phase of germination. Damage done at this stage is often irreparable, unlike damage at later phases of germination.It appears to disrupt mobilisation of seed reserves and the growth of the radicle, as well as causing abnormalities in embryonic structures. However, if the seed is able to initially take up water at a higher temperature, then subsequent dehydration, chilling and rehydration do not have the same effects: the process that has formerly been activated is irreversible and the likelihood of chilling damage to later stages is greatly reduced (Maguire 1977).

The loss of viability recorded in this experiment appeared to be due to metabolic disruption rather than microbial action, since very

Table 4.2 The viability of buddleia seed after 10 weeks storage, fully imbibed, at low temperatures.

Replicates	Percentage germination of control seeds	Total percentage germination of seeds pre-treated at 4/10°C for 10 weeks
A	72	58 (11)
B	77	50 (17)
C	82	54 (15)
D	79	63 (21)
x	77.5*	56.25 (16)*

* significantly different when compared using a t-test: $p<0.001$

Numbers in brackets denote number germinating during pre-treatment, and are included in the total figures.

few seeds showed evidence of fungal infection at the end of the viability test. Consequently, the 27% decrease in viability is a conservative estimate of the expected losses in the field. Repeated drying and wetting, which would probably occur in the ground, would fracture seed coats and damage the embryo by allowing water to leak in and metabolites to leak out. Saprophytic fungi would be likely to invade at these sites and microfloral activity around the seed would be increased by the extrusion of metabolites into the surrounding soil (Bewley and Black 1978).

The implication of all of this for buddleia seed is that if they are initially rehydrated at the higher spring or autumn temeratures then injury to the seed during cold weather or sudden frosts is minimised and repairable. Conversely, if they commence imbibition during a cold spell of weather, many seeds lose their viability and seedlings may develop abnormally. The late flowering and ripening of many seeds on buddleia means that if it was not retained on the parent over the winter, having no dormancy mechanism it would be subject to initially low imbibitional temperatures in a typically cold, wet November in Britain, or at the end of the season in native China.

4.7 The maintenance of viability of seed stored on the bush.

Having established above the costs of overwintering on the ground, the consequences of overwintering on the parent plant will be considered, by comparing the viability of the seed which has been kept outside on the shrub for 5 months with seed which was collected immediately after it had fully formed on the plant, in the previous

November.

Seed heads were collected from plants at the Dry Sandford site on 28 November 1981 and stored dry for 5 months. Further seed heads were collected from the same plants on 27 April 1982, and seeds from both harvest dates were used in the standard test procedure for viability.

The results, shown with a t-test on the means in Table 4.3, indicate that there is a significant difference in the viability of the 2 sets of seed ($p<0.01$). It had been expected that any differences would show reduced viability in the seed stored outside, where fluctuating weather conditions might cause some damage to the seeds. However, this is clearly not the case; the seeds kept in warm, dry and constant laboratory conditions showed a marked decrease in viability which their counterparts outdoors did not.

It was noticed during the course of the tests that laboratory stored seeds showed a higher degree of fungal infection than those collected freshly from the field. It may be concluded from this that storing all the seeds together in the plastic box provided excellent conditions for the spread of fungal spores over the exterior of many of the seeds. This does not occur on the bush, where seeds are only stored in small groups. If a seed capsule does become infected then the spread of the fungus is probably checked by the distance between the seed heads. Tightly enclosed in their capsule and borne high above the ground, the seeds are safer from the risk of microbial attack than they would be lying on the ground.

A similar situation arises for deciduous plants which retain their senescent leaves overwinter. Otto and Nilsson (1981) suggest

Table 4.3 A comparison of the viability of buddleia seed stored overwinter in the laboratory and on the plant.

Replicates	Percentage germination of laboratory stored seed	Percentage germination of seed stored on the plant in the field
A	84	93
B	85	95
C	89	91
D	87	99
x	86.25*	94.5*

* significantly different when compared using a t-test: $p<0.001$

that they do this because overwintering in moist soil conditions leads to greater losses from micro-organisms and soil fauna than occur on the plant.

4:8 The influence of oxygen supply on germination.

Another factor governing the germination of many species is the supply of oxygen to the seed. Many germinate only at or near normal atmospheric conditions of 23% oxygen. However, others germinate at much lower oxygen tensions, some even requiring anaerobic conditions. Morinaga (1926) showed that of 70 species tested, 48 could germinate under water and 18 performed as well in these conditions as they did with a normal air supply. While germination under water is not anaerobic, since the water contains some dissolved oxygen which diffuses into the seed, it does represent a much reduced supply, particularly when the water is boiled to remove the air, which is what Morinaga did. Under more precise conditions imposed by Harel and Mayer (1963) on lettuce seed, 96% germinated in an atmosphere of 20% oxygen in nitrogen, and 58% were still capable of germination in only 5% oxygen. Cucumber seed can germinate at even lower levels: there is no significant difference in the germination obtained at 2% and at 20% oxygen concentrations (Siegel and Rosen 1962).

The ecological impact of these different degrees of tolerance depends on the situation in which the plant is found. Soil oxygen rarely falls below 19%, although this may be lower in the atmosphere immediately around a seed, due to microfloral and root activity (Karssen 1982). The only natural situations in which generally low

oxygen concentrations are prevalent are in waterlogged soils and marshes. The absence of buddleia from waterlogged soils, or even those which retain a lot of water, such as peat and fen soils, was noted in Chapter 2, so an investigation of the oxygen requirements for germination of buddleia seeds should establish whether this is a factor in their absence from these soils.

Whatman No. 1 filter paper was cut into 3 cm wide strips and double thicknesses were moistened with 2 ml distilled water. One hundred seeds were arranged evenly over the strips which were then placed inside wide-necked 150 ml glass bottles laid on their sides. The filter papers were suspended between the neck of the bottle and a wedge of "plasticine", placed at the bottom of the jar, to prevent them touching the glass. A further 1 ml of water was added to each bottle in order to maintain a moist atmosphere, then they were air-tight sealed with "subaseals". The atmosphere of each bottle was flooded with different proportions of oxygen and nitrogen, using aerosols of calibration gas mixture, made up to precise specifications of 18.1%, 11.95%, 5.0%, 1.05% and 0% oxygen in nitrogen, by Phase Separations Ltd. Each treatment was replicated 3 times, using 1982 seed throughout.

The gases were injected with a large syringe needle, inserted through the subaseal. Air was removed from the bottle at the same time using a second needle which was attached to rubber tubing that led into a water trap. Figure 4.4 shows the experimental apparatus in use. As the atmosphere was replenished from the aerosols, the gas forced out was collected and measured in the water trap until 200 ml had been replaced, which was more than the volume of the bottle. This flushing

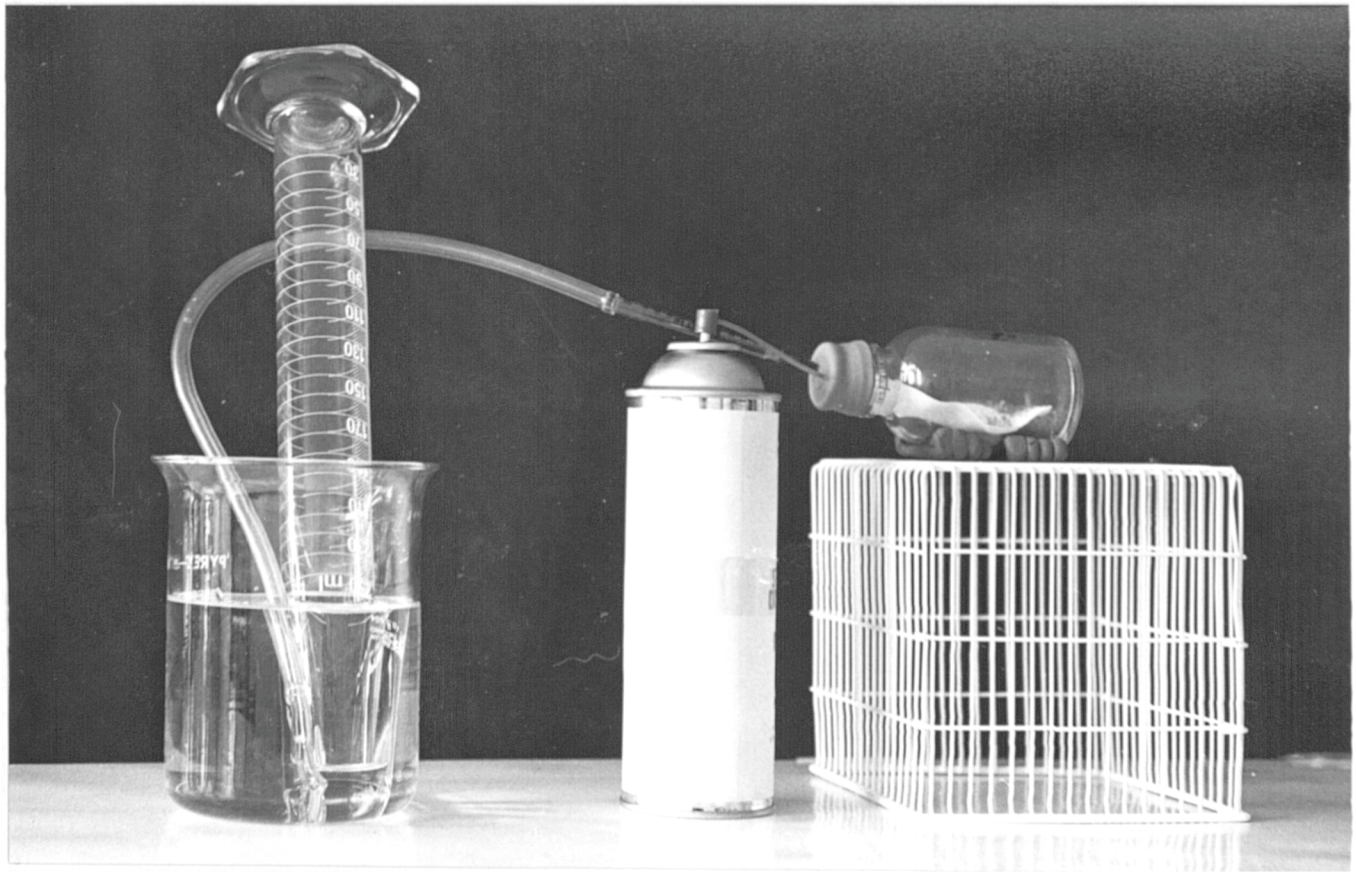


Figure 4.4 The experimental apparatus used to test germination at different atmospheric oxygen concentrations.

with gas was repeated immediately when the experiment was set up, to ensure that all the air had been replaced by the gas mixture. Thereafter the atmosphere was replaced daily with fresh injections, to ensure that the oxygen supply to the seeds remained constant. The gas was injected in short bursts at high pressure, with the 2 syringe needles inserted to different distances and angled in opposite directions, in order to minimise the chance of gas passing directly between them.

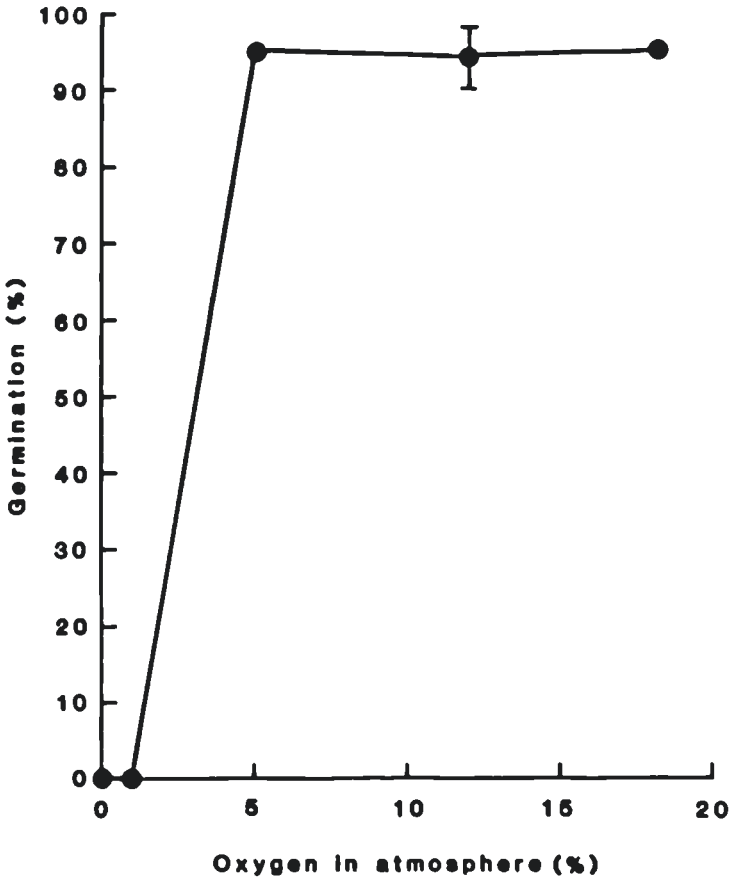
The experiment was carried out over 10 days until all viable seeds appeared to have germinated. The levels of moisture were regularly checked and replenished as necessary, using another syringe. The number of seeds which had germinated was recorded 3 times during the experiment: the results are presented in Table 4.4 and Figure 4.5. From these it is clear that buddleia is not capable of germinating under anaerobic conditions, or very low oxygen tensions (1.05%). However, there is no significant difference in the final germination recorded for concentrations at or above 5% oxygen. Table 4.4 shows that the rate of germination is slightly slower at 5% than at the 2 higher levels, but after 1 week the percentage germination was no longer significantly different. The decline in germination which must occur between 1% and 5% oxygen concentrations may be gradual or sudden (Mayer and Poljakoff-Mayber 1982): unfortunately it was not possible to repeat the experiment at intermediate tensions in order to establish this. Nevertheless, the results of this experiment indicate that oxygen is not limiting the colonisation of water-retentive soils in Britain.

While it is difficult to relate these data to the soil

Table 4.4 Mean germination of buddleia over a 10 day period, at 5 different oxygen concentrations.

Oxygen concentration (percentage)	Mean germination after 5 days (%)	Mean germination after 7 days (%)	Mean germination after 10 days (%)
18.1	83	92	95
11.95	86	91	94
5.0	76	88	95
1.05	0	0	0
0	0	0	0

Figure 4.5 Total germination at a range of 5 atmospheric oxygen concentrations.



atmosphere in the field, because of the modifying actions of microbes mentioned previously, the figures can be related to those found for ecologically similar species. The list of species germinating under reduced oxygen conditions reported by Morinaga (1929) included Antirrhinum majus (Scrophulariaceae), Artemesia absinthium (Compositae), Oenothera biennis (Onagraceae), Solanum dulcamara (Solanaceae), Melissa officinalis (Labiatae) and Daucus carota (Umbelliferae): all species typical of wasteland, often marginal and well-drained habitats (Clapham, Tutin and Warburg 1962). Buddleia seems to fit into the same pattern as these wasteland species. However, such sites are generally well-drained and aerated and so the significance of this adaptation is unclear for these plants.

4:9 The effect of pH on germination of buddleia seeds.

Having found no evidence of reduced oxygen supply, typical of water-retentive soils, affecting the germination of buddleia, it is also possible that the lower pH of these sites is responsible for preventing the plant from establishing itself there. The soil analyses described in section 2.3 demonstrate that beneath existing stands of buddleia soils vary between a pH of 6 and 8. But as no sites were found with lower pH values, and as the alkalinity of a soil in the field rarely exceeds a pH of 8, it is impossible to assess the impact of pH on the germination of seed from this data alone. So, an experiment was carried out to test whether the pH of the soil solution has an inhibitory effect on germination. The opportunity was also

taken to look for an upper limit to pH tolerance.

A modification of the usual germination test was used, replacing the distilled water with solutions of a particular pH. Rather than introducing a range of different buffer materials, with additional problems of osmotic potential, it was decided to make up solutions to pH 4, 5, 6, 7, 8, 9, 10 and 11, using distilled water with 0.1M H_2SO_4 and 0.1M NaOH as the adjusting solutions. The solutions were changed every 2 days, together with the lower of the 2 sheets of filter paper, to minimise drifts in the pH of the unbuffered solutions.

The results of the experiments are shown in Figure 4.6. Analysis of variance was carried out on the final totals, but no significant differences appeared. Figure 4.6 shows that the rates did not vary much at any pH; the slowest to germinate were those at ph 6 and 7, but these ultimately produced the highest totals. The final totals at the extremes of pH tested were slightly lower than for the middle of the range, but the germination was still over 70%. So the direct effect of pH on the germination of buddleia appears to be negligible and can be discounted as a factor limiting the distribution of buddleia. However, this simple experiment takes no account of other factors associated with pH in the soil, such as aluminium and ferrous ion toxicity, which may be limiting in natural acidic soils.

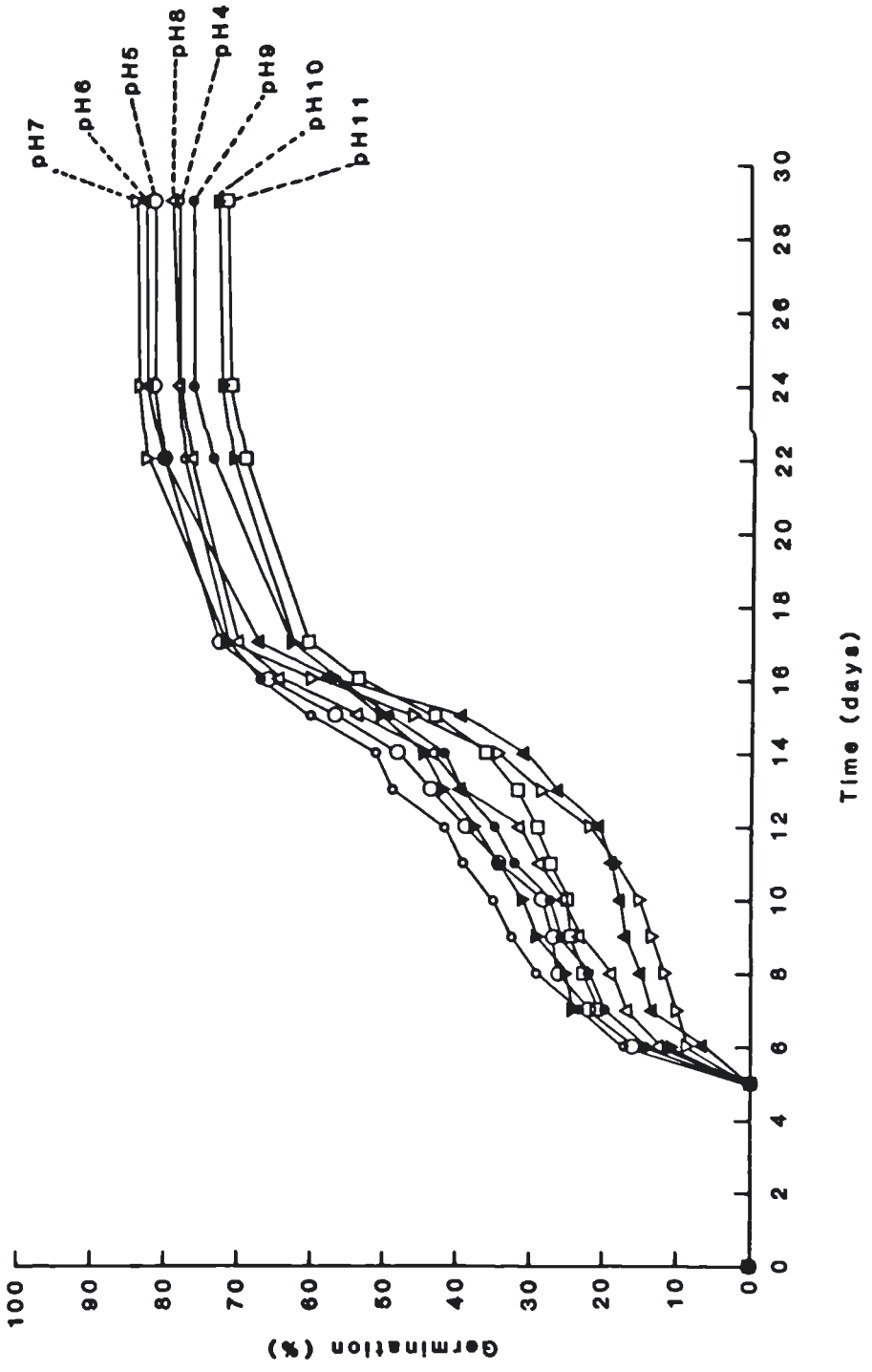


Figure 4.6 Germination at a range of 8 substrate pH levels.

4:10 The effect of depth of burial of seed on the emergence of seedlings.

Seedlings with small food reserves have to be able to penetrate substrates before these reserves are totally depleted. Consequently, they must germinate near the surface of the soil so that they can begin actively photosynthesising as soon as possible and minimise the expenditure of energy in elongation of the hypocotyl, using it instead in root extension. As the germination test in section 4:2 showed, the buddleia seedling in total darkness diverts its energy away from root growth, so that even if the cotyledons do reach the surface, the stunted roots may be unable to take up sufficient water to supply the newly emerged seedling.

To assess the impact which the depth of seed burial has on the successful emergence of buddleia and to determine the maximum depth from which seedlings can emerge, the following experiment was carried out.

Sixteen seed trays were filled to the same level with fine grade "vermiculite". , and 100 seeds were scattered over the surface of each tray. These were then covered with more vermiculite to achieve the following depths of burial: 1.5 cm, 1 cm, 0.5 cm and uncovered, with 4 replicates of each treatment. The trays were placed under a mist propagator unit in a heated greenhouse, with natural lighting, on 15th June 1982. The number of seedlings which emerged was recorded after 4 weeks and again after 7 weeks, since those at greater depths would take longer to emerge. No further emergence occurred after this period.

The mean figures for seedling emergence at each depth of burial are shown in Figure 4.7. The emergence of seedlings from unburied seeds was the control value for the experiment; by comparison with this only half the seeds successfully produced seedlings when they were buried under 0.5 cm of vermiculite. This rapidly falls to less than 10% emergence when the seed is buried 1 cm deep, and to less than 1% at a depth of 1.5 cm. So it can be extrapolated from Figure 4.7 that no seed successfully emerges from depths below 2 cm.

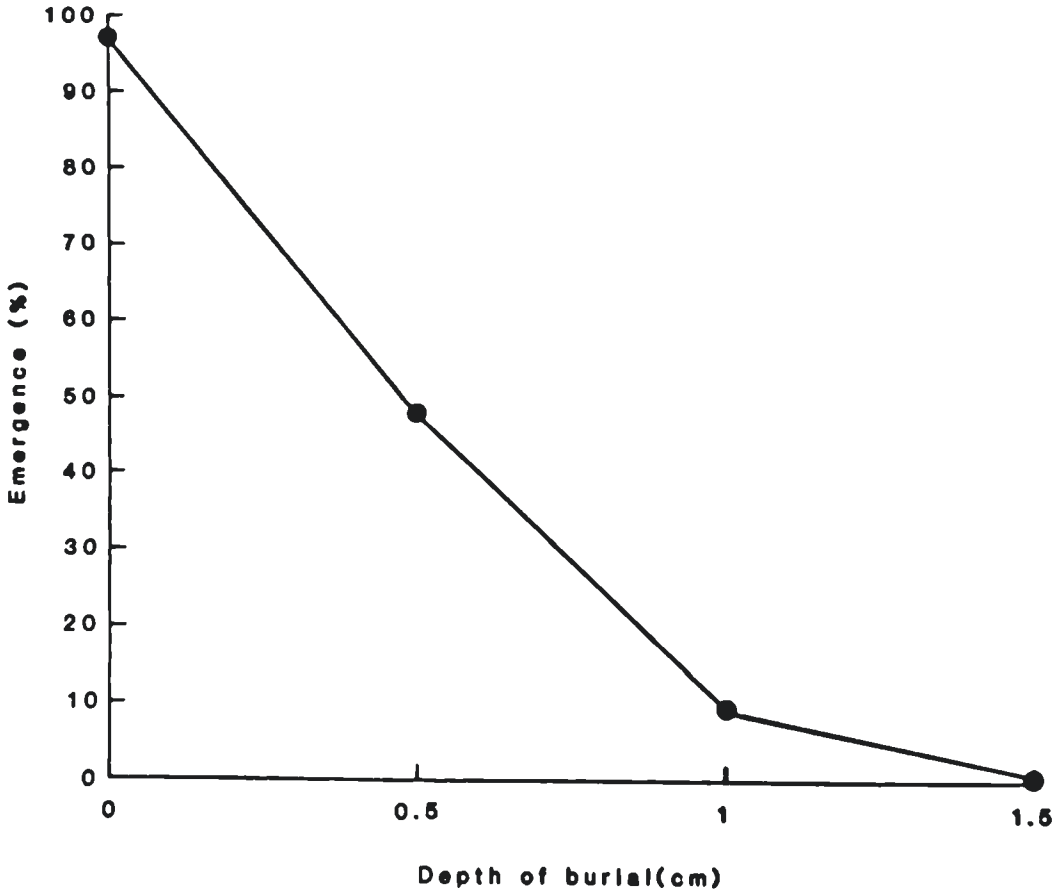
The use of a fine growth medium and of mist propagation means that the experimental conditions favoured any possible emergence from depth. So these figures repesent the maximum soil distances that the seedling can grow through successfully. In the field there is likely to be a poorer water supply, and more energy will have to be diverted into radicle extension than is the case here. So the limiting figure will be even shallower: considerably less than 50% will be able to penetrate the surface from 0.5 cm depth.

These results have demonstrated that buddleia seed is highly sensitive to burial. In the absence of dormancy the indications are that burial is disastrous for the majority of seeds, unless they are rapidly returned to the surface by soil disturbance, or they fall into a crevice wide enough for light to penetrate to the seedling.

4:11 Allelopathy.

It was suggested in section 2.9 that the reason for the absence of seedlings beneath the adult plant might be an autotoxic effect of the parent plant or allelopathy, a subject generating considerable

Figure 4.7 Total numbers of seedlings which emerged after burial at different depths, in trays of irrigated vermiculite.



debate (Harper 1977). Many plants certainly produce toxic substances which inhibit the germination and growth of other species and often of their own offspring; the latter is known as autotoxicity. However, most of the investigations to date have involved making an aqueous or alcohol extraction of parts of the plant and then applying this to seeds in the laboratory. The relevance of these findings to the field situation is not clear, and the paths by which allelopathic interactions naturally occur are not known for any species. There is evidence of toxic effects from liquid and gaseous exudates from living and dead plant material, and of microbial activity releasing toxins from previously non-toxic material, but the extent of their impact in a natural situation, where leaching and further microbial activity may rapidly immobilise them, is probably not significant in many instances (Harper 1977).

Bearing in mind these problems in interpreting positive evidence of toxicity, it was decided to investigate living and dead leaves, and roots, to see if autotoxins were readily leached out. All the plant material was collected at the Folly Bridge site in Oxford.

A root extract was prepared by mixing 100 g freshly collected roots with 100 ml distilled water and leaving them to soak for 48 hours, stirring the mixture periodically. A similar preparation was made using 100 g fresh leaves. The 2 extracts were then filtered through Whatman No 40 paper and the extractant used to replace distilled water in a modification of the standard germination test. A third preparation was carried out by packing a 1 m column with senescent leaves taken from beneath the plants. A peristaltic pump was used to pass a steady stream of distilled water down through the

column over a 24 hour period, and the collected, filtered leachate was then used to replace distilled water in a third set of dishes. A fourth set of dishes were set up with distilled water as controls. All of the extractants were dark-coloured and pungent-smelling, suggesting the concentrations were greater than would actually occur in the field.

The results are presented in Table 4.5, together with t-tests which show that there is no significant difference in germination under any of the experimental conditions and that under control conditions. It was also noted that the cotyledon development and subsequent growth of the seedlings appeared normal in all treatments. However, there is no evidence of autotoxicity in buddleia: the absence of seedlings beneath the adults cannot be attributed to chemical inhibition.

Many apparently autotoxic interactions have subsequently proved to be physical responses of plants competing for limiting resources and not attributable to allelopathy at all. For example, the creosote bush Larrea divaricata is widely spaced in its native Californian desert, with the seedlings which germinate around the adult bush dying at an early stage, particularly directly beneath the parent. Went (1955) attributed this to allelopathy, but work on L. divaricata shoot and root extracts showed that there was no inhibitory effect and that the mortality of the seedlings was apparently not due to toxins but to some physical factor such as drought, heat or disease (Knipe and Herbel 1966). The study by Woodell et al. (1969) demonstrated that the density of bushes was correlated with rainfall, and that at high rainfall levels the bush is

Table 4.5 The mean germination of buddleia seeds using extractants from root and leaf tissue as the moisture source.

Plant fraction	Mean germination (%)	Standard deviation	t-value	significance
Live leaves	94.75	1.26	0.66	$p>0.01$
Dead leaves	95.25	3.20	0.112	$p>0.01$
Roots	95.75	2.63	0	$p>0.01$
Distilled water control	95.75	2.75	-	-

clumped while at low rainfall levels it is regularly spaced. The distribution pattern appears to be a consequence of root competition for water and the death of the seedlings reflects the competitive advantage of the parent plants.

There is a striking similarity between the distribution patterns described by Woodell et al. (1969) and those observed in the natural populations of buddleia in Sussex, as described in section 2:9, especially as there is no evidence of autotoxicity in either species. But it would be unwise to draw too many parallels between such ecologically dissimilar species. Although both are shrubs, they have evolved in totally different climates and habitats. Nevertheless, the investigations so far have not considered the influence of water on the success and survival of any stage in the life cycle of buddleia; investigations of this may provide evidence of a factor which limits its distribution.

4.12 A summary of the findings on germination.

The following responses have been demonstrated by buddleia:

1). The seeds germinate well at a range of temperatures betwenn 10 and 30 ^{o}C particularly if there is a fluctuating temperature.

2). Regardless of the external temperatures, there is no light requirement for germination.

3). Germination is not limited by low oxygen concentrations until they fall below 5% of the total atmosphere and the conditions in the field are unlikely to drop to this level.

4). The seed will germinate equally well in acid and in alkaline

environments.

5). There is no innate dormancy.

6). The viability of the seed remains high when it is stored overwinter on the bush.

7). Even when stored in the imbibed state through winter temperatures, over 50% of the seed retained viability, although about 20% of that amount had germinated prematurely, during the cold period, and losses due to microbial activities were not included.

8). There is no evidence of any autotoxicity in buddleia.

All of the above represent non-limiting factors in the germination of buddleia. Even the loss of imbibed seed is not likely to be a major factor for a species which sheds its seed in the spring. The only positive constraint which has been recorded here is the effect of the depth to which the seed falls in the soil. Even under the very moist conditions of the mist propagator, in which root growth is minimal and shoot growth much greater than in the field situation, 50% of the seeds failed to produce emergent seedlings from 0.5 cm below the surface. This may explain the absence of any dormancy mechanism and the long retention of the seed on the bush: when the seed drops it must germinate as soon as possible. Any form of dormancy mechanism would increase the risk of it becoming buried too deeply in the soil to emerge. Similarly, if the seed fell to the ground in the autumn and did not germinate before winter, the it would be subject to a lot of surface disruption, from the action of frost as well as animals, and again it would be likely to be buried. Alternatively, if it did germinate when it fell, the risk of chilling injury to the seed or seedling would be high.

CHAPTER 5: THE EFFECT OF WATER STRESS ON GERMINATION AND SEEDLING SURVIVAL.

5:1 Introduction.

So far the effects of all the major environmental variables influencing the germination of buddleia seed have been assessed in the laboratory, except for moisture. The response to moisture levels is particularly important for seeds in the upper layers of the soil which are subject to greater fluctuations and longer periods of drought. Variation in the moisture content of the soil occurs not only because of the soil type and location, but also between different times of the year and different soil horizons. Soil may be classified as xeric, mesic or hydric, according to the total water content over a prolonged period, but this gives relatively little information on the availability of water for seeds at the particular time and location where it is required for germination.

For a small structure such as the buddleia seed, availability is determined by a combination of osmotic factors, the binding of water to soil colloids, the capillary forces in the soil and hence the texture and compostion of the soil, and the degree of competition with other plants and animals which also require water. In cold weather the freezing of water obviously makes it unavailable, so that winter can present as much of a drought problem as summer. In coastal areas the salinity also affects the water availability, through a decrease in osmotic potential (Mayer and Poljakoff-Mayber 1982).

From the distribution of buddleia described in Chapter 1, it is apparent that the seeds are frequently subjected to any or all of the

above stresses, particularly since they are confined to the upper layers of the soil where conditions are most extreme. Hence, an investigation of the responses of the main developmental stages to water stress should yield information to explain buddleia's success in certain habitats and failure in others.

5:2 The choice of medium used for studies of water stress effects.

The choice of medium for subjecting seeds to a range of water stresses poses a number of problems. The heterogeneity of the soil and the difficulty of measuring the moisture content in the area of the seed are well known: as Heydecker (1977) points out, incomplete seed/soil matric contact greatly extends the effective diffusion path of the water and lowers the potential around the seed, compared to that in the bulk of the soil. Consequently, measurements of diffusion and water potential may bear little relation to that experienced by the seeds.

Many workers have therefore chosen a totally artificial medium in order to achieve a replicable and precise measure of the moisture conditions in which germination occurs. This is achieved by using solutions of different concentrations to create different osmotica in which the seeds are germinated. The most widely used osmoticum at present is polyethylene glycol (PEG), a high molecular weight polymer which is metabolically inert, excluded from the seed by the size of its molecules and thus avoiding the possible problems of toxicity which occur with ionic solutions. The use of similar media also facilitates the comparison with data for different species: comparative responses are important with such a variable parameter as

moisture.

Throughout these investigations PEG-"6,000" was used, as supplied by Sigma Chemical Co., U.S.A. The molecular weight is an approximate value: since it is a polymer the individual molecules may vary by at least 1,000 units either way, but the majority are around the mean figure. In fact the molecular weight has recently been respecified as 8,000 by Sigma, who state that while no change has occurred in the product, improved analytical techniques have indicated this as a better estimate of the molecular weight.

As a polymer it is also unstable in solution, the molecules breaking up in time to produce a more concentrated solution of smaller molecules. This is particularly so in solutions with high original concentrations, since the molecules are more likely to become tangled and break repeatedly (Thill et al. 1977). This presents problems since the osmotic potential of a solution is directly related to its concentration. In order to establish the stability of the solutions at the concentrations to be used in these experiments, the osmotic potentials of freshly made solutions were measured at 4 hour intervals over a 20 hour period, using a Knauer vapour pressure osmometer. The solutions were prepared at concentrations calculated from the equation proposed by Michel and Kaufmann (1973), for PEG-6000:

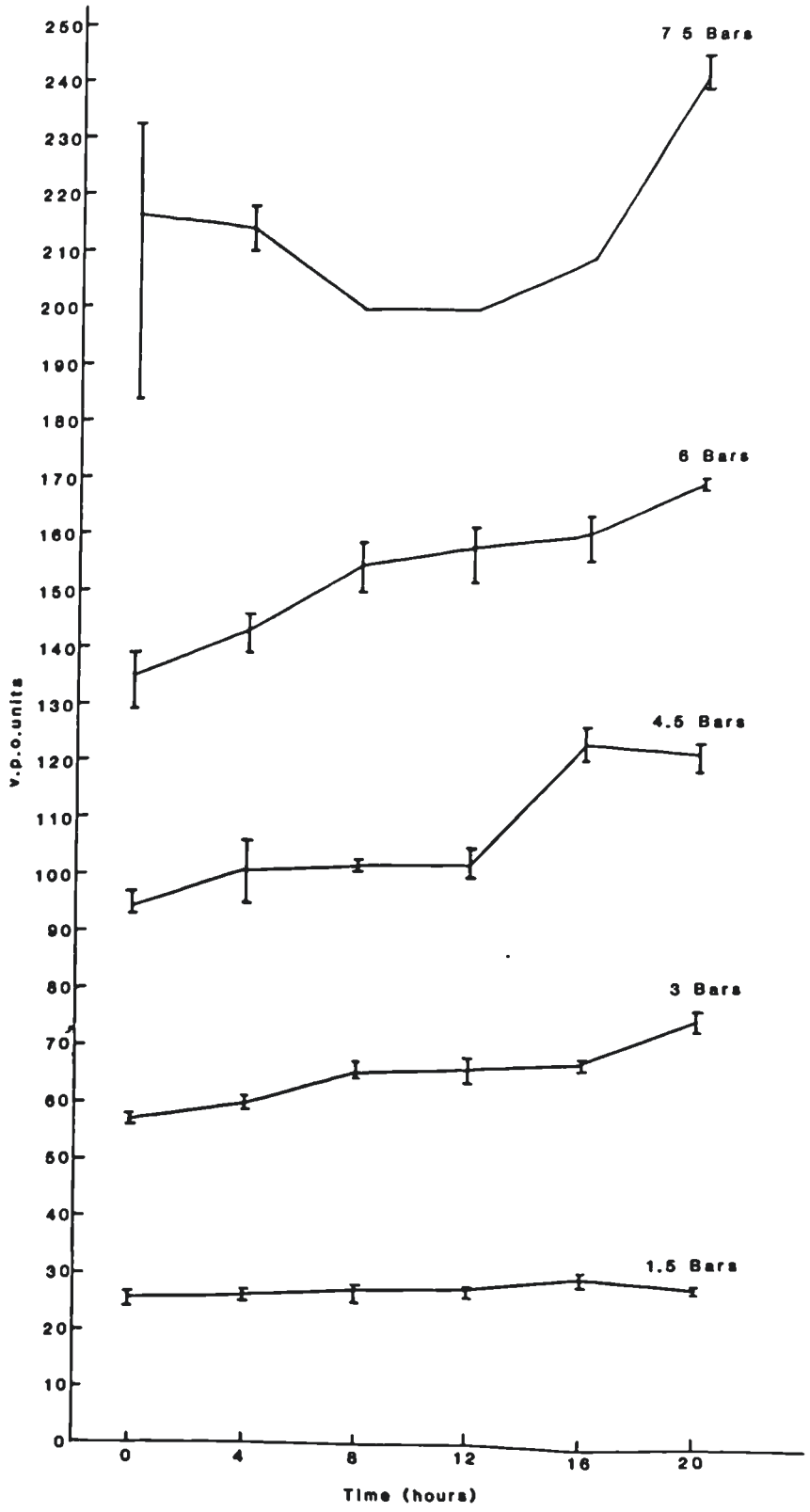
$$\psi_s = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, CT \quad (8.39 \times 10^{-7})\, C^2T$$

where C= Concentration

T= Temperature

The curves obtained from these measures are shown in Figure 5.1 and indicate that there is no overlap between the 5 solutions over this period, although there is increased depolymerisation at the

Figure 5.1 Changes in the osmotic potential of 5 concentrations of PEG-6000 solution as a function of time.



higher concentrations. Consequently, to minimise these changes it was decided to prepare fresh solutions to replace the osmoticum every 24 hours.

A further problem associated with the use of PEG as an osmoticum is the reduction of oxygen supply to the seed and consequent effects on germination. This is particularly so with higher molecular weight solutions and with greater concentrations, since the solutions become more viscous and cause more impedence to the passage of oxygen (Mexal et al. 1975). However, these authors suggest that the effects are not significant at concentrations of PEG-6000 lower than that which gives a potential of -7 bars. As the solutions used in this experiment did not exceed this value, and since buddleia is not sensitive to a 75% reduction in oxygen supply, there is no reason to suppose there is a complicating effect on germination at different osmotic potentials.

5.3 The germination of buddleia seeds under moisture stress.

The seeds used were formed in 1981 and had been stored under air tight conditions for a year. Each treatment was replicated 4 times with seeds set up in a modification of the standard germination procedure (as described in section 3:2). The distilled water in this test was replaced by 5 ml of PEG-"6000" solution at the appropriate concentrations to produce osmotic potentials of -3, -4, -5 and -6 bars, with a distilled water control of 0 bars. The filter paper bearing the seeds was placed on top of 2 more papers. Every day the filter papers bearing the seeds were transferred into new petri dishes containing 2 more filter papers moistened with 4 ml of freshly prepared PEG solution. Some polymerisation occurred in the residue of

the solution on the transferred paper, but was minimised by daily replacement of the solution in new dishes.

The germinated seeds were counted and removed daily, before transferring the remainder to the new dishes. The experiment was carried out over a period of 7 weeks, although after the first 4 weeks the solutions were changed only on alternate days.

The results are presented in Figure 5.2; unfortunately, the viability of the 1981 seeds had declined over the 12 months after their collection (more than with seeds from previous years), such that the germination of the control was only 79%. Nevertheless, the progressive decline in both the rate and the total germination which occurs with increasing water stress is apparent in Figure 5.2. As seeds take up water the seed coat stretches,decreasing its resistance so that water enters more readily (Hadas 1976). Thus, germination is slower but can still occur at -4 and -5 bars. As figure 5.2 clearly shows, germination cannot be achieved when the osmotic potential of the external solution is -6 bars. The critical value at which germination is inhibited varies between species and cultivars: more vigorous seed stock germinates under greater water stress than less vigorous seed of the same species (Bewley and Black 1982). However, examples of the variability between species are shown in Table 5.1.

The species shown in Table 5.1 are taken from the literature and represent an assortment of plant groups. Only one of those shown has germination inhibited by an external potential of -6 bars and that is the soybean. It is worth noting that seeds of Picea abies lose their ability to germinate under water stress as the seed ages, a phenomenon which may be widespread in ageing seeds. Most of the other species show a marked reduction in rates and total

Figure 5.2 Rates of germination over a 6 week period in response to a range of water stress conditions, created with different concentrations of PEG-6000 solution.

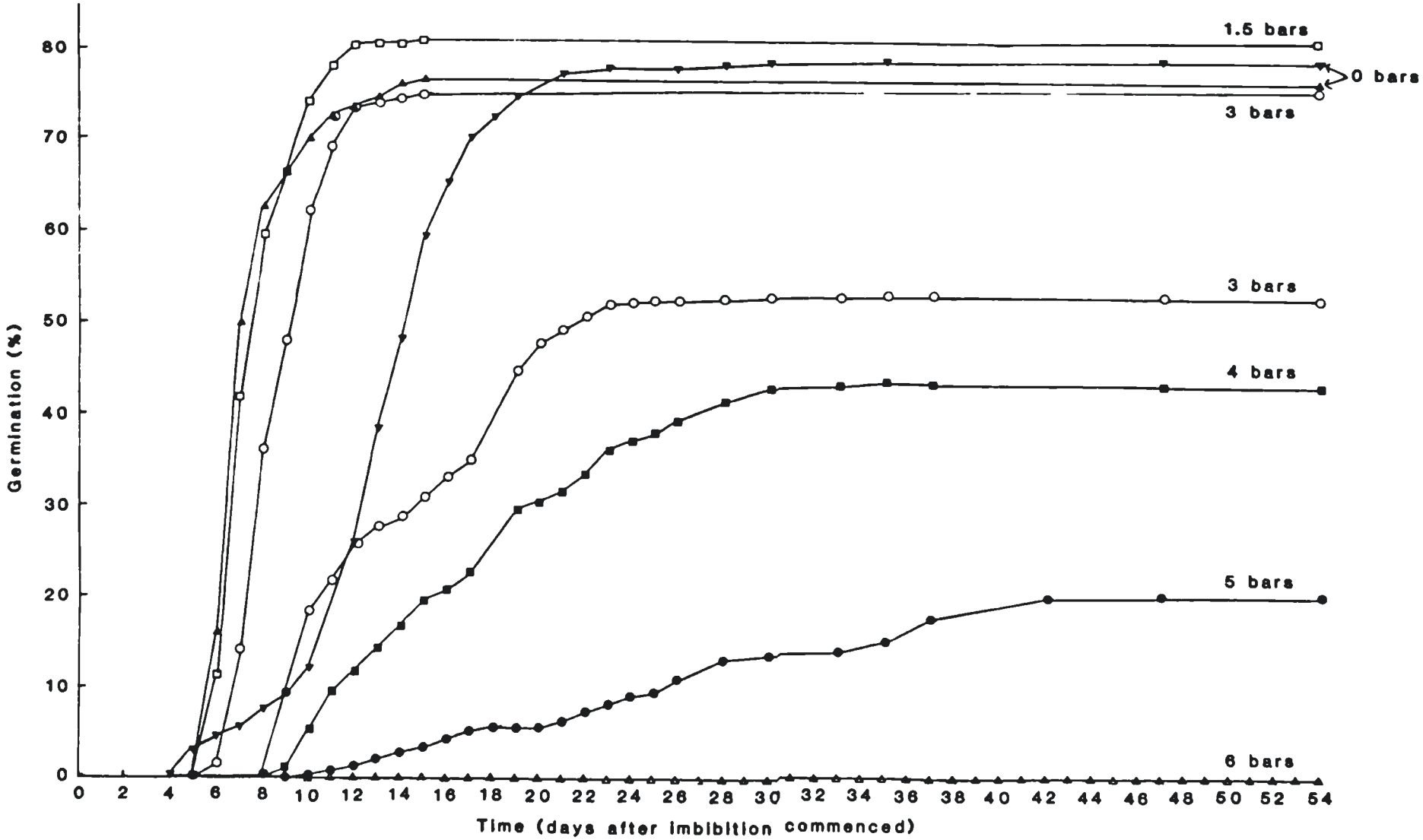


Table 5.1 Germination responses of various species to a range of osmotic potentials, induced with P.E.G. solutions.

(Data from Bewley and Black 1978, Tesche 1975, Redente 1982, and Whisenant and Ueckert 1981 and 1982.)

	Osmotic Potential (bars)					
Species	0	-3	-6	-8	-10	-12
Sorghum sudanense (Gramineae)	+	+	+	+		
Taraxacum agg. (Compositae)	+	+	+	0		
Rumex crispus (Polygonaceae)	+	+	+	0		
Datura stramonium (Solanaceae)	+	+	+	0		
Glycine max (Leguminosae)	+	+	0	0		
Pinus sylvestris (Pinaceae)	+	+	+	0		
Cupressus lawsonii (Cyperaceae)	+	+	+	0		
Picea abies - fresh seed (Pinaceae)		+	+	+	0	
Picea abies - stored seed (Pinaceae)		+	+	0	0	
Anisacanthus wrightii (Acanthaceae)	+	+	+	+	+	+
Hymenoxys odorata (Compositae)	+	+	+	+	+	+
Hedysarum boreale (Leguminosae)	+	+	+	+	+	+

(+ indicates successful and 0 indicates no germination.)

percentages of germination at this point, but many of them continue to germinate at reduced percentages, even at considerably lower potentials. This is particularly so with Anisacanthus wrightii. Hedysarum boreale and Hymenoxys odorata, which are all native shrubs of arid regions of North America. This presumably is an adaptation which enables them to germinate under reduced soil moisture conditions. This is even true of Anisacanthus wrightii which is found growing on the rocky banks and flood plain of streams in southern Texas, reminiscent of the native habitat of buddleia in China. Buddleia occurrs in a more mesic situation: although seasonal fluctuations in the water levels would be expected in both of these areas, it would seem unlikely that the water level drops to the same extent in the foothills of Central China as it does in arid areas of North America.

Bearing this in mind, it is not surprising that buddleia seeds are not able to germinate at less negative water potentials. For a species which in its native habitat is so close to a good water supply, there is no obvious advantage in developing a capacity for germination in limiting conditions. Similarly, there would be no pressure to develop a rapid imbibition and germination process: the seed would be well supplied with water over prolonged periods, so that it could germinate without the risk of rapid soil drainage and dehydration.

In fact this may be a useful mechanism whereby the plant avoids premature germination. To support effective and rapid growth of the seedling, plants need to "predict" their environment to some degree. The requirement of a prolonged period of imbibition ensures that the seed will have to be in a fairly stable environment, with a good

water supply, given the low water potential of the seed, before it eventually germinates. This is the sort of habitat that a river bank provides, and is also found at the base of damp walls, near drainpipes and guttering, and presumably on coastal cliffs, despite the increased ionic content of the "soil" solution in both these habitats. Seeds which land away from regular water courses are not so likely to germinate immediately, but have to wait for sufficient rainfall for the seed to imbibe gradually, possibly over a period of weeks.

Seeds can support a considerable amount of metabolic activity at water potentials which are too low to permit germination: such seeds are said to be at the "brink" of germination, which only ensues when more water becomes available (Hegarty 1978). Holding seeds at the "brink" is a practise known horticultually as "priming", and makes use of the metabolic activity occurring at reduced water potentials. The seed is imbibed in solutions of high osmotic potential and progresses through Phase II of germination, the non-visible activation stage, so that it is ready to germinate immediately after sowing (Heydecker 1977). Many can be maintained in this state for considerable periods without damage, although others rapidly lose viability or are induced into secondary dormancy.

It was shown in section 4.6 that buddleia seeds may maintain over 50% viability after imbibition for 10 weeks in cold and dark conditions, so it is reasonable to assume that holding the buddleia seeds at the "brink" for several weeks would not cause a total loss of viability. If the seeds are imbibed at low soil moisture tensions, or over a prolonged period with intermittent drought, then the seeds may actually germinate in a much shorter period than expected when soil

moisture becomes adequate for Phase III of the process to occur. However, the possibility of the seeds becoming dormant should not be dismissed.

5.4 The effect of water stress on pre-emergent seedling growth.

Once seeds have germinated the problem of obtaining water begins. Until the seed commences cell elongation and division it is desiccation-resistant; after emergence of the radicle the seedling can be described as desiccation-sensitive: it has reached the point of no return (Evenari *et al.* 1971). If the water supply fails only a few desert species can simply arrest development; for most species the radicle must continue to grow to obtain water for the developing seedling or it will soon die.

Having stated in section 4.2 that germination and growth were under separate physiological controls it is reasonable to expect some species to show a differential response to water stress in each of these stages. Work by Hegarty and Ross (1978, 1980/81) indicates that for many species the osmotic potential against which the plant can remove water from the soil becomes more negative once the growth phase has begun. In fact Hegarty (1978) suggests that the initiation of germination is the most osmotically sensitive phase of germination. To investigate the different responses to water stress between the germination and growth phases of buddleia seeds the following experiment was carried out, following the methodology of Hegarty and Ross (1978).

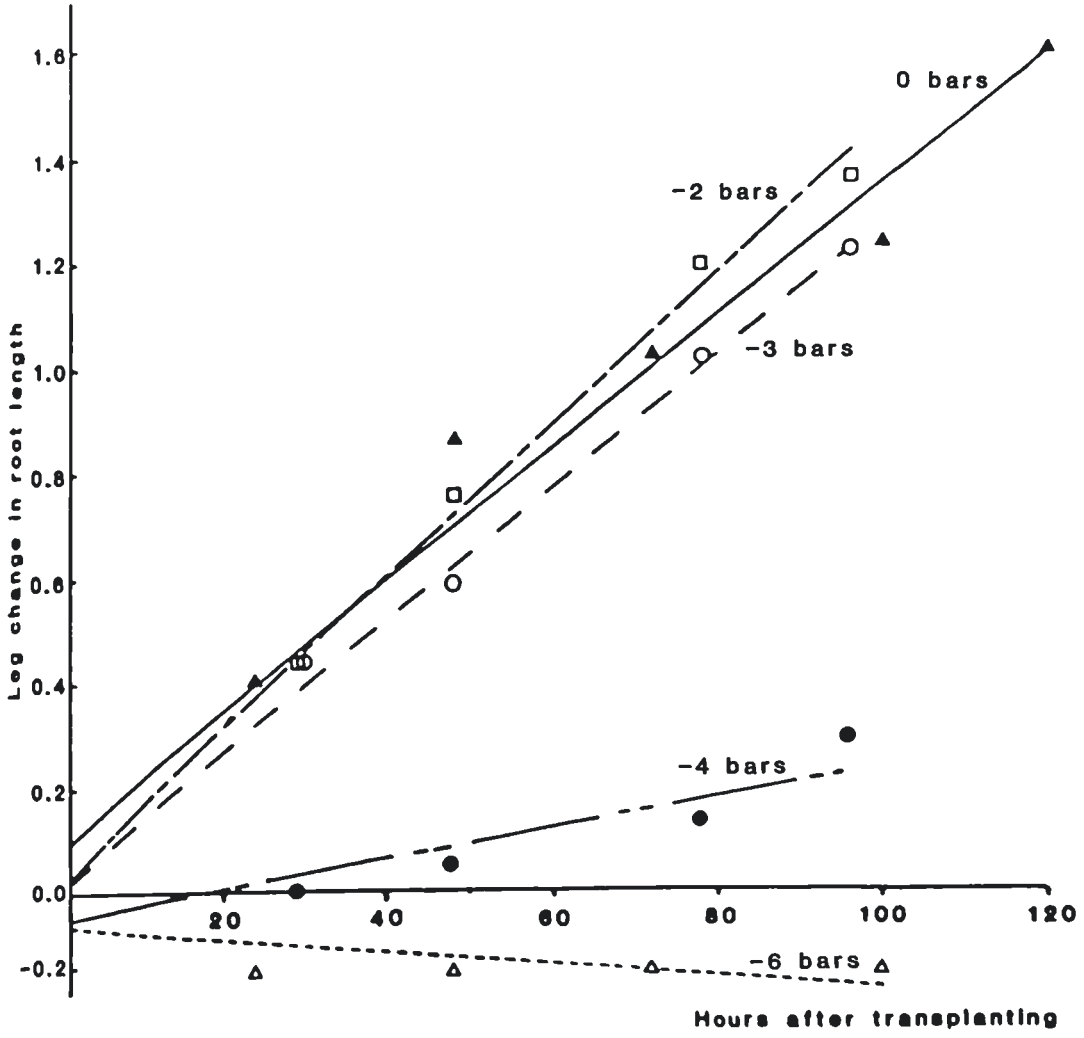
About 100 seeds were spread on filter papers, moistened with distilled water, and left in the growth cabinet under standard

conditions until they had germinated. Three sheets of filter paper were placed in each of 5 petri dishes and moistened with 5 ml PEG-"6,000" solution made up according to the equation of Michel and Kaufmann (1973), to produce osmotic potentials of -2, -3, -4 and -6 bars, with a distilled water control of 0 bars. The newly germinated seeds were then transferred onto these dishes, 10 to each dish, and the length of the radicle protruding from each one was measured on transferral, using a Vernier microscope. The length of the radicle was measured on 4 occasions during the following 100 hours, and a further measurement was made of the distilled water control, 120 hours after transplanting.

The changes in root length over this period were calculated from the original measurements and expressed as $\log_e$, so that they could be plotted as a function of time, as shown in Figure 5.3. The slopes of the regression lines therefore represent the rate of growth at each water potential. It is therefore possible to compare the results between treatments, regardless of the original size of the individual radicles, which was variable.

The slopes of the lines in Figure 5.3 show that there is effectively no difference in the growth rate of the roots not under moisture stress (0 bars) and those growing in water potentials of -2 and -3 bars. However, the growth rate shows a marked decline at -4 bars, with very little extension of the radicle after 48 hours, as compared with at least 2 mm growth in the 3 less stressed treatments. This is the time interval after which Hegarty and Ross (1980/81) recorded the responses of all the seedlings they investigated. Most notably, seedlings subjected to a water potential of -6 bars did not grow at all, even after 96 hours; in fact they

Figure 5.3 Growth of the newly-emergent root at a range of different osmotic potentials, created with PEG-6000 solutions.



shrank, giving a negative growth rate in Figure 5.3. Soon after they were transplanted, some of the water from the hydrated radicle appears to have been drawn out in response to the osmotic potential of the surrounding PEG solution, causing an overall decrease in length.

There is no evidence of an increase in the ability of the newly-emerged seedling to overcome more negative osmotic potentials than that which permits the initiation of germination. Figure 5.3 shows that germination was reduced by over 50% at a water potential of -4 bars, which is the same potential at which root growth was more than halved. This result is in contrast to the data given for various genera by Hegarty and Ross (1980/81); they found that most of the species tested were able to grow at much higher water potentials than those which permitted germination. They concluded from these data that seeds held under moisture stress are effectively in a form of dormancy, since they are being prevented from germinating by a "subtle block", in the form of a higher water potential requirement for the initiation of elongation than would be required to support the developments preceding or succeeding it.

However, as with other possible dormancy mechanisms, buddleia has presumably evolved in a situation where the environmental pressures were not strong enough for it to develop any delaying mechanisms once the initial imbibitional needs had been satisfied. Seeds are retained on the parent until conditions are favourable for germination, and in the short term the water supply on the river bank is unlikely to be limiting, provided they are shed early enough in the spring. Thus there is no necessity for seedlings to develop drought-hardiness until later in the year.

Visual monitoring of the seedlings left in the various PEG

solutions showed that the inhibitory effects subsequently increased. After 3 weeks those at -4 bars had grown very little more, while the seedlings at -3 bars had roots 1-1.5 cm long, but no new leaves. The plants at -2 bars had 3-4 cm roots, with lateral branches, and most had one pair of leaves, while the control seedlings under no osmotic stress had well-branched roots, 5-6 cm long, and a second pair of leaves.

Buddleia seeds have been demonstrated to be drought sensitive throughout the initiation and processes of elongation and of division, and until their growth is well advanced. Progress at these phases is limited by the same water potential, which means that in order to achieve successful germination and emergence of the seedling, there must be a plentiful water supply over a prolonged period. However, adult plants are usually established on well-drained substrates which are likely to dry out rapidly during dry weather. So at some point during the early stages of its growth the seedling must become much less susceptible to water stress if it is to survive and continue to grow during the dry summer months.

Unfortunately, it is not possible to carry out experiments on older plants with the simple osmotic system used in the previous studies. As Heydecker (1977) points out, there are 3 major problems associated with the use of PEG solutions which are likely to have much more impact on seedlings than they will on seeds: a) The oxygen supply to the roots will be reduced. b) There is no component which is comparable to the impedence caused by hydraulic fluxes outside the seed. c) A concentration gradient may be established if the PEG builds up around the root, as the water is withdrawn from the solution in this region. Therefore a system which combines the elements of the

soil system with the controlled conditions of a laboratory experiment was used to monitor the effects of drought on later stages of seedling development.

5.5 The effect of drought on post-emergent seedlings.

In the preceding studies the influence which soil moisture has on the survival of seedlings has been confined to the earliest stages after germination, before the seedling has emerged from the soil. Once the shoot reaches the surface, the radicle must take up enough water to support the formation and extension of transpiring leaves, so that the plant can achieve and maintain net positive assimilation. Consequently, it may be assumed that this constitutes a further period when the availability of soil moisture is critical. It will presumably continue to be so until the seedling becomes established, although the critical value for survival may progressively change.

To test the validity of this assumption for seedlings at different stages of development, and to establish the minimum level of moisture supply which supports seedlings at these stages, the following experiments were set up in the laboratory.

A system was devised by Kaufmann (1969) using the realistic situation of growing the plants in soil, but controlling the moisture supply in that soil with an osmoticum which was separated from the soil by a semi-permeable membrane. This idea was developed further by Humphries *et al.* (1982), and the experimental apparatus used in this experiment was a modification of their design.

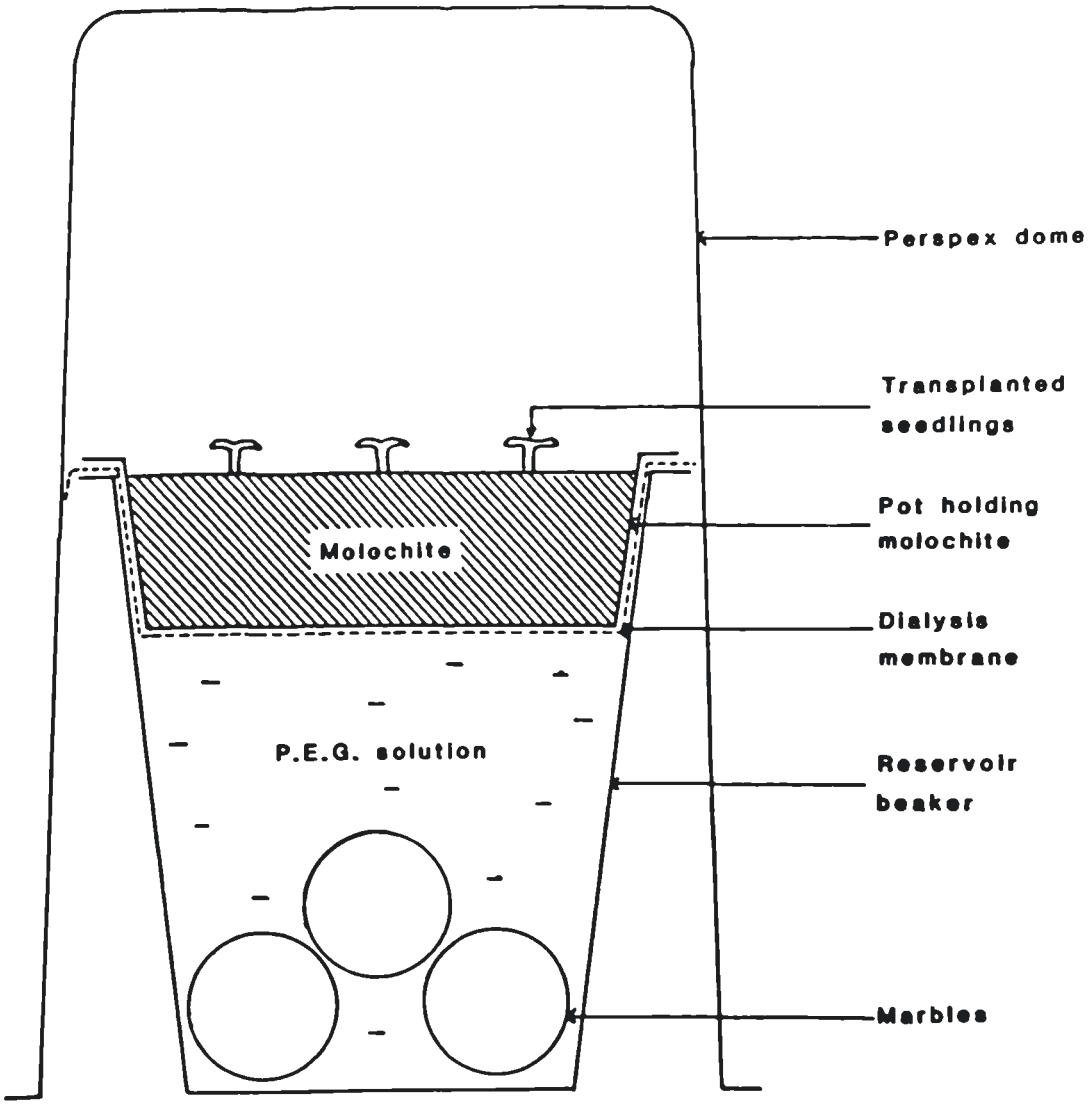
As discussed in section 5.2, it is difficult to relate the data from simple osmotic experiments to data from systems that approximate

to a soil situation. The water potentials achieved in the latter include many components apart from the matric potential simulated by the osmotic solutions. Hence it is impossible to make a direct comparison of the responses of buddleia at various stages of development to specific potentials. However, by growing the plants in an inert soil substitute and using PEG solutions to exert different osmotic potentials, it was possible to gradually reduce the substrate moisture content. The system can thus be used to ascertain the effects of various water stresses applied at different rates to buddleia seedlings at two different ages. This in turn should indicate the stage at which seedlings develop resistance to water stress.

It was initially decided to monitor and compare the survival of 2 sets of seedlings, 7-day old and 14-day old plants, hereafter respectively referred to as group 1 and group 2 seedlings. Thirty six experimental pots were set up, using PEG-"6,000" solution as the osmoticum. Five different concentrations were used to create osmotic potentials of -1.5, -3.0, -4.5, -6.0 and -7.5 bars, using the equation proposed by Michel and Kaufmann (1973), together with a distilled water control of 0 bars. As in previous experiments the solutions were freshly prepared and changed daily to ensure that depolymerisation did not significantly alter the osmotic potential of the solution.

Each experimental pot, as illustrated in Figure 5.4, consisted of a plastic beaker which acted as a reservoir for the PEG osmoticum, into which were fitted smaller pots of substrate, containing the experimental seedlings. These pots were made from the top halves of similar plastic beakers with a piece of fine muslin glued over the

Figure 5.4 A diagramatic repesentation of the experimental pots used to investigate seedling mortality responses to substrate water stress.



base, to hold in the substrate. The substrate used was "molochite", an inert china clay waste product which is supplied in different particle sizes, by English China Clays Ltd., St.Austell, Cornwall. Grade 3 has particles of 0.25-0.5mm diameter, similar to a medium sand, and was used in all these pots. The pots of molochite were separated from the PEG solutions by a sheet of "Visking" dialysis membrane, which acted as the semi-permeable membrane. The osmoticum was added until it reached the top of the reservoir beaker when the plants were in place, but without crossing the dialysis membrane, which had been cut to allow a generous margin overlapping the sides of the beaker. This was to prevent the PEG from polluting the substrate and altering the balance of the osmotic potential across the membrane, and to avoid deleterious effects of direct contact between the PEG and the plant roots. Each reservoir contained 6 glass marbles to take up some of the volume of the beaker and so minimise the amount of PEG used.

At the start of the experiment, 100g of molochite was weighed into each of the small pots, which were then placed in 50% strength A.R.C. nutrient solution until they were at field capacity. Seedlings which had previously been grown on filter paper moistened with distilled water, in petri dishes, were then transplanted into the pots of molochite. Ten seedlings were transplanted into each pot in a regular and evenly-spaced pattern. One set of seedlings had germinated 2 days previously and had no leaves, just a pair of cotyledons, with roots less than 0.5 cm long. The other set had germinated 9 days before they were transplanted and had developed the first pair of true leaves, and had roots with laterals over 1 cm in length.

The pots were left for 5 days in an environmental cabinet under

a 16 hour lighting regime at 25/18°C, and watered constantly from beneath with half strength A.R.C. nutrient solution. This was to give the seedlings time to recover from transplant trauma. The pots were then fitted onto the reservoirs of PEG-"6,000" solutions in the experimental set-up described previously.

The atmosphere in the environmental cabinet was dry and resulted in rapid evaporation and desiccation of plants without a plentiful water supply. In order to slow down the rate of desiccation and look at the effects of reduced soil moisture while minimising the complicating factor of a drying atmosphere, each experimental pot was enclosed under an individual perspex dome. Measurements of the relative humidity in the domes were made using cobalt chloride paper and a "Lovibond 1000" comparator. These showed a range of 60-95% humidity over all the domes, the pattern of decreasing atmospheric moisture reflecting the decreasing soil moisture in the experimental pots. It is reasonable to assume that this is a similar situation to that which would prevail in the field: if soil moisture is low, atmospheric moisture is similarly reduced.

A soil moisture curve was constructed for the molochite substrate using a Gallenkamp soil tensiometer. The tensiometer pot was buried in a container of grade 3 molochite which was then saturated with water and allowed to drain freely to reach field capacity. Readings were taken at this point and on 6 further occasions over the following week, and at the same time samples of approximately 100 g of molochite were removed from the same depth as the pot. These samples were weighed before and after drying in an oven at 100°C, so that the percentage moisture content could be individually determined. Relating the figures for percentage moisture to the readings obtained

with the tensiometer, it is possible to prepare a soil moisture characteristic curve, shown in Figure 5.5.

Since the molochite particles are uniform in size and comparable to medium grade sand, it is not surprising to find that the water was held at relatively low tensions. The small amount of available water initially held is rapidly removed so that it soon falls below 2% moisture content. Below this there is little more available for plants to take up, even with steep water potential gradients into the root. Consequently, plants growing in molochite at about 2% moisture content are subjected to moisture stress.

For consistency, the availability of water in the soil is referred to in the discussion of results as percentage moisture content of the substrate. However, it should be noted that this figure applies only to the substrate used, and to relate the data to any other soils it is necessary to convert to an estimate of the soil matric potential, using Figure 5.5. For example, 2% soil moisture content is equivalent to a soil matric potential of 0.19 bars.

Figures 5.6(a) and 5.6(b) show the progressive decline in substrate moisture content in the experimental pots that was achieved with each PEG osmoticum, with the point at which 50% mortality occurred in each treatment indicated on the curves. Unlike the work reported by Humphries et al.(1982) no equilibrium point was achieved; even the distilled water control continued to lose water steadily, up to the end of the experiment. The reason for this is not obvious but probably reflects continual water loss by evaporation, despite the perspex dome, which exceeded the rate of replenishment from the reservoir. Looking at the molochite moisture curve in Figure

Figure 5.5 Moisture characteristic curve for molochite grade 3.

(0.25 - 0.5 mm diameter particles)

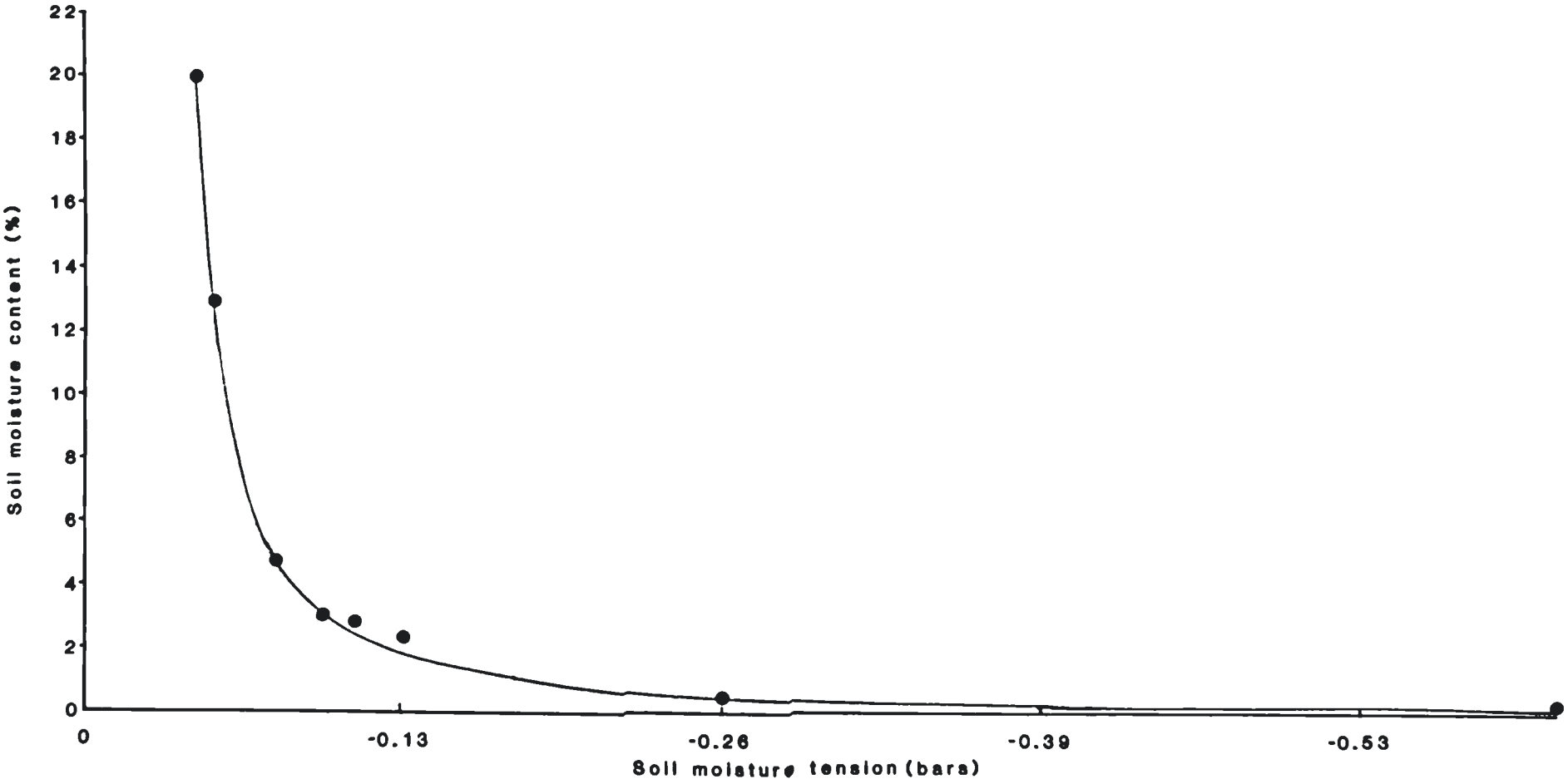


Figure 5.6(a) Substrate moisture depletion curves in group 1 experimental pots.

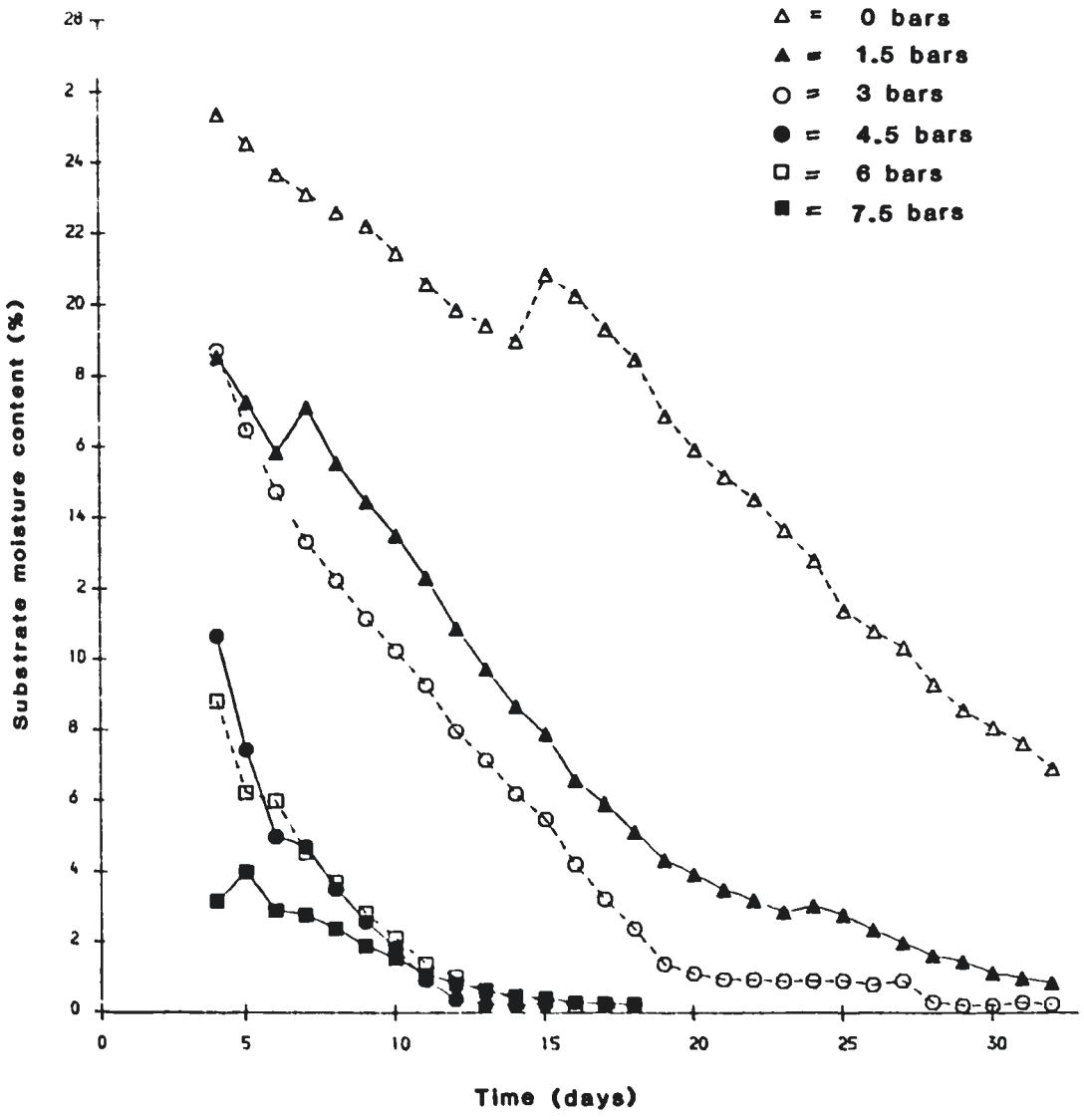
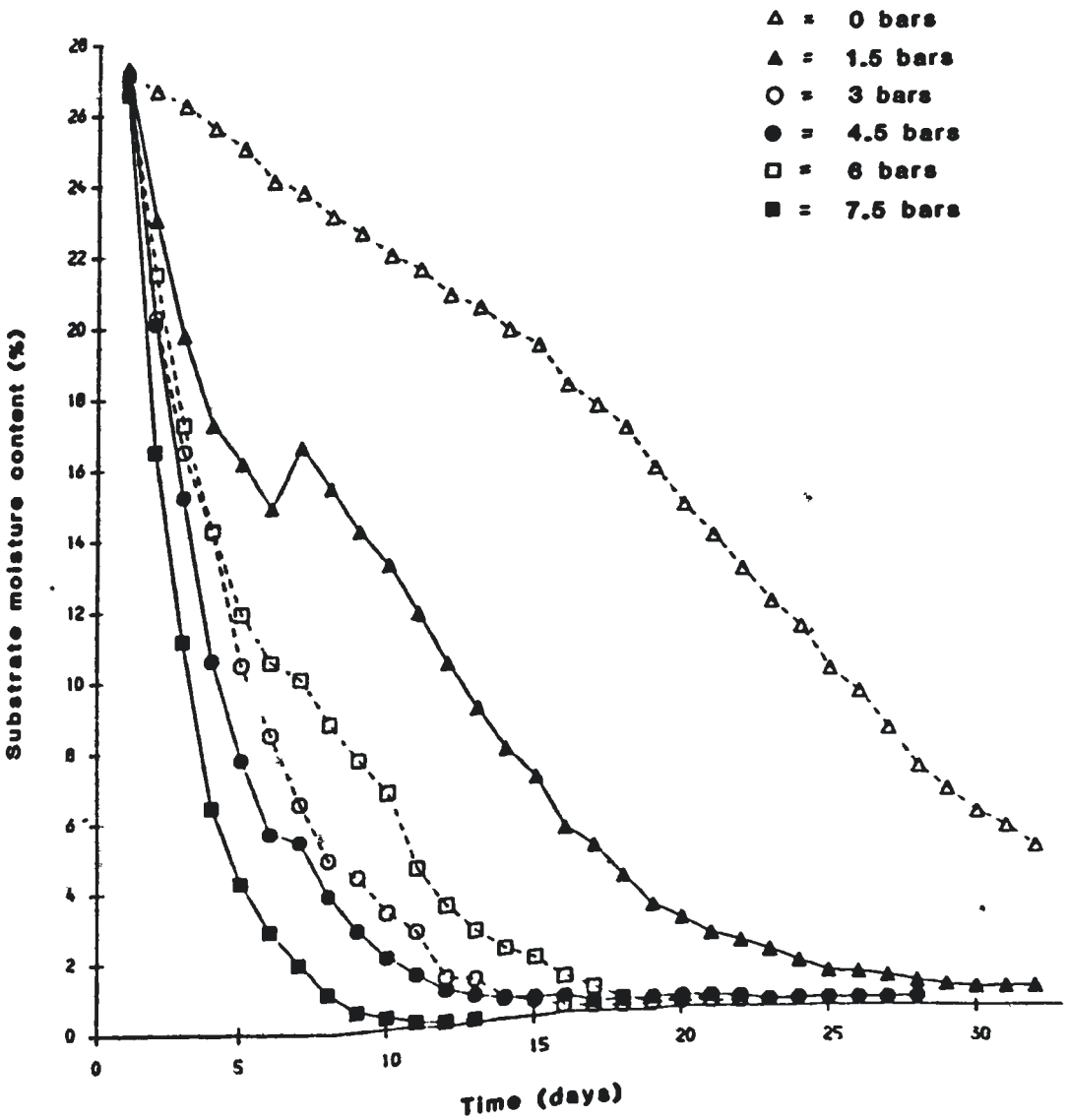


Figure 5.6(b) Substrate moisture depletion curves in group 2 experimental pots.



5.5, it is unlikely that the substrate is sufficiently moisture retentive for any feasible explanation based on slower rates of water loss than with the sand used by Humphries *et al*.

Figures 5.7(a) and 5.7(b) show the survivorship curves for the seedlings of the 2 groups over the 32 days of the experiment. Apparently it is not just the level of the substrate water but also the rate at which it dries out that is important. To facilitate a direct comparison between the responses of the 2 groups of seedlings, 1 survivorship curve from each group, together with the appropriate substrate moisture curves, has been drawn on the same pair of axes. Figure 5.8 shows the effects of a gradual decrease in water availability and Figure 5.9 demonstrates the outcome of a rapid decrease, on the survival of seedlings of both ages.

Figures 5.8 and 5.9 show that the soil moisture level at which 50% mortality occurred was between 1 and 2% in all 4 cases: in fact, as Figures 5.6(a) and 5.6(b) show, the substrate moisture level at which 50% mortality occurred generally only exceeded 2% when it dried out fast. Secondly, the effects of moisture stress were apparent much earlier in the group 1 seedlings, even though the rates of desiccation were similar in both groups. This is particularly apparent from Figure 5.8: the group 1 seedlings started to die after 8 days, when the moisture level was still about 13%, but the group 2 seedlings were all still alive after a further 10 days, when the water content had fallen to about 3%, and a third of the group 1 seedlings had died. All of the group 2 seedlings had developed some degree of tolerance of water stress and could withstand a drop in substrate water content to 4%, which was not the case for the group 1 seedlings.

The results in Figure 5.9 show the other end of the survivorship

Figure 5.7(a) Seedling survivorship curves in group 1 experimental pots.

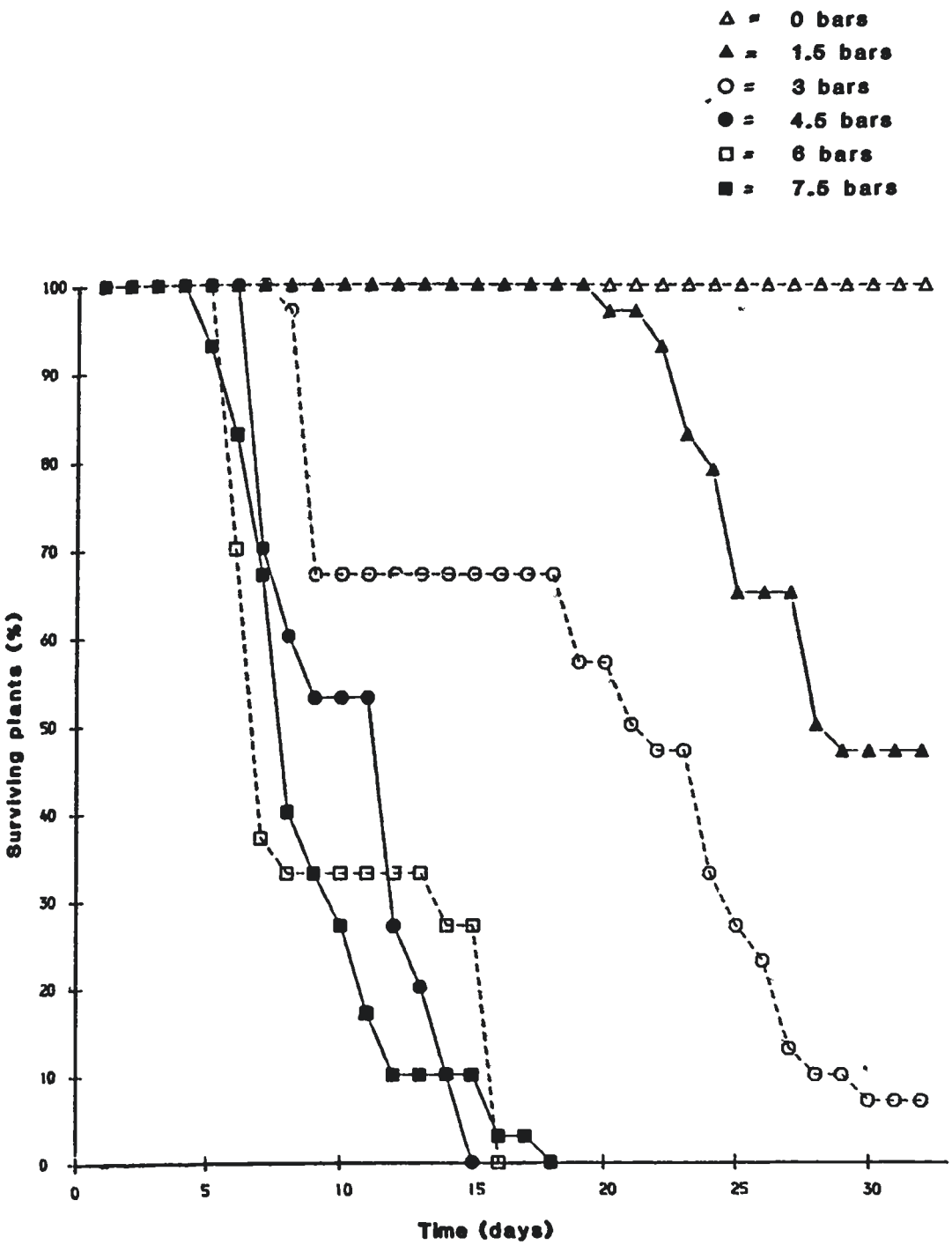


Figure 5.7(b) Seedling survivorship curves in group 2 experimental pots.

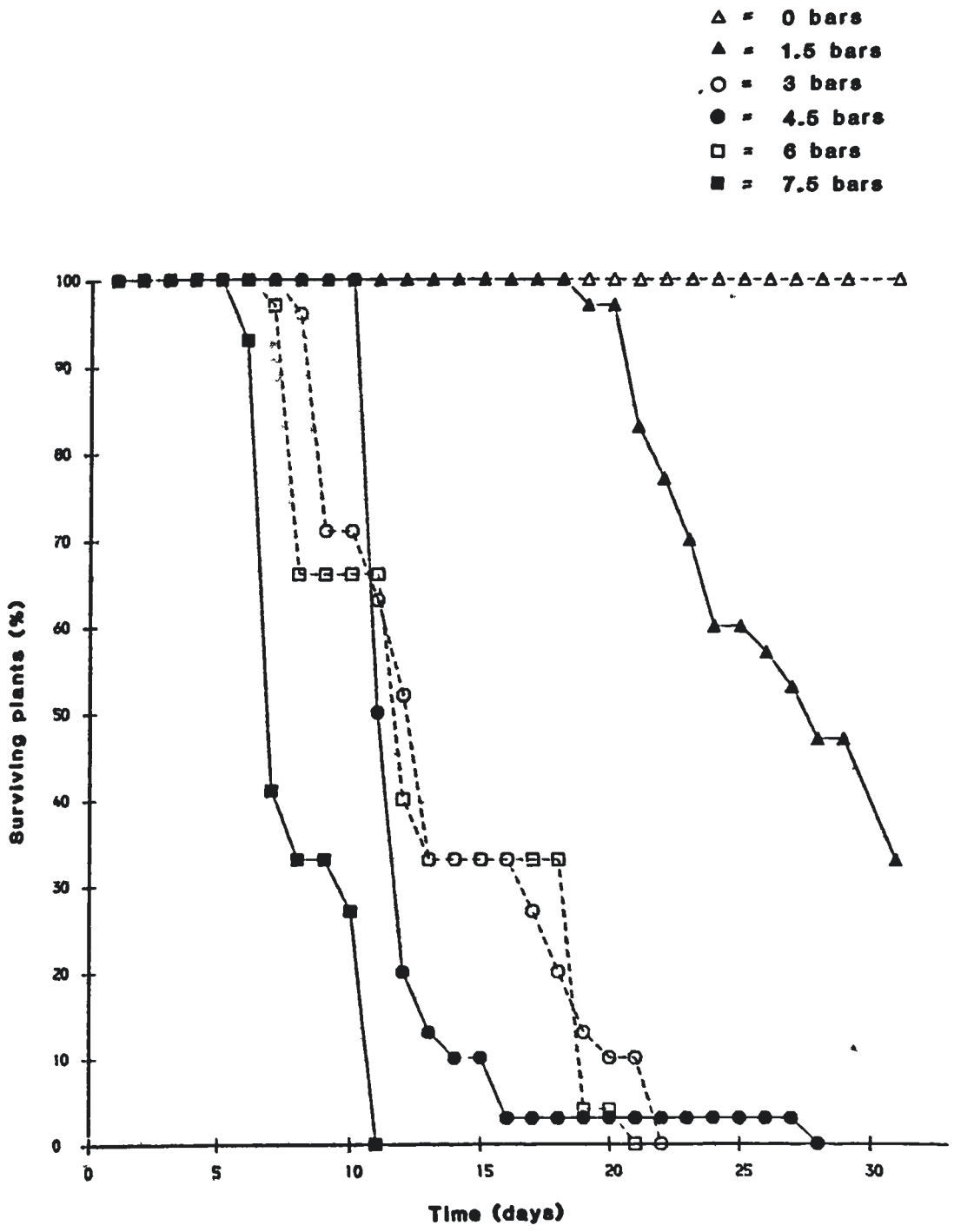
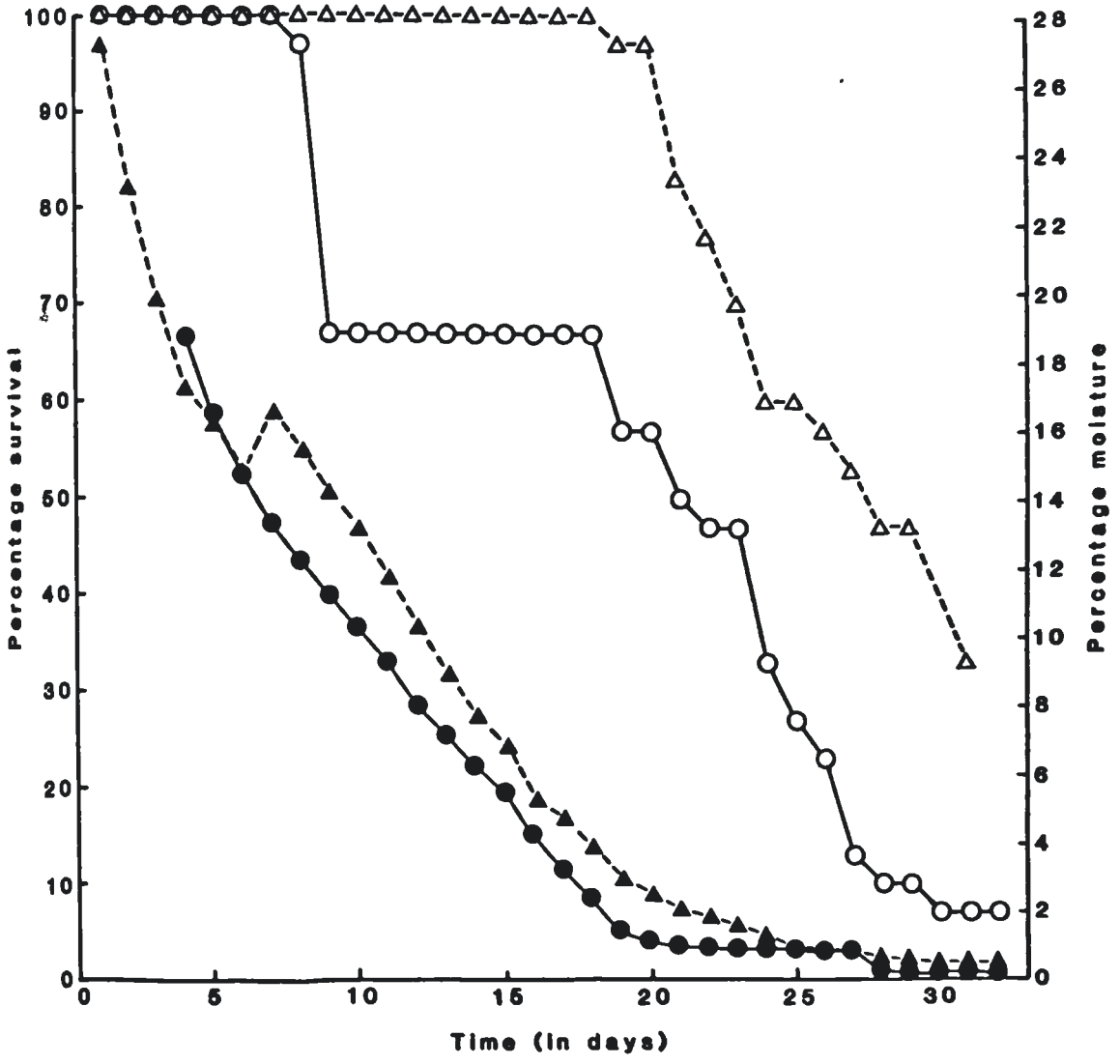
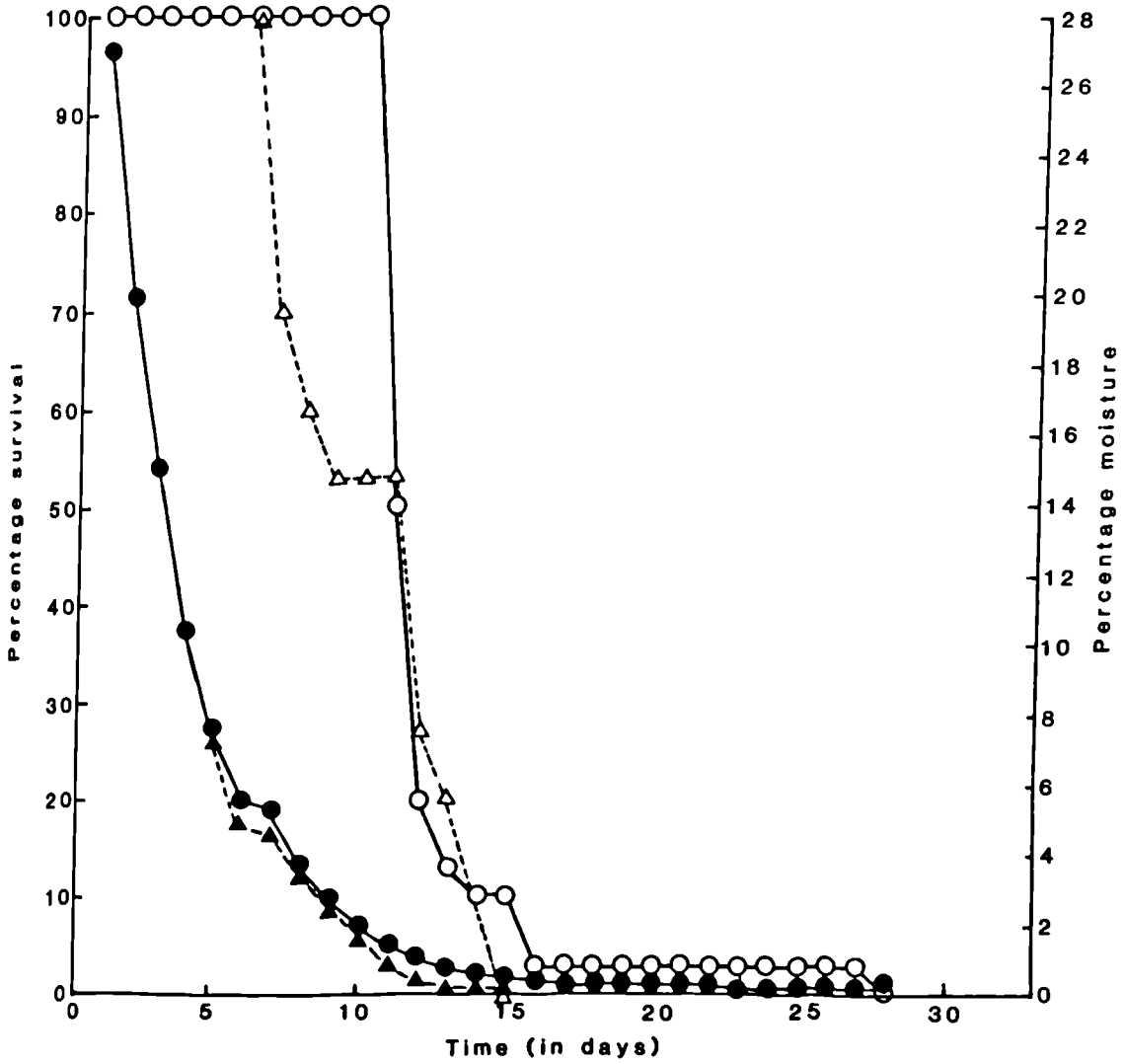


Figure 5.8 Comparative survival of seedlings from groups 1 and 2 in response to gradual depletion of substrate moisture content.



● Substrate moisture content : group 1 seedlings
○ Survival (%): group 1 seedlings
▲ Substrate moisture content: group 2 seedlings
△ Survival (%): group 2 seedlings

Figure 5.9 Comparative survival of seedlings from groups 1 and 2 in response to rapid depletion of the substrate moisture content.



▲ Substrate moisture content: group 1 seedlings
△ Survival (%): group 1 seedlings
● Substrate moisture content: group 2 seedlings
○ Survival (%): group 2 seedlings

curve: in this case both sets of pots dried out much faster and the majority of seedlings in both groups died within 15 days. Again the onset of mortality in group 2 seedlings was delayed for 4 days, although the rate was then so accelerated that it rapidly caught up with group 1. At day 15 the last of the group 1 seedlings died, but the survivors from group 2 stayed alive for a further 12 days despite the substrate moisture level having fallen to below 1%. This indicates that these seedlings had developed a high tolerance to drought by the time they were 4 weeks old, but that none of the 3 week old seedlings was capable of withstanding a similar degree of water stress. The group 1 seedlings in Figure 5.8 were also capable of persisting in substrates with a water content below 1%, but in these pots this level was not reached until they were 4 weeks old.

The data from these experiments are consistent with the findings of Humphries _at al_ (1982), who showed that the critical level of moisture for 1 week old buddleia seedlings is below -2.8 bars. However, these experiments have also demonstrated that for the first 4 weeks following germination, the survival of buddleia seedlings under conditions of low water availability depends on the rate as well as the extent to which the substrate dries out. Many seedlings never reach the stage of becoming drought resistant, dying before the moisture content falls to the expected critical level, but there is a progressive increase in their drought resistance with age. Those seedlings which are initially better equipped to cope with limited water supplies, either by their genes or their environment, develop a significant degree of drought tolerance approximately 4 weeks after germination is completed. The level of moisture, in this substrate, which is critical for most seedlings is between 1 and 2%,

which is a matric potential of -0.13 to -0.19 bars, but the drought tolerant individuals which are 4 weeks old can survive for at least 10 days when it is below 1%, or -0.13 bars.

It should be noted that the moisture levels in the control pots also fell progressively and that none of the seedlings in these pots died but continued to grow and develop new leaves throughout the experiment. Few of the experimental plants grew much in the later stages of the experiment, but the fact that they persisted for prolonged periods without growing or conspicuous physical damage indicates that they had developed into stress tolerators (sensu Grime 1979). Further growth would not only require water for synthesis, but would increase the demands made by the plant to support its metabolism. Hence it is advantageous for a plant with a limited water supply to conserve water until it becomes plentiful again. This does not apply to species capable of supporting rapid and extensive root growth, but for a small-seeded species such as buddleia with its limited food reserves, such growth is not possible until much later in its development.

5:6 Conclusions about the effects of water stress on seeds and seedlings.

The implications for the seedlings in the field is that the soil moisture content is critical in determining when germination occurs and that it occurs only if a high moisture level is sustained over several days. For at least 5 days following germination the moisture supply must not fall below the critical level that permitted germination to occur, if the seedling is to develop sufficiently to

emerge from the ground. The first 4 weeks after germination are crucial for the survival of the seedling: the plants which survive to this stage are thereafter capable of persisting even when available water is scarce.

CHAPTER 6: THE FATE OF THE SEEDS AND SEEDLINGS IN THE FIELD.

6:1 Field investigations.

For buddleia, the 2 most critical elements of the environment are water supply and the depth of seed burial. Below 10°C temperature may have some influence while light and oxygen are not important.

Most of this work involved single factor variations in the laboratory, but in the field the situation will involve many inter-relating factors. However, it is possible to follow the fate of the seeds and seedlings in the field and, adding this to the laboratory data, to suggest reasons for the observed patterns of persistence, emergence and survival.

In Chapter 3, the formation and dispersal of buddleia seeds was monitored. The next stage in the life history to be investigated is the fate of the seeds on and in the ground. Do they germinate immediately or can they persist in the soil for a period before germinating? If the latter, then what happens to the seeds while they are in the soil? The final study is a detailed survey of the emergence and survival of seedlings over an 18 month period, on 2 sown experimental sites outside Oxford.

6:2 The existence of a soil seed bank incorporating buddleia.

Flowering plants have been divided into 2 groups by Grime (1979): firstly, those in which the seeds all germinate soon after they are released and secondly, those which become incorporated into a bank of viable but ungerminated seeds. The latter group are

divisible into "transient" and "persistent" seed banks; in a transient seed bank the seeds do not remain viable for more than 1 year, but in the persistent bank some of the seeds are there for at least a year and often longer. The seeds may simply be quiescent and remain in the bank as a result of inappropriate conditions for germination, or they may possess innate or secondary dormancy. Losses from the seed bank occur as a result of germination, fungal or bacterial attack, seed predation and ageing effects.

The following studies were designed to show whether buddleia is capable of forming either a transient or a persistent seed bank, to determine the rate of depletion from such a bank, and to suggest the major causes of these losses.

6:2:1 Testing for a persistent buddleia seed bank in the field.

The severity of the 1981/82 winter caused the death of the aerial parts of many adult buddleias, including all of the plants at Site B. None of these produced flowers in the 1982 season, so that no new seed was shed during 1983. Consequently, if seeds were found in the soil during that year, they could be classed as having persisted in the soil for at least a year, and would thus be evidence of the existence of a seed bank.

On 4 May 1983 soil samples were collected from beneath 5 adult plants at site B. The top 2 cm of soil and litter were removed before the samples of similar size were dug out to different total depths of 5-12 cm. The soil was spread out on trays, ready to be placed under a mist propagator unit in a heated greenhouse. The volume of the soil in each tray was noted and the buddleia seedlings recorded as they emerged over the next 2 months.

The total numbers for each tray and the depth from which the sample was removed are shown in Table 6.1. The results demonstrate conclusively that buddleia seeds are capable of persisting in the soil for at least one year. The figures in Table 6.1 also suggest that the majority of seeds were in the top 5 cm of soil: those samples including soil from greater depths had less than two thirds the number of seeds, in the same volume of soil, as the sample taken only from the upper layers. Since the soil is sandy, with little structure, it is to be expected that small seeds are soon washed into the top layers of soil. However, further burial is usually dependant on the activities of soil fauna (Harper 1977), and the fact that fewer seeds are found in the lower horizons probably reflects the paucity of invertebrates in this sandy soil.

The number of seedlings emerging in the trays was high: an average of 96 seeds successfully emerged from 1000 cm^3 soil. This reflects the density of the seed rain directly beneath the parent shrub, reported in section 3:6. This demonstration of the persistence of large numbers of viable seeds in the soil suggests that the reason for the absence of seedlings beneath these plants is not simply the result of the seeds germinating and dying soon after emergence, as a consequence of intense competition for resources.

6:2:2 The fate of buddleia seeds in an experimental seed bank.

To establish the rate at which seeds are lost from the seed bank and to determine the major factors responsible for these losses, known numbers of seeds were buried at sites D and E and subsequently retrieved and tested, at intervals over a period of 6

Table 6.1 Seedling emergence from soil samples collected at site B.

Sample	Number of seedlings	Approximate soil volume	Depth from which sample extracted
1	160	1,500 cm^3	6-15 cm
2	250	1,500 cm^3	5-7 cm
3	135	1,500 cm^3	6-15 cm
4	80	1,500 cm^3	5-12 cm
5	95	1,500 cm^3	5-12 cm

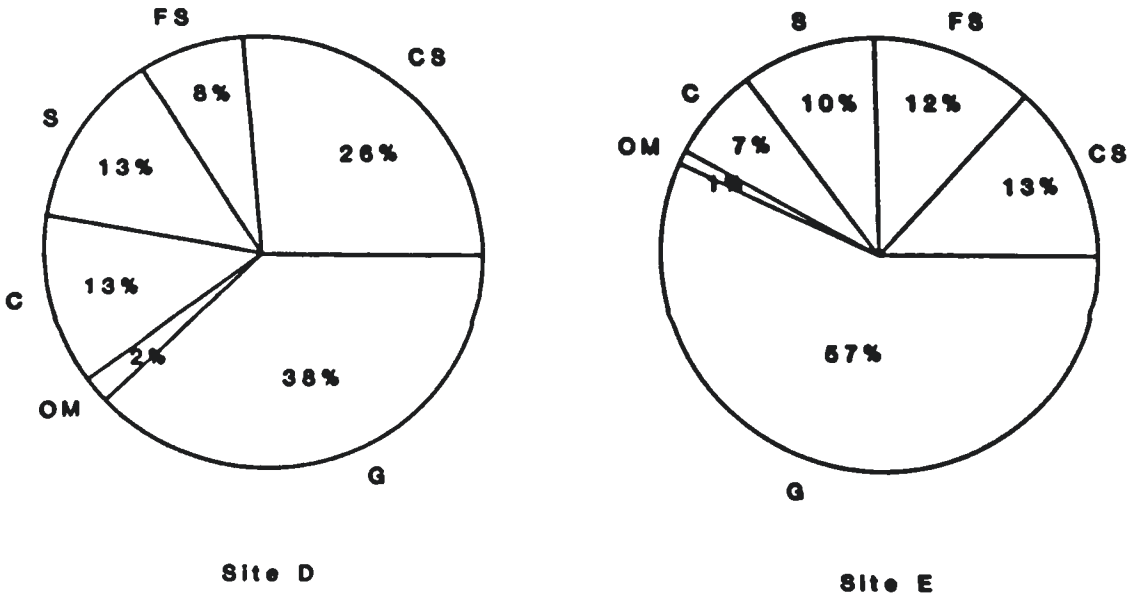
months. Site D is a gently sloping cliff top in an abandoned sandstone quarry at Forest Hill, 5 miles east of Oxford, and is currently used as a car distribution depot by the owners. Site E is on the side of the Banbury/Oxford railway line, at the disused station at Islip, 5 miles north of Oxford.

The substrate at site D is sand, the thin covering of top soil having been removed together with the existing vegetation before any experimental studies commenced. At site E the substrate was composed of limestone chippings, at least 5 cm deep in most parts, which overlay cinders and sandy dust. Figure 6.1 shows the proportions of the different particle sizes in samples taken from both sites. These sites were chosen since they represent typical sites which buddleia might colonise: the shrub had previously grown on the quarry floor at site D, but was removed by the owners. Seeds used in this experiment were collected from plants at site B, in February 1982. They were known to possess high viability, despite the severity of the winter, from the tests reported in section 4:7.

The containers in which the seeds were buried in the soil and retrieved easily were made from plastic petri dishes. Discs of 6 cm diameter were removed from the centres of the lids and the bases of the dishes. A piece of graded mesh was stuck over the hole in the lid, firmly sealed at the edges so that nothing could enter. A fine mesh was glued to the base of the dish, allowing water to drain out freely, but preventing anything other than micro-organisms from passing through it. Four mesh sizes were used:

1. Simon Textile monofilament nylon bolting cloth, N35, aperture 35, with an open area of 16%.
2. Simon Textile monofilament nylon bolting cloth, 38GGN, aperture 500, with an open area of 50%.

Figure 6.1 The proportions of different textural classes in substrate samples from sites D and E.



3. Nybolt monofilament grit gauze, 20GG,aperture 1000, with an open area of 58%.

4. Black P.V.C. mesh (Gardening quality), aperture 8x5 mm.

Eighteen dishes of each catagory were prepared. The type 1 mesh was used for all the bases. An example of each type is shown in Figure 6.2.

The dishes were filled with about 60 g of soil taken from either site D or E. This soil had previously been dried and autoclaved, to ensure all the containers provided the same sterile conditions at the start of the investigation. Anything which was subsequently found in them would have entered from the surrounding soil. One hundred seeds were mixed with each 60 g soil before placing it in the container, the sides of which were then sealed with P.V.C. tape to prevent entry of soil organisms. The finished container is shown in Figure 6.3. Ultimately, 36 dishes were buried at each site, incorporating 4 different mesh grades, making 9 replicates of each treatment.

The dishes were buried in a grid pattern using random coordinates to determine the position of each dish. The bases were at a depth of 5 cm and the mesh tops approximately 3.5 cm below the soil surface. They were buried on 12 March 1982, when natural dispersal was also occurring. Three replicates of each treatment were removed from each site after intervals of 2, 3 and 6 months, again using random coordinates to decide which dishes to take.

The containers were opened back in the laboratory and the seeds were extracted by flotation. Most of the seeds were easily visible and rapidly removed, but the last 15-20% were more difficult to locate. They were transferred directly onto moistened filter paper in new petri dishes, and their viability assessed by recording

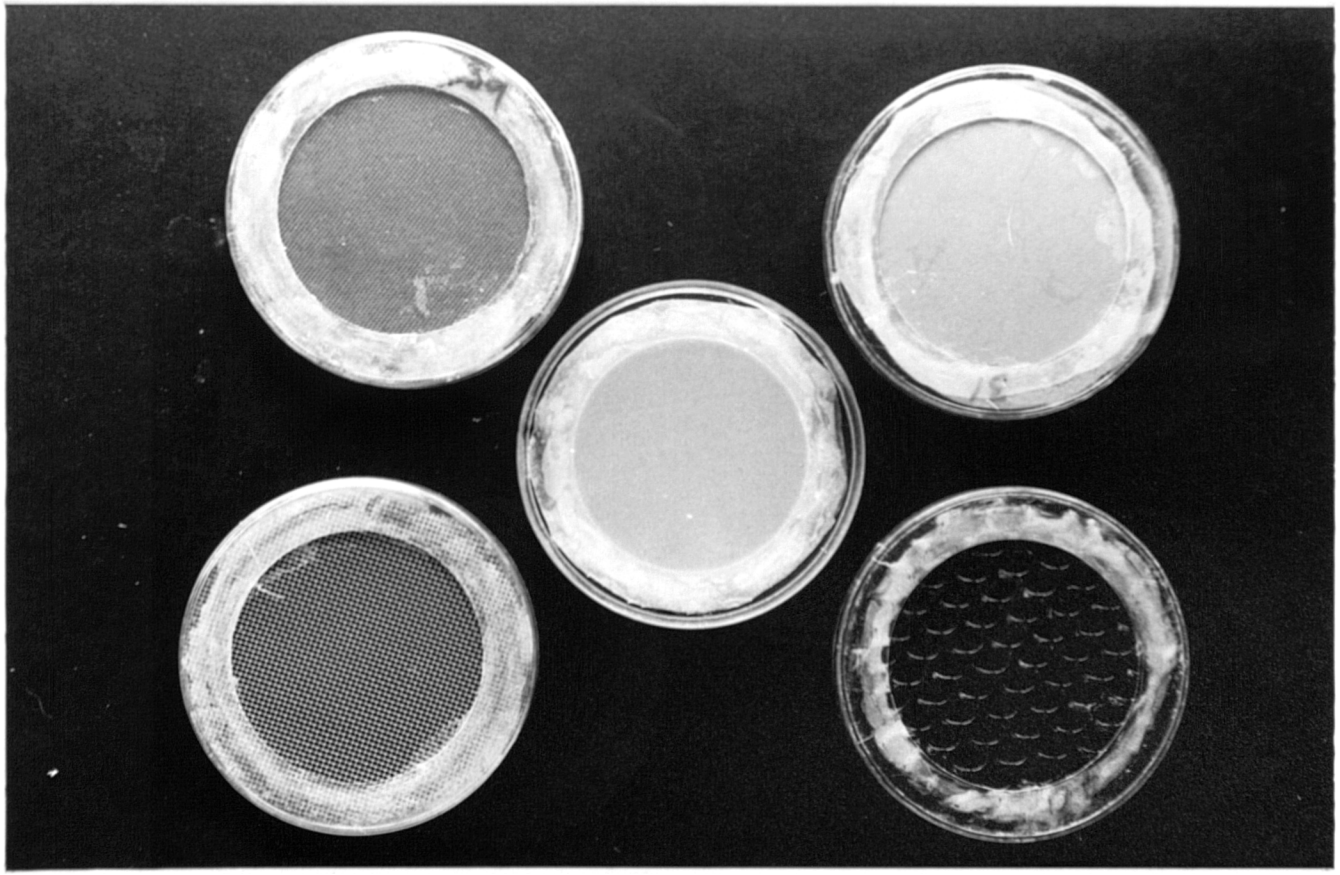


Figure 6.2 The base, surrounded by lids of the 4 mesh types used in the seed burial containers.

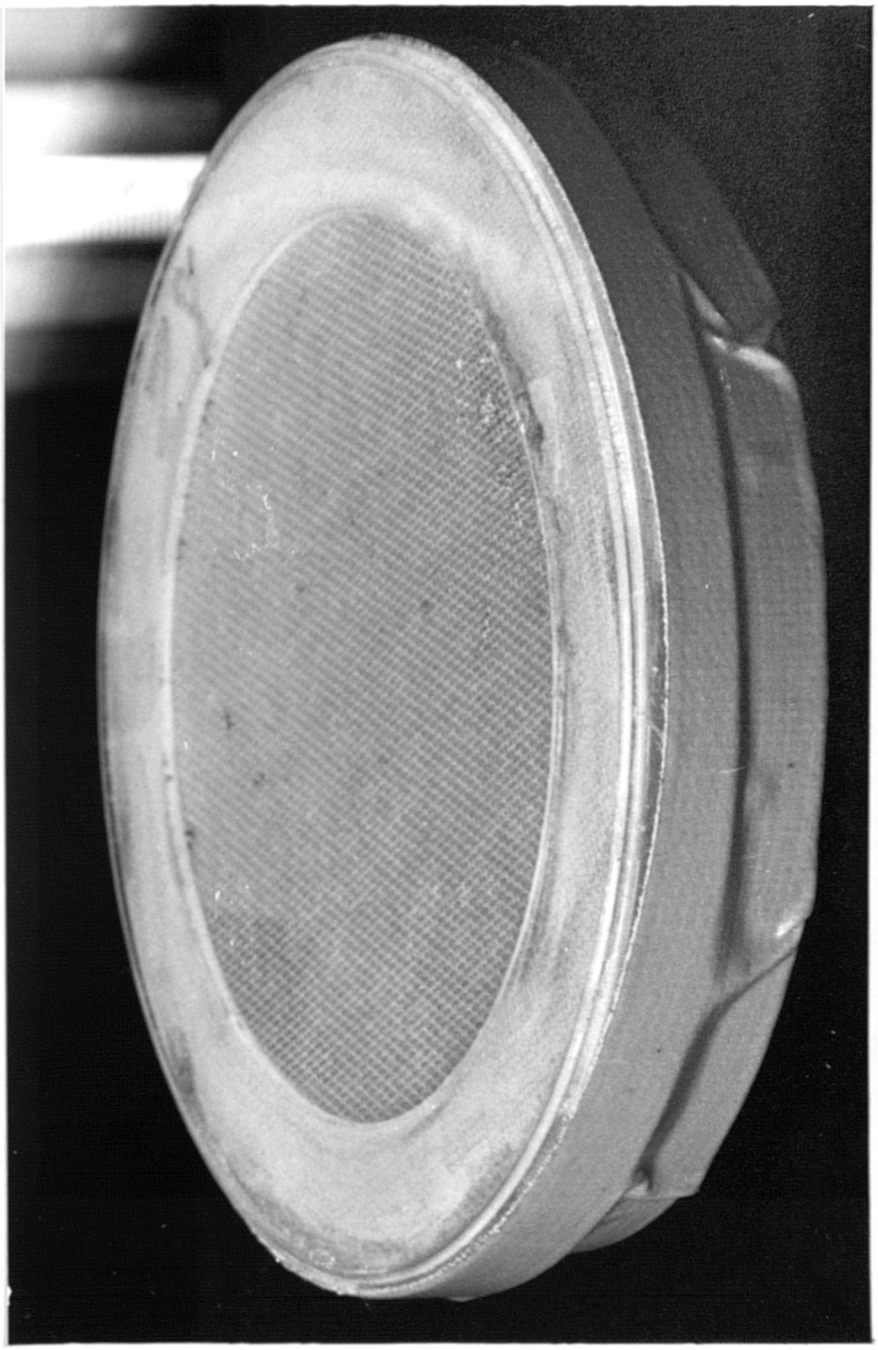


Figure 6.3 A sealed container, ready for burial.

the percentage germination after 3 weeks in the environmental cabinet.

Figures 6.4 (a-d) show the mean numbers of seeds recovered from site D after 2, 3, and 6 months of burial. Figures 6.5 (a-d) show similar results for seed burial at site E. It is apparent from these figures that on both sites most of the seed which was recovered was viable. Considering first the effect of mesh size on the persistence of seeds: the seeds recovered from grade 1 containers showed remarkably little change over the 6 month study period. The numbers of seeds retrieved and their viability remained constant at site D and only decreased slightly at site E.

The lower number retrieved from site D was typical of the results from all container types: it may reflect the difficulty of recovering seeds from the sandy sustrate compared with the stony soil at site E. Alternatively, it may be that conditions at the site were suitable for some seeds to germinate and the losses were due to this or to enhanced microbial activity. Whichever of these is applicable, it is apparent from these results that microbial activity and unsuccessful germination alone did not account for the loss of many seeds from the soil.

As the mesh size increased, so more types of soil organism could enter the container. The effects of this became more marked the longer the seeds remained in the soil. After 2 months there was no significant difference in the numbers of seeds left in each type of container, at either site, and at site D there was still not much difference after 3 months. However, at site E, the decrease in seed recovery with increasing mesh size was more noticable at this stage, and after 6 months there was a conspicuous difference at both sites, although it was always greater at site E.

Figure 6.4 (a – d) Depletion of a bank of viable seeds buried at site D, over a six month period.

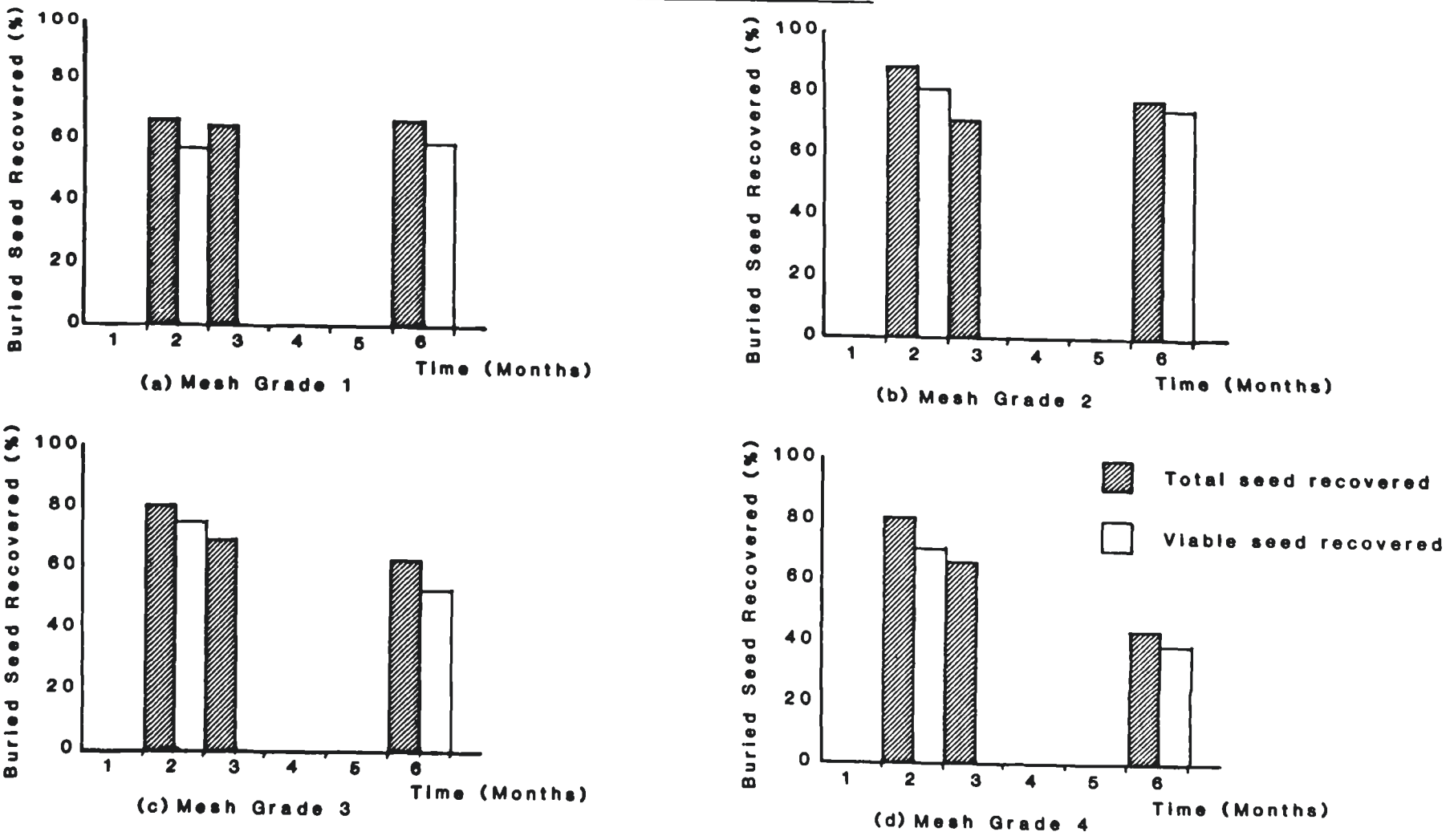
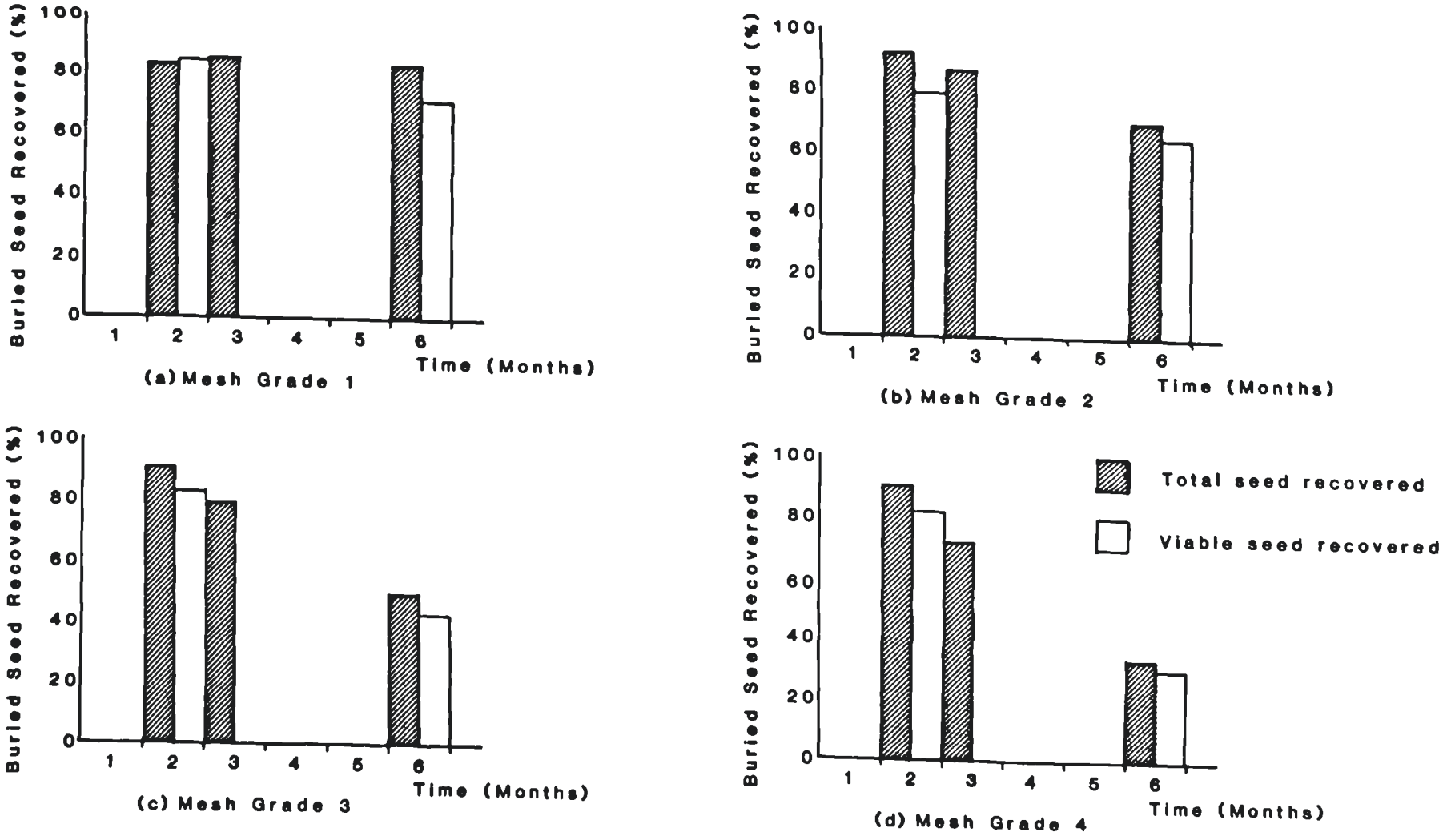


Figure 6.5 (a – d) Depletion of a bank of viable seeds buried at site E, over a six month period.



Mesh grades 2 and 3 were fine enough to exclude earthworms and other large invertebrates which might be expected to have most impact by predating or redistributing seeds. The results in Figures 6.5 (b-d) show that at site E the small invertebrates had an important impact on the seed bank, as great as that of the larger fauna allowed to enter through mesh 4. This implies that animals such as earthworms were not particularly important at this site. However, as Figures 6.4 (b-d) show, at site D the finer meshes did not produce noticable effects on the seed bank: it was only with grade 4 mesh that there was a significant decrease, which may signify that at this site there was more earthworm activity.

It is the grade 4 containers which approach a normal soil situation, although the passage of any organism through the container would be impeded by the fine mesh base. It would have to enter from the top and leave by the same surface, which may have deterred some potentially significant animals. This base was necessary to prevent the seeds from simply washing away into other parts of the soil, which they might well do in such loosely structured soil. This would create errors in the results since it would not be removing them from the seed bank, only from the sample. Nevertheless, the impedence will mean that the estimate of seed bank depletion which may be made from Figures 6.4 (d) and 6.5 (d) is conservative.

In order to generalise about the rate of disappearance of seeds from the soil, the mesh size 4 data from both sites can be combined. The mean number retrieved after 6 months is about 40%, and if this rate continues then no viable seeds would remain in the soil after 1 year. However, this extrapolation takes no account of the seasonality of seed loss. Seed activity in the soil

was only monitored between March and September, but over the winter the rate may change. For example, the invertebrate activity which appears to be so important decreases in cold weather, while the loss of viability, which is a comparatively minor component in this study, may increase with colder and wetter weather. Accepting that there may be these seasonal fluctuations in rates at which seeds are lost, it seems unlikely that they substantially alter the estimate of seed persistence.

Finally, by comparing the data on viable seeds recovered from the grade 1 and grade 4 meshes, it is possible to determine the relative importance of the 2 groups of agents investigated here. The grade 1 data show losses due to inherent properties of the seed: premature germination and loss of viability, as well as microbial activity. The grade 4 data show these losses and also those due to invertebtrate activity. Hence the difference between these 2 figures will indicate how much of the seed disappearance is due to animal activity. Figures 6.4 (a and d) indicate that at site D only about 21% of the losses to the seed bank are due to invertebrate action alone and over 40% are due to microbial action, premature germination and declining viability. This is reversed at site E, as Figures 6.5 (a and d) show. Here, some 43% of the seeds are removed by invertebrates, while only 27% are lost from the other causes.

In conclusion, most of the buddleia seeds are unlikely to persist in the soil for much more than 1 year, but the results discussed in section 6:2:1 indicate that some viable seed can still be found after longer periods. Several factors contribute to the removal of buddleia from the seed bank, but which is the most important depends on the characteristics of the soil. On the stony substrate used in this study the activity of invertebrates was the

dominant factor, but on the sandy site this was less important than the effects of the environment on the seed and the activities of mico-organisms.

6:3 Conclusions: the fate of buddleia seeds in soil and the kind of seed bank formed.

The results of the 2 seed bank investigations indicate that there is some mechanism whereby the seeds avoid germination. For most of the year the ground temperature at a depth of several cm will approach that on the surface, and the moisture content will usually be higher.There is therefore no reason to suppose that when seeds on the surface are able to germinate, those buried do not also do so, unless some other factor prevents it (see section 7:1).

The experimental study of the fate of seeds in the soil was in progress at sites D and E at the same time as the survey of seedling emergence from surface sown seed, described in section 6:5. Many of the latter seeds germinated during the spring and summer, while those which were buried remained ungerminated in the soil. This suggests that buddleia seeds develop some form of induced dormancy when buried. The fact that this was not detected in the laboratory indicates that it does not require any factor other than light to break the dormancy.

Work by Wesson and Waring (1968) demonstrated that in 3 species whose germination was normally unaffected by light, burial of seeds induced photo-sensitivity. The physiological processes responsible for this change are not yet fully understood, but probably involve the production of a gaseous inhibitor by the seeds themselves. The degree of photo-sensitivity certainly

increases with depth of burial and with the higher soil moisture content they experience as they move deeper into the soil.

If buddleia develops a photosensitive response to burial, then this would explain the observed persistence of the seeds in the soil, which has not been accounted for by any of the other factors investigated.

Work by Thompson and Grime (1979) has made it possible to consider in more detail the type of seed bank that buddleia forms. They have subdivided the "persistent" and "transient" types and thus recognise 4 major categories.

1. Species with transient banks present during the summer.
2. Species with transient banks present during the winter.
3. Species with persistent banks, the seeds of which germinate soon after release, but some of which fall into a persistent bank.
4. Species with persistent banks, few seeds of which germinate initially, but which form a large and seasonally stable bank.

Having already established that buddleia is persistent, the question to be answered is do most of the seeds germinate immediately or are they mainly retained in a bank? The indications from the experimental study are that only a small proportion of the seeds buried remain in the seed bank for 1 year. The type 4 seed bank is described by Thompson and Grime as being generally large compared to the annual seed production. This is not the case with buddleia.

Conversely, type 3 is characterised by a small seed bank, and the species are often small seeded, with germination only over a restricted range of temperatures and tending to be inhibited at lower ones. Many of the species are also unable to germinate in

darkness, which may be important in maintaining dormancy in the soil over prolonged periods. This description fits well with the observed behaviour of buddleia seeds, particularly if they do exhibit induced photosensitivity. Other species which belong to this group are Arabidopsis thaliana (Cruciferae), Poa annua and P. trivialis (Gramineae), and Epilobium hirsutum (Onagraceae), all species which are frequently found in similar habitats to buddleia.

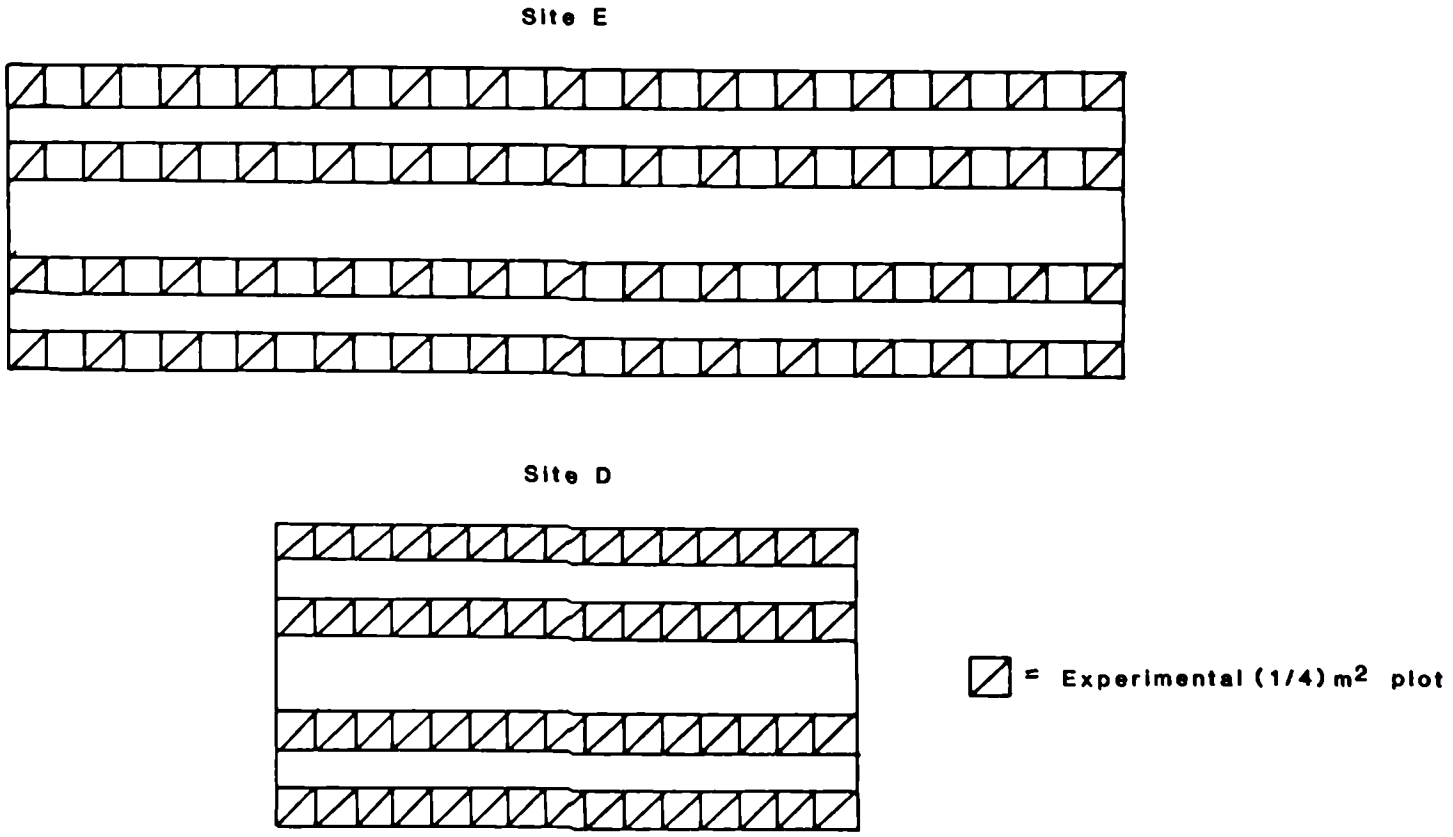
6:4 Survey of seedling emergence and survival on 2 experimental sites.

6:4:1 The design and techniques used in the survey.

The following survey was carried out over the period between 31 August 1981 and 9 December 1982, at sites D and E, as in the previous experiment.

The vegetation and the top 2 cm of soil were removed from an area measuring 1.5 x 4 m at site D, and 1.5 x 8 m at site E. These areas were then divided into 4 rows of $(0.25)^2$ m plots, arranged as shown in Figure 6.6, so that there was access to each plot for recording the emergence of seedlings. The rows of plots were separated from each other by strips of green plastic lawn edging, buried to a depth of 2-3 cm. This created a barrier which had 2 functions: firstly, to prevent the seeds from invading adjacent plots and secondly, to prevent the sandy soil at site D from creeping down the slope. Thus, 2 sides of each plot were bounded by these barriers and the other 2 sides were separated from neighbouring plots by a gap of 0.25 m.

Figure 6.6 The layout of the experimental plots at sites D and E.



The 60 experimental plots at each site were numbered and then 5 plots at each site were each randomly sown with 400 buddleia seeds at monthly intervals, throughout the year between 31 August 1981 and 1982. The 5 plots to be used each time were chosen using random number tables. To ensure constant experimental conditions, each site was cleared of any new vegetation immediately before the buddleia seeds were distributed over it. There was no attempt to cover the seeds or to water them in: they were just left on the surface as they would be in a natural situation. The subsequent emergence and survial of the seedlings was then recorded, first at 3 or 4 day intervals, but subsequently, as it became apparent that the changes in the population took place more slowly, at weekly intervals.

In order to record the fate of individual seedlings and so build up an accurate estimate of the proportion of the original seed input which had emerged in total, the position of each seedling on the plot was mapped on each visit. This was achieved using a specially constructed wooden frame, shown in Figure 6.7. This frame supported a $(0.25)^2$m piece of perspex, 4 cm above the ground, with a sheet of acetate film fastened over it, on which the positions of the seedlings were marked with a waterproof pen. There was a separate sheet for each plot,and on subsequent visits to the site the emergence of new seedlings and the disappearance of old ones were recorded. These results were transferred onto a master sheet following each visit.

To overcome the problem of parallax, a 2 cm grid was etched over the perspex sheet, and over a second piece of perspex which was then attached to the lower surface of the wooden frame. Lining up the 2 grids in a consistent manner when inspecting any part of the plot minimised the risk of recording the same seedling twice.

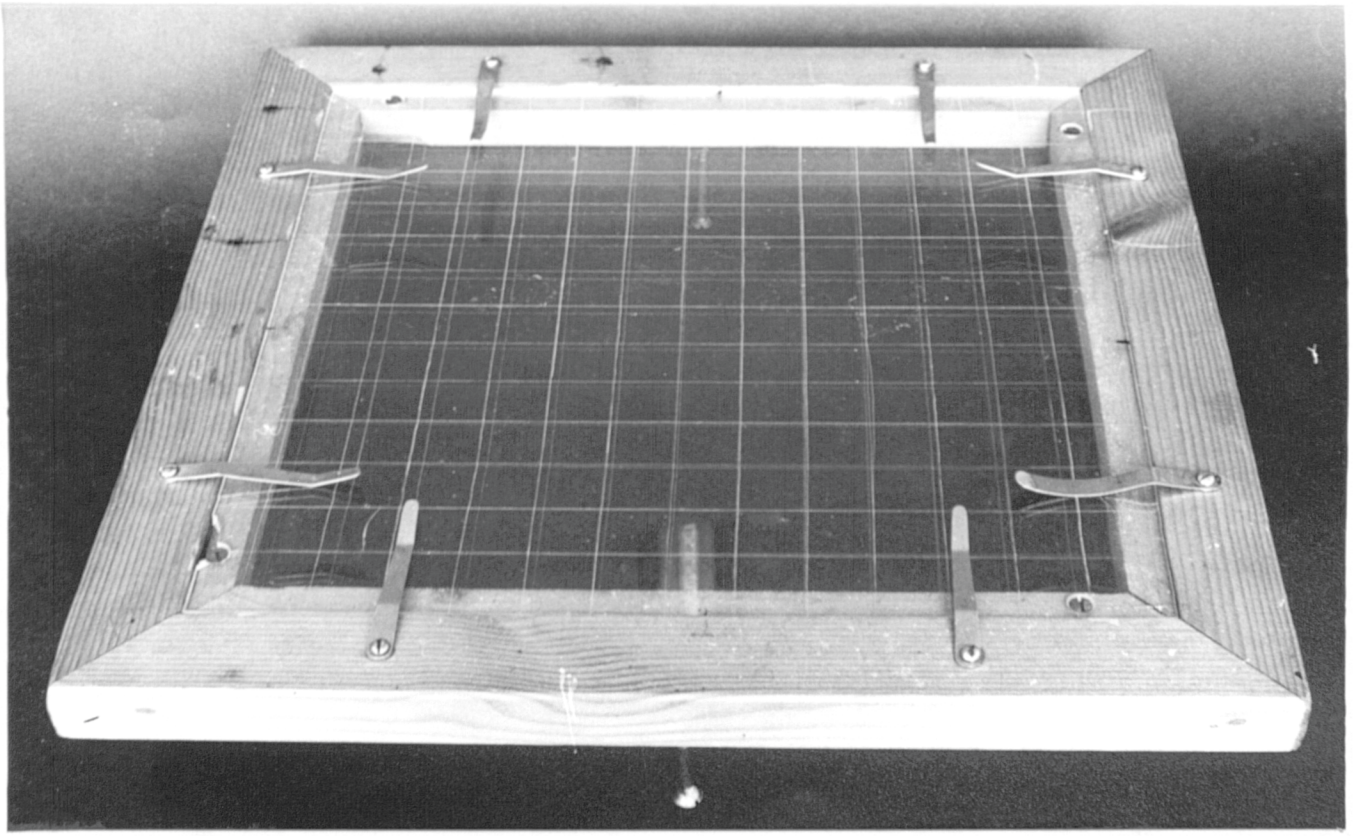


Figure 6.7 The quadrat frame used for recording the emergence and persistence of seedlings on permanent experimental plots.

Furthermore, the position of the frame was marked on the green plastic edging at each plot on the first recording to ensure it would be replaced in an identical position each time.

Measurements of temperature and moisture were obtained in the field and related to the observed patterns of emergence and survival. Since site D is close to a Thames Water Authority weather station, their weekly rainfall records were used for this site. However, site E is several km from the nearest meteorological station and is known to experience local differences in rainfall from site D, so a rain gauge was used at this site to record the daily rainfall.

To summarise the method, at each site 2,000 buddleia seeds were distributed over a total of 5 plots, at monthly intervals ocer 1 year. Subsequent emergence and mortality were recorded for each plot. The resulting data on the timing and quantity of seedling emergence and mortality were then related to meteorological records. The seedling data from site D are presented in Figures 6.8 (a-d) as 3 curves, (with results from all 5 plots pooled in each case) one showing cumulative emergence, the second showing the cumulative mortality, and the third showing the number of surviving seedlings. Figures 6.9 (a-e) shows a similar set of curves for site E.

The weekly rainfall records (1 September 1981 - 13 December 1982) from site D were used for the histograms in Figures 6.8 (a-d), and from site E, for the histograms in Figures 6.9 (a-e). The temperature records shown in both sets of figures are the minimum ground level temperatures for the period, supplied by the Radcliffe Meteorological Station, in Oxford. It should be noted that where the total emergence was less than 100, the y-axis scale has been doubled for clarity; otherwise the scale is constant throughout.

Figure 6.8 (a - d) The emergence, mortality and survival of seedlings from 2000 seeds sown at monthly intervals, on experimental plots at site D.

Figure 6.8(a) Seeds sown on 31.8.81 and 30.9.81.

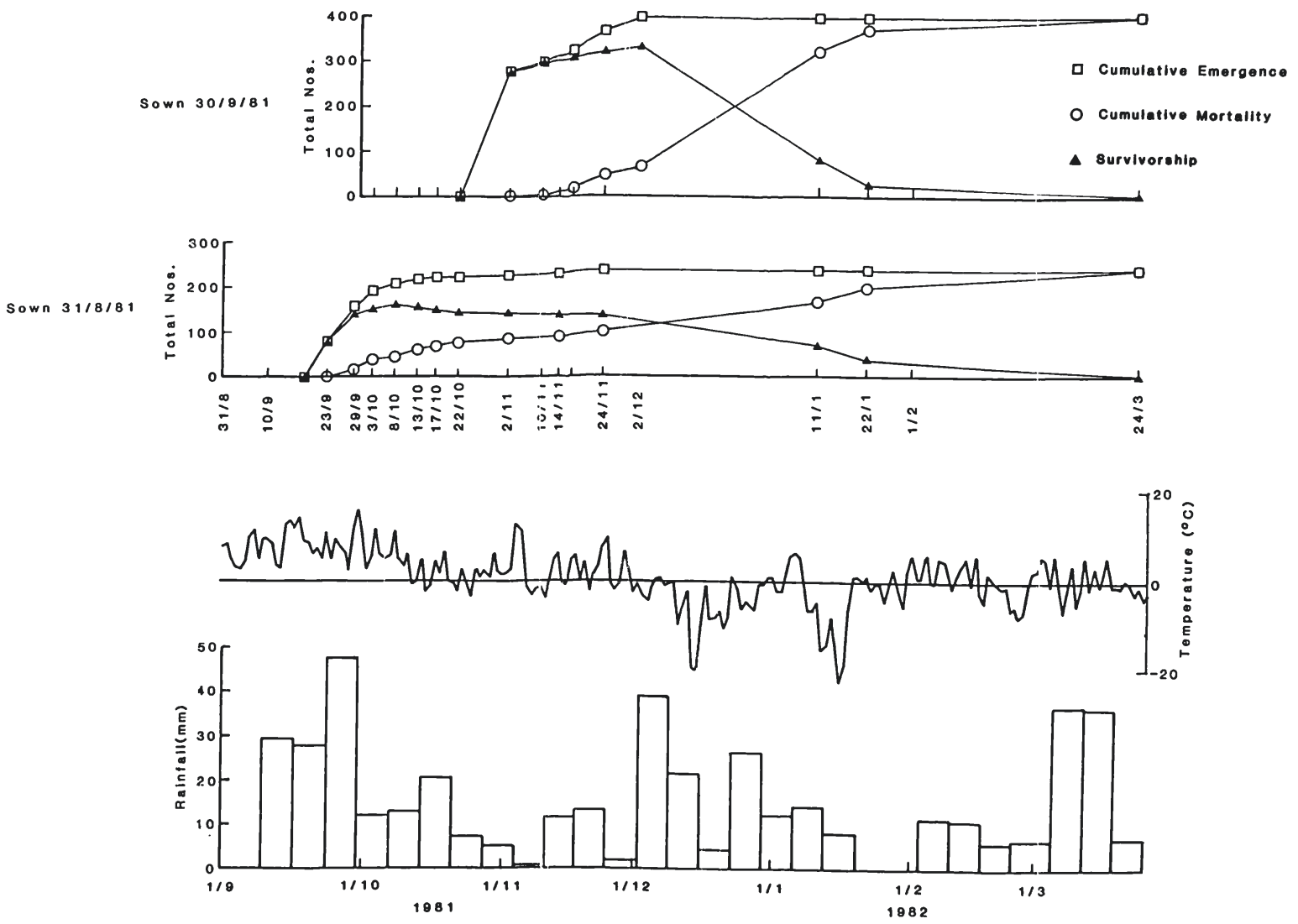


Figure 6.8(b) Seeds sown on 30.10.81, 30.11.81, 28.12.81 and 3.2.82.

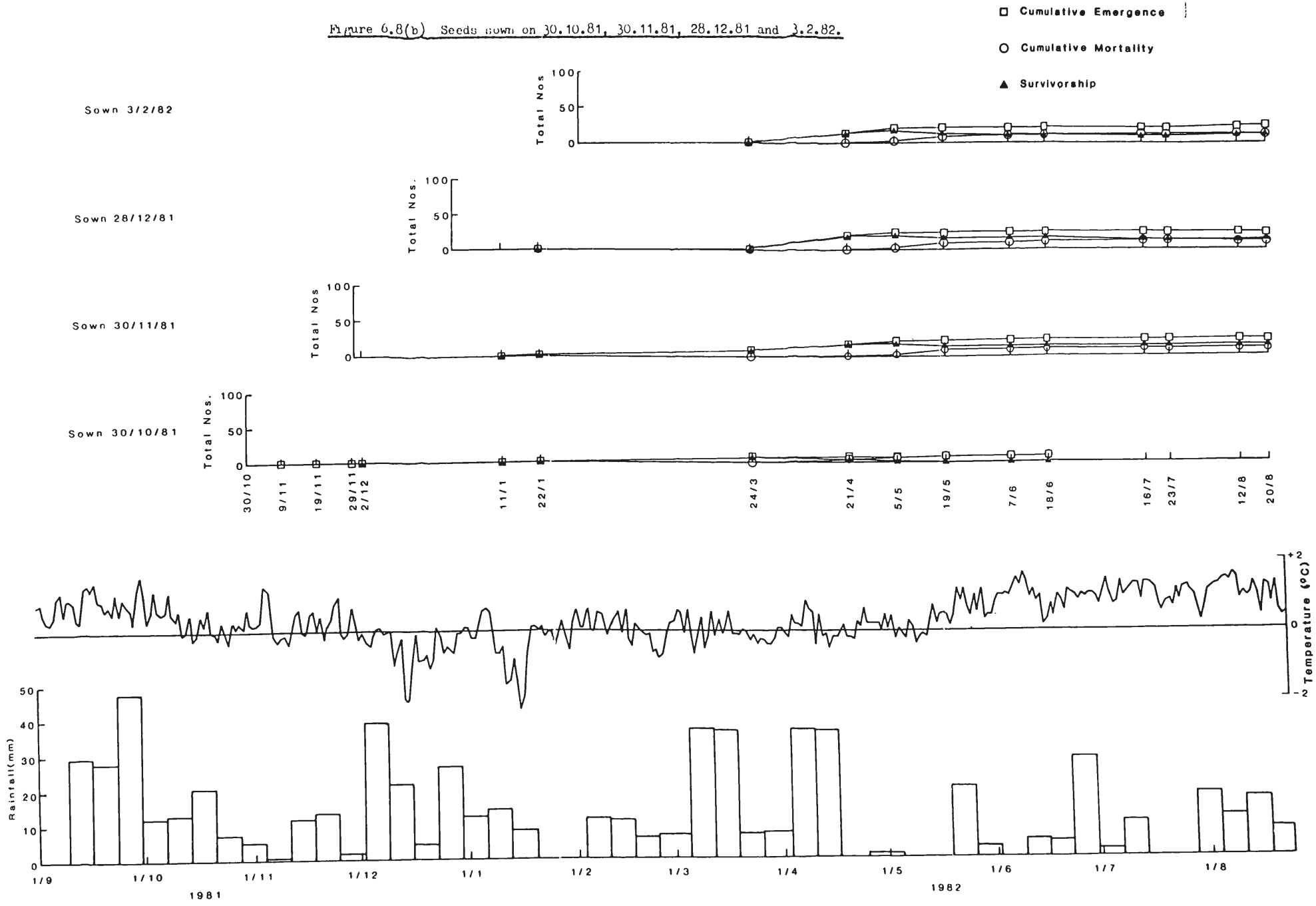


Figure 6.8(c) Seeds sown on 5.3.82, 2.4.82 and 4.5.82.

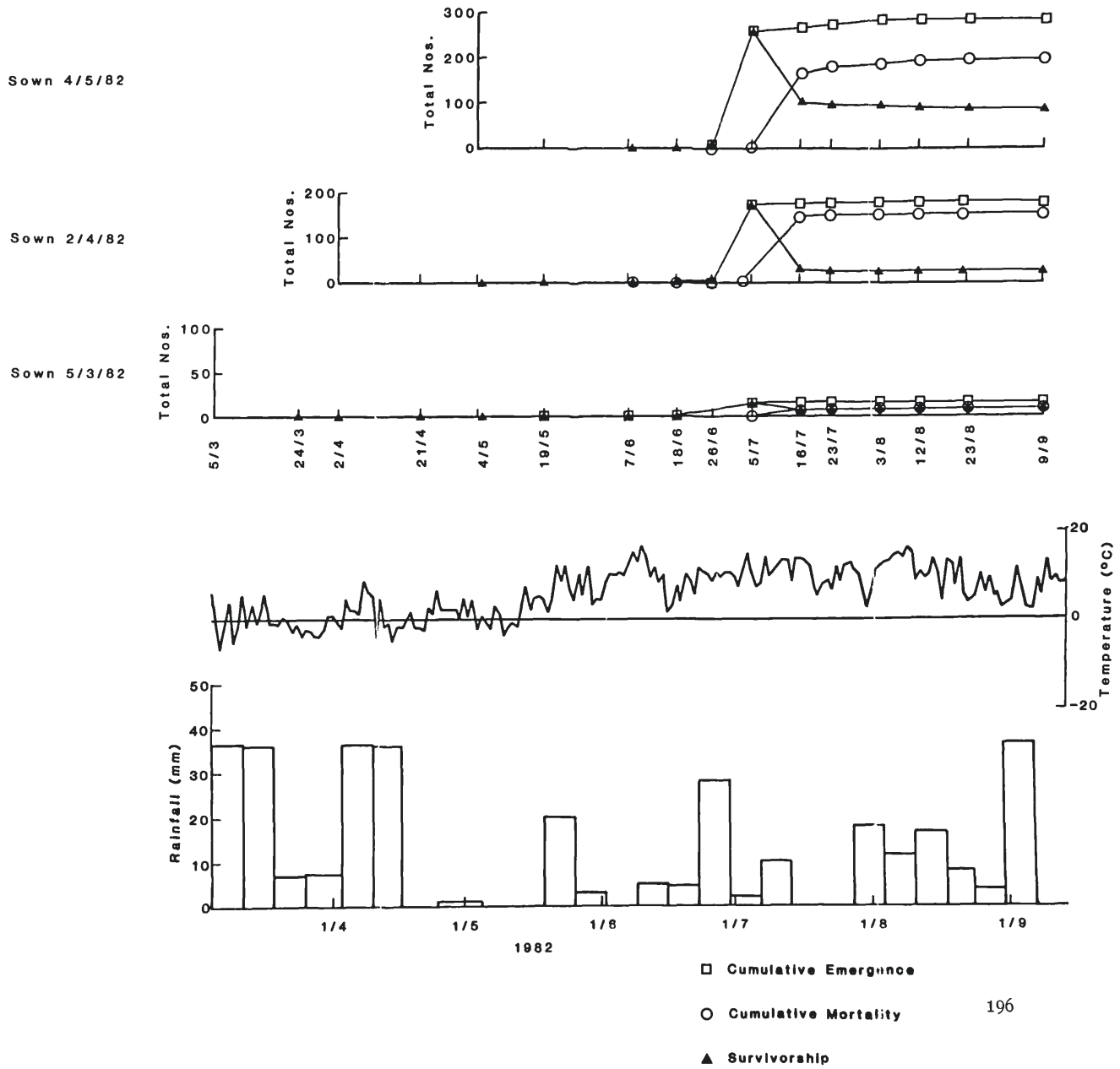


Figure 6.8(d) Seeds sown on 2.6.82, 7.7.82 and 5.8.82.

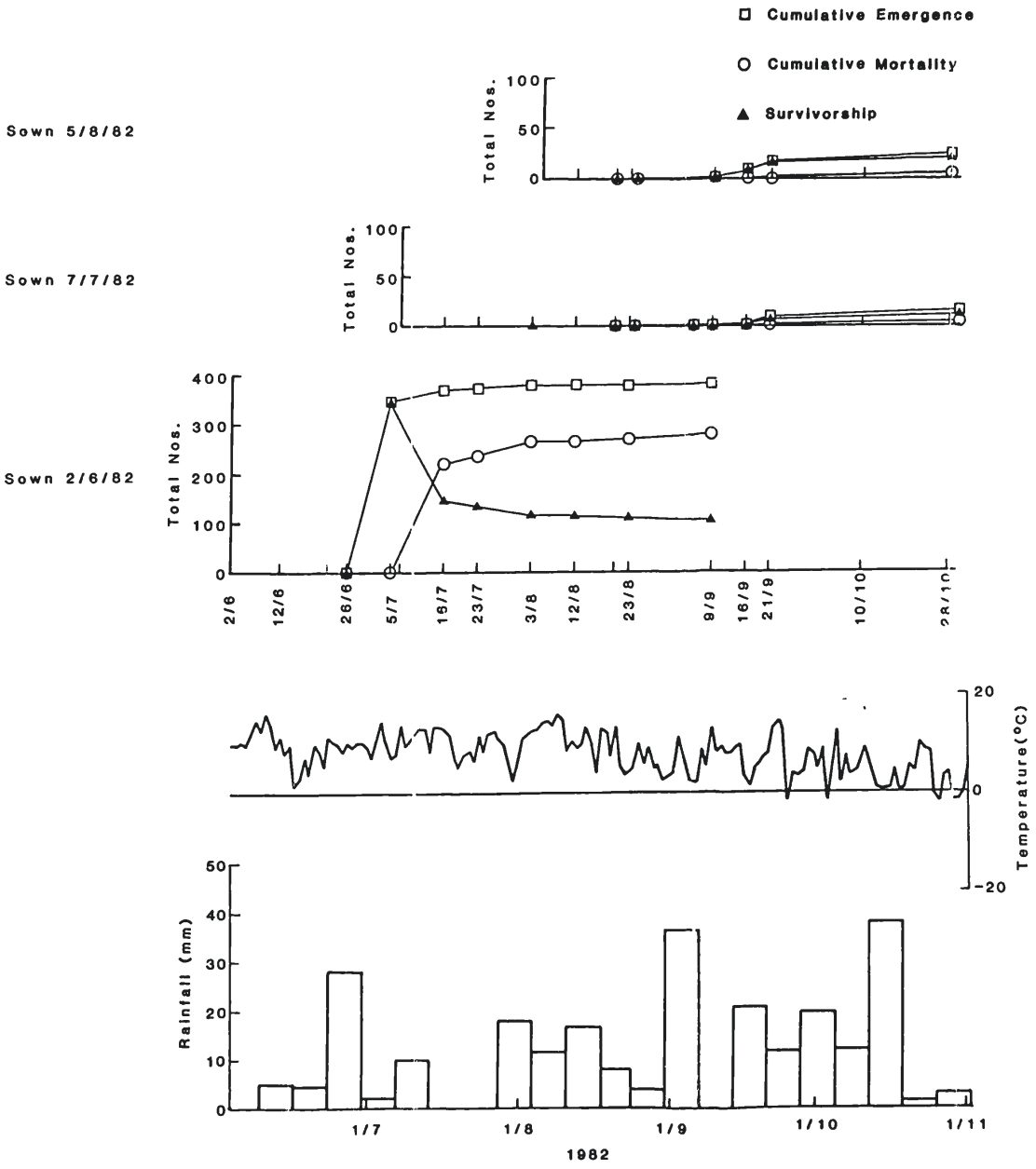


Figure 6.9 (a - e) The emergence, mortality and survival of seedlings from 2000 seeds sown at monthly intervals, on experimental plots at site E.

Figure 6.9(a) Seeds sown on 31.8.81.

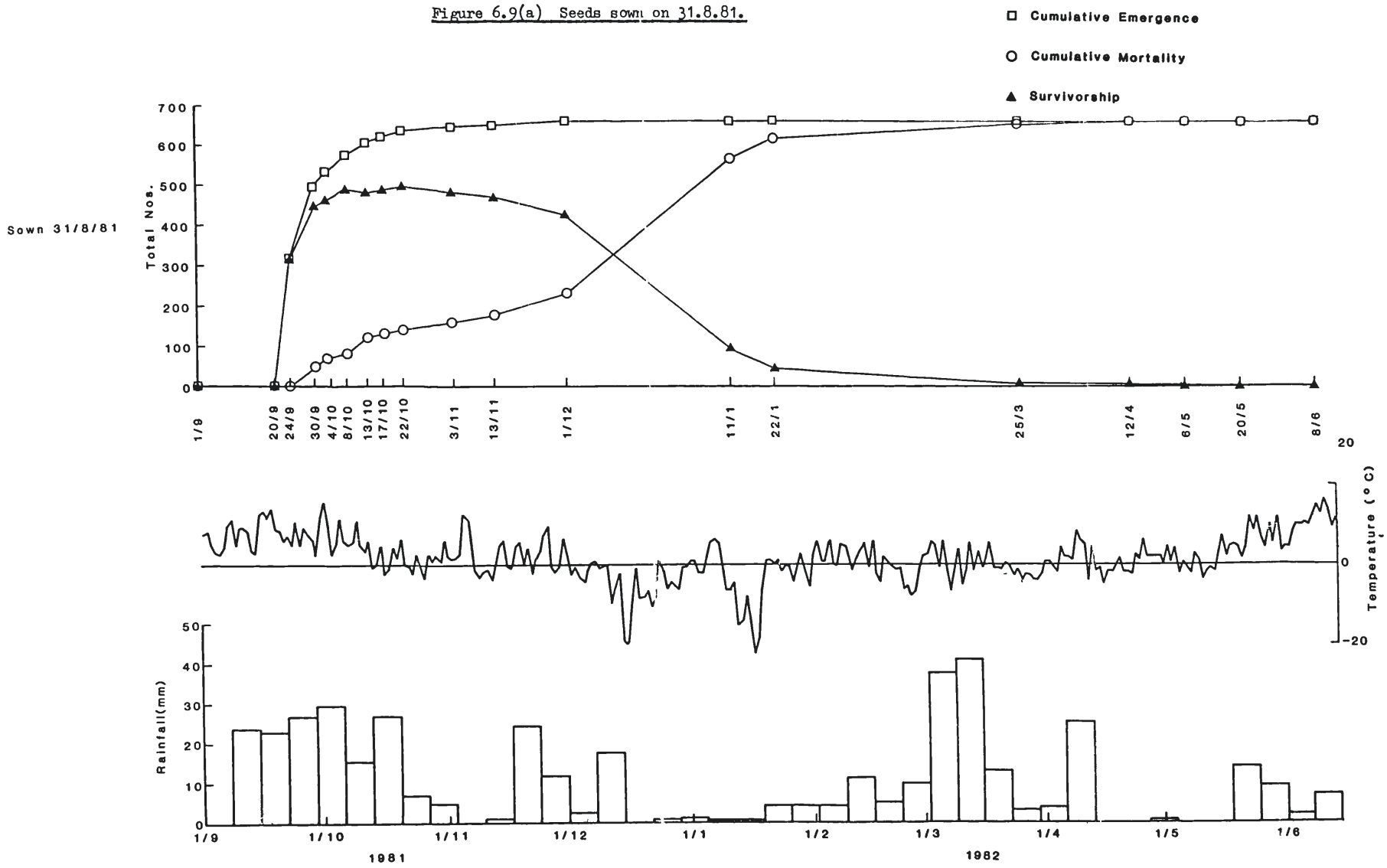


Figure 6.9(b) Seeds sown on 30.9.81.

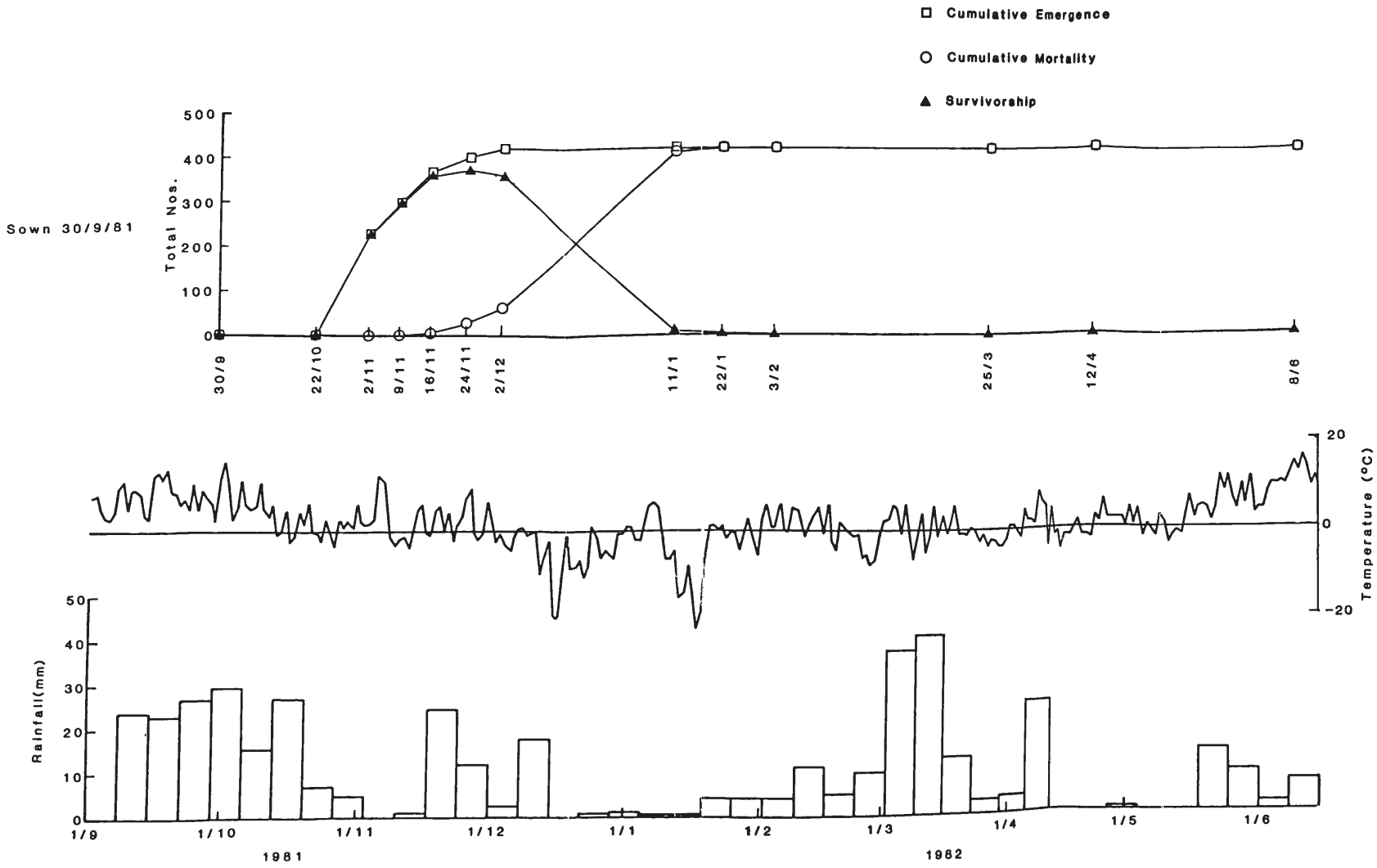


Figure 6.9(c) Seeds sown on 30.10.81, 30.11.81, 28.12.81 and 3.2.82.

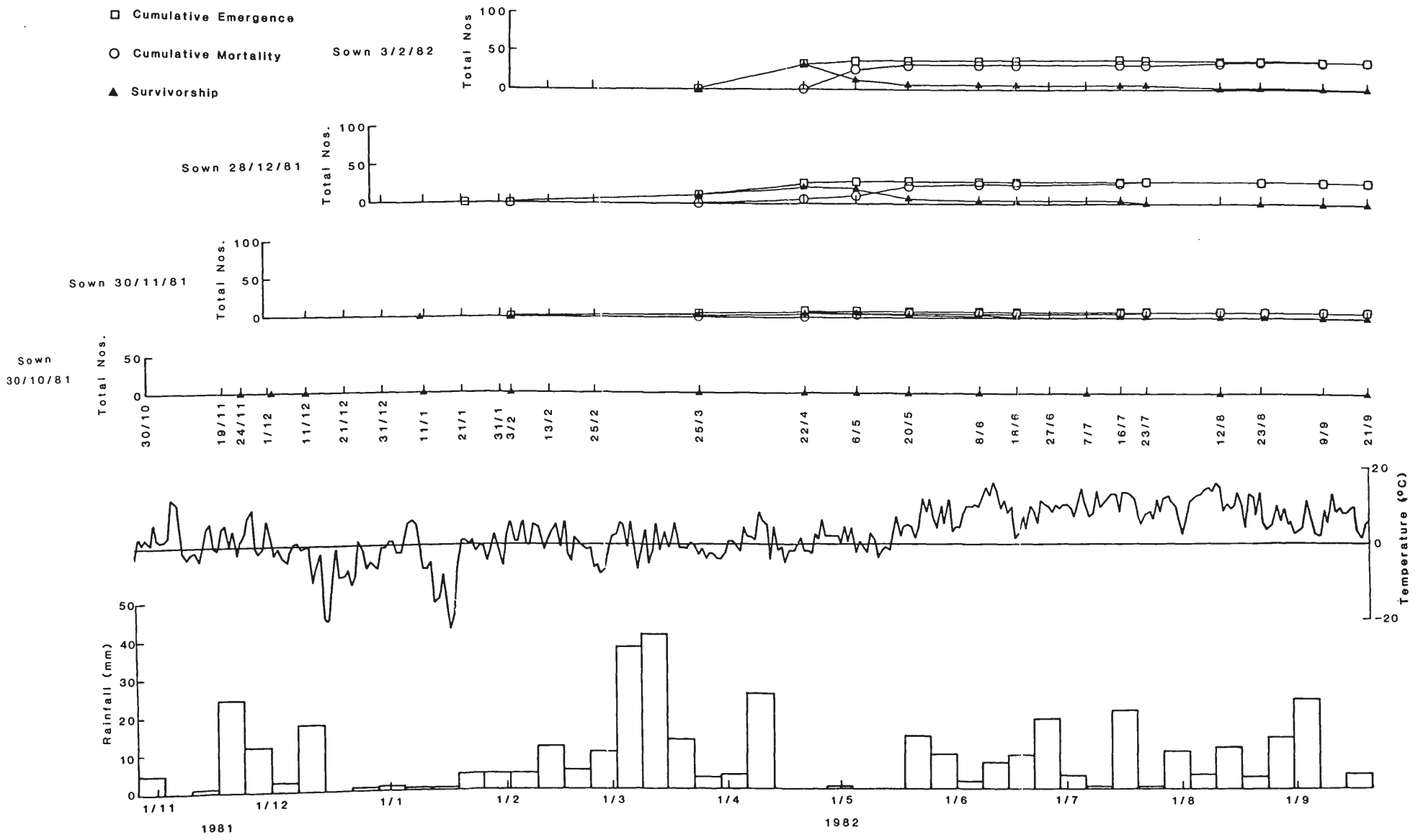


Figure 6.9(d) Seeds sown on 5.3.82, 2.4.82 and 4.5.82.

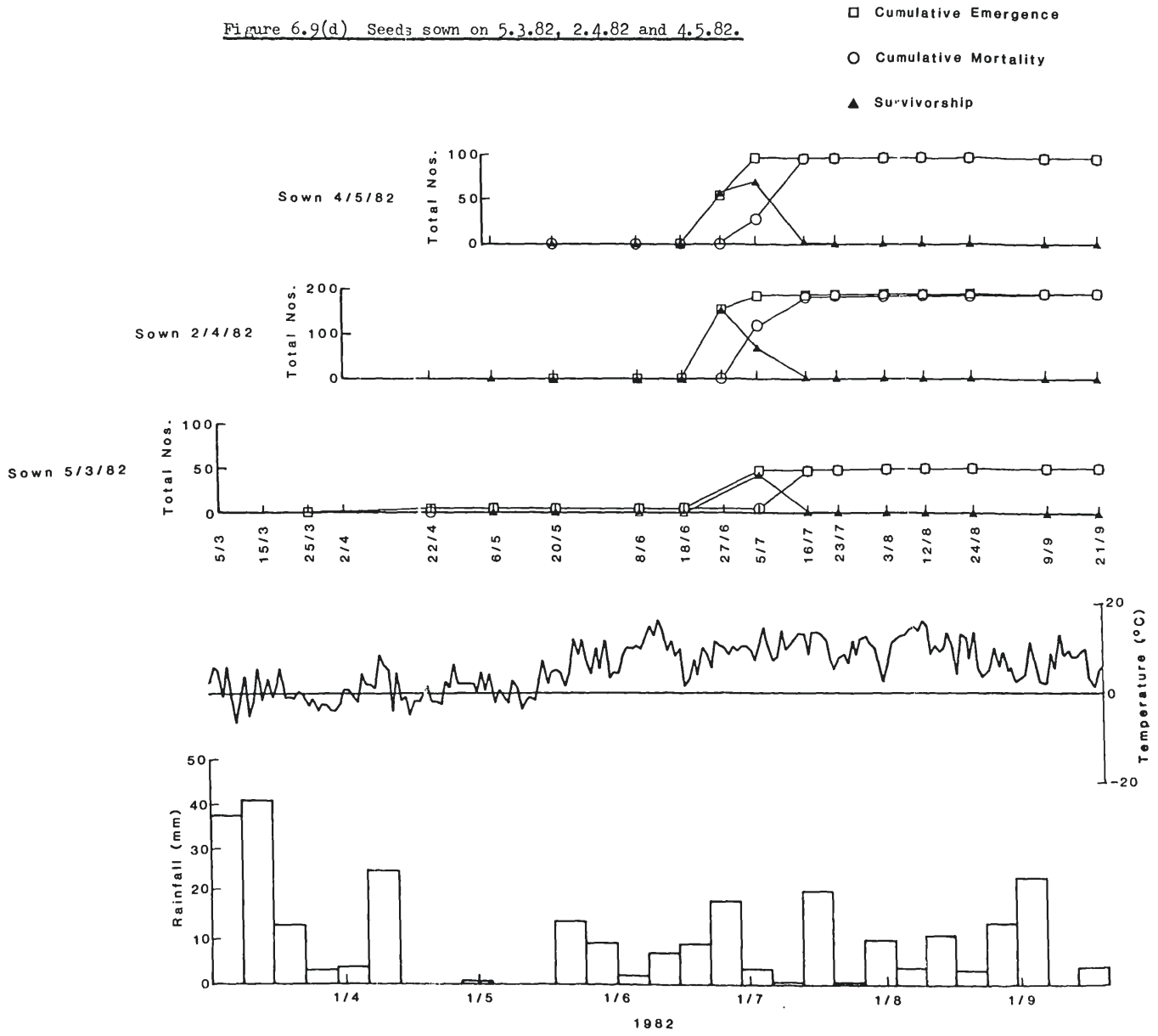
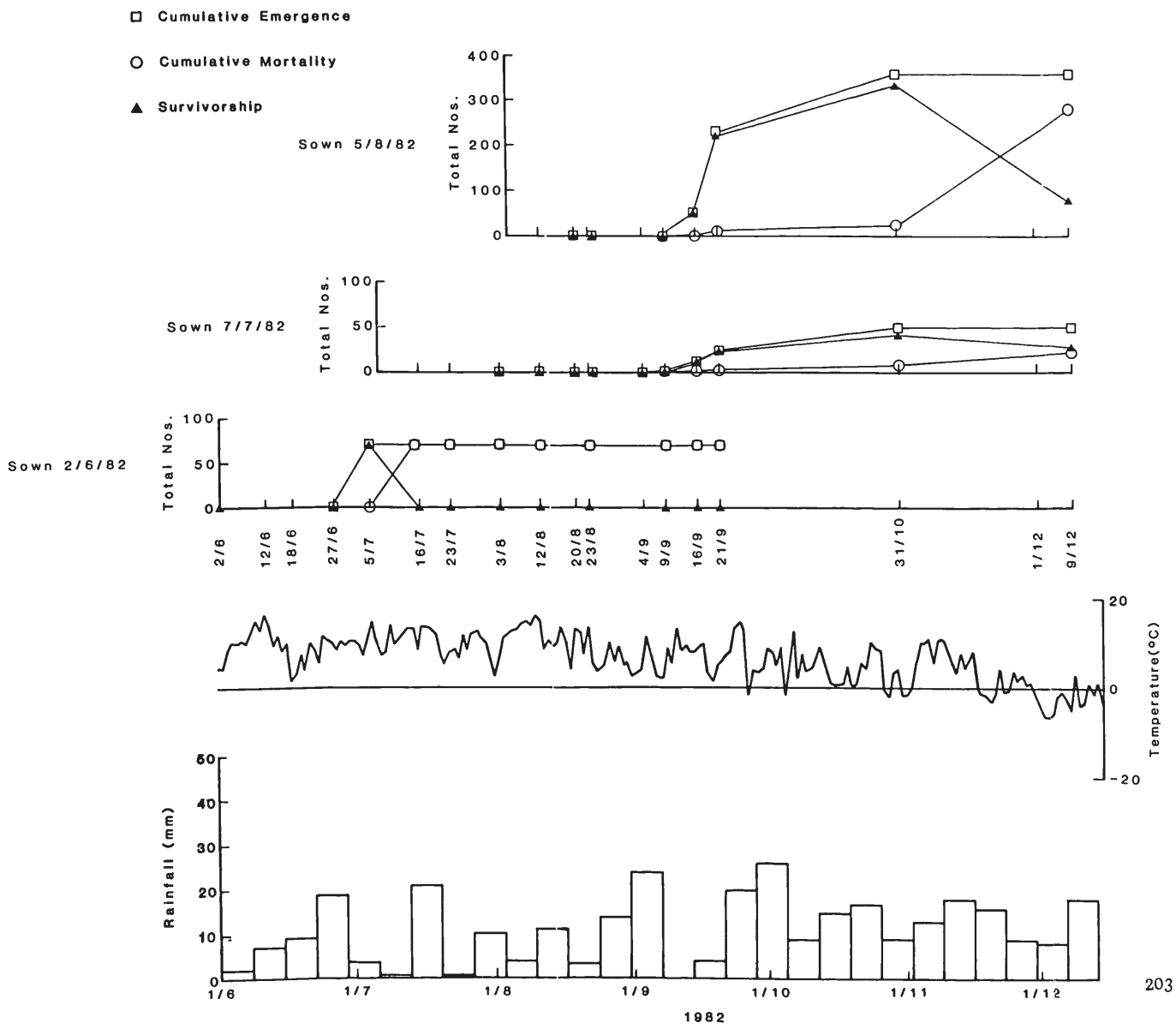


Figure 6.9(e) Seeds sown on 2.6.82, 7.7.82 and 5.8.82.



The results have been grouped as shown in Figures 6.8 and 6.9 to make it easier to compare the results of the different sowing times. A general inspection of all the figures reveals 2 striking features. Firstly, there are only a few periods in the year when seedlings emerge, regardless of the date of seed distribution. Secondly, almost all the seedlings emerge together, with no second flush occurring on any of the plots.

6:4:2 Discussion of the observations at site D.

The first curve in Figure 6.8(a) shows that the seeds sown on site D at the end of August 1981 do not produce seedlings for 3 weeks. Since there was no rain in the first week, it can be assumed that there was insufficient water in the soil for the seeds to become fully imbibed. However, the rain which fell plentifully throughout the rest of September provided adequate water for germination and growth to proceed without further interruption. Within 2 weeks of the first rain about 100 seedlings had emerged, and this continued for a further 2 weeks.

The minimum temperatures throughout this period were over 2°C, so there was no risk of frost damage to young plants. Similarly, there was plenty of rain to ensure conditions remained moist, so the steadily increasing mortality shown in the second curve cannot be explained by the direct effects of either temperature or drought. A possible explanation is that the seedlings were attacked by fungal pathogens, which cause widespread "damping-off" in the seedlings of many species (Augspurger 1983), and particularly the etiolated seedlings of species with small reserves (Grime and Jeffrey 1965). Later in the year the mortality

is more likely to have been caused by the abnormally low temperatures. As the temperature drops, the water in the top soil horizons freezes and thus is unavailable to plants. When this occurs for a prolonged period it creates a physiological drought, which may kill the seedlings even if they can withstand the low temperatures *per se*.

Only about 12% of the seeds scattered over the plots emerged, which is much lower than from seeds sown the following month, shown in the second set of curves. Some of the seeds may have blown off the plots or been removed by animals, but the most likely explanation is that the heavy rain washed a lot of the loose sand down the slope, taking with it many of the seeds. These were buried in the pile of sand which collected against the barrier at the bottom of each plot: most of the seedlings which did emerge subsequently, were on this material. In the following months most of the loose material was removed from the surface of plots before scattering the seeds, to minimise this problem.

The emergence of seedlings on the August-sown plots ended remarkably early when compared with the results from the second set of curves. In these plots, sown a month later, emergence is only just beginning at the time when it has reached a plateau for the earlier seeds. Further emergence on the August plots must be limited by the lack of quiescent seeds: some may have been removed, but either the rest have become dormant or they have died, perhaps having germinated but unable to emerge.

The seeds sown at the end of September produced a similar set of curves to those from the previous month, but they appeared over a much longer period, which reflects the lower temperatures and intermittent rainfall in the following months. There were no

seedlings until the beginning of November, when at times temperatures were starting to fall below freezing point. The rainfall continued to be high for 3 weeks after sowing, but then dropped, until the first week of November when there was no rain at all.

Emergence had started over 1 month after the seeds were sown, despite the plentiful water supply; the metabolic processes responsible for germination and emergence were being slowed by the low temperatures, but not sufficiently to stop them altogether. It was only when the temperatures dropped far below freezing point over long periods in December that the appearance of new seedlings finally stopped. This prolonged season of emergence was unexpected, but fits with the results reported in section 4:4, which demonstrated that although it was slow, germination could still occur at low temperatures provided there was sufficient diurnal fluctuation.

The other notable feature of this set of results is that many of the seedlings appear to be undamaged by frequent exposure to sub-zero temperatures, even during the first 4 weeks after germination, which was shown in section 5:5 to be the most sensitive stage of development. How long this tolerance would have lasted if the temperatures had not fallen steeply is not known. It is possible that the seedlings could have withstood a milder winter than this one, but unlikely that they would have developed to the physiological age at which they are drought-resistant and able to withstand prolonged freezing of the soil water.

As with the previous set of seeds, the low winter temperatures appear to have been the major mortality factor for these seedlings: none survived until the spring.

The first set of curves in Figure 6.8 (b) shows that the seeds sown at the end of October were too late for germination to occur until the following spring. There was still enough rain to support emergence of the second set of seedlings, so the limiting factor was probably not moisture, but temperature. The seeds might have germinated initially, but then been damaged by the low temperatures, which would account for their poor emergence in the following spring.

All of the sets of curves in Figure 6.8 (b) show the same pattern: all the seeds sown between November and the end of January overwintered without emerging, and when they did appear in the following March, only a small proportion of the original seed innoculum produced seedlings. The seeds may have germinated prematurely and then died in the cold weather, or lost their viability as a consequence of prolonged storage as imbibed seeds at low temperatures. The few that did produce seedlings must have overwintered in the resistant dry form. They did not germinate until there had been heavy rain and a rise in temperature.

The seeds sown in October and November started to appear in mid-March, but no seedlings were recorded until April from those plots sown at the end of December and the beginning of February. There is no obvious reason why they should take significantly longer to emerge, unless those sown earlier had undergone part of the germination process before the winter and were effectively "primed" ready to complete germination as soon as conditions became suitable (see section 5:3). Unfortunately there are no records between 24 March and 21 April, so the seedlings recorded on the latter date could have emerged at any time between these visits. If the previous explanation of the emergence dates is correct, then they would be

expected to start appearing soon after the other seedlings in April, before the end of the second period of heavy rainfall.

The small number of seedlings which emerged on these plots suggests that the losses are heavy if the seeds overwinter on the ground, which highlights the advantages of retaining the seeds on the parent plant until spring. As reported in section 3:3, the natural season of seed dispersal is between January and June, reaching a peak from March to May. Thus only the last of the sowing dates in this set of plots represents a typical distribution time. However, having noted that buddleia seed is capable of forming a persistent seed bank, it is possible that following disturbance of the soil, some seeds may be found at the surface during the winter, so the figures are still relevant to field situations.

Having pointed out the costs of overwintering on the ground to the seed population, the curves in Figure 6.8(b) show that there are also benefits. Although the number of seedlings was low, a high proportion of these survived the droughts in May and July and were still alive at the end of 1982. The significance of this will be discussed in conjunction with the rest of the data, in section 6:4:4.

The low mortality reflects the availability of soil moisture over the weeks after the seedling has penetrated the surface. The laboratory studies of water stress, reported in section 5:5, demonstrated that the seedlings do not become drought tolerant until about 4 weeks after germination. Since they are likely to emerge several days after germination in the field, it can be predicted that those seedlings which have grown for at least 3 weeks in moist environmental conditions have developed drought tolerance. The survival curves in Figure 6.8(b) confirm this: over 70 cm of rain

fell during the first 2 weeks of August, which meant that the soil moisture levels were high for at least 2-3 weeks after emergence, and the seedlings which remained alive through the dry weather in late April and early May were those which had appeared in late March and early April.

The results of scattering the seeds during the 3 months March, April and May, when natural dispersal is greatest, are shown in Figure 6.8(c). The seed sown at the beginning of March did not germinate after the heavy rain which fell in 2 periods over the following 6 weeks. This was unexpected, but may be a consequence of the low temperatures, which were still below freezing point on many occasions. The seeds sown in previous months had a long time in which to initiate the metabolic processes of germination, but at these slow metabolic rates, the seeds sown in March perhaps could not complete germination until after the second period of wet weather. If this is so, then without a proper root system the seedlings may not have obtained enough water to emerge and establish themselves before the surface of the soil dried out at the beginning of May. This would also explain the small number of seedlings which eventually appeared in July: the rest of the seeds having unsuccessfully germinated at an earlier date.

As Figure 6.8 shows the seeds sown in April and May did not produce seedlings until after further heavy rain had fallen in the last week of June. The rapid appearance of seedlings, only 7 days after most of the rain fell, indicates that the seeds must have been primed to the point of germination by the rain in May, but that the soil must have dried out so rapidly that development was arrested until there was a further input.

The combined effects of dry weather and higher temperatures

after the beginning of May on the well drained soil at site D led to rapid drying of the soil throughout the summer, so that even when these seedlings did manage to reach the surface, the majority died within 10 days. However, a second short spell of rainfall in the second week of July seems to have provided sufficient moisture for some of the seedlings to stay alive, and after this these seedlings were also able to resist the drought which occurred over the last 2 weeks of July. It is possible that these more tolerant seedlings had been closer to germinating after the rain in May and were therefore able to germinate after the light rain in the middle of June, and thus could progress through the sensitive early weeks after germination when the water supply was plentiful. If this had happened, then the seedlings would have been about 4 weeks old by the time the soil dried out again.

The low total emergence of seedlings from the April seeding is probably also a result of unsuccessful germination earlier in the year. The freshly scattered seeds were subjected to the April rains, initiating germination, which may then have been completed after the rain in May, but in the dry spell that followed the seedlings were probably unable to emerge, and so eventually died.

The final set of curves from the plots at site D (Figure 6.8(d)), record the fate of the seedlings which emerged from seeds sown in the first weeks of June, July and August. The pattern of seedling emergence from the June sowing of seeds is similar to that from the May sowing, except that in the latter they started to appear by the third week in June and then peaked in early July, while the June seedlings did not appear until a week later. The rapid emergence after the heavy June rain again indicates that the seedlings had begun to germinate earlier in June, even though there

was not enough moisture at that stage for them to complete the process.

As with the May seedlings, the majority died within 10 days, but most of those which were still alive in the third week of July persisted until the autumn, surviving the drought at the end of July. Again this suggests that these seedlings had developed a degree of tolerance, although the seedlings could barely have been 3 weeks old when the water supply became limiting. It seems that some of the seedlings were initially better fitted, by either their structure or their micro-environment, to withstand the initial drying of the soil, and were able to maintain sufficient growth and development in this period to become tolerant.

The total number of seedlings was higher from the June sowing than from any month since those sown in September in the previous year. Seedlings had appeared from both sets within 1 month of sowing, and neither had been subjected to the intermittent wetting and drying of the substrate during the period of germination and pre-emergent growth, which occurred in all the other months. It appears that maximum emergence is achieved when these are continuous processes, but it is unlikely that the losses result from damage to the ungerminated seed: they are probably the consequence of dehydration of the newly germinated seedling (Bewley and Black 1983).

The other 2 sets of curves in Figure 6.8(d) show that remarkably few of the seeds sown at the beginning of July and August appeared as seedlings. Such emergence as did occur started in the middle of August in the July sown seeds, with a further small emergence in the middle of September, when the August seeds were also starting to appear. There is no obvious explanation for the

lack of seedlings on these plots. The charts show that over 50 cm of rain fell, in an even spread over the month, and temperatures remained high. It is possible that they were so high that the water in the top few cm of the soil evaporated rapidly after rainfall. While there is a good correlation between the meteorological data and soil moisture availability during the cooler seasons, this may not be so strong in the summer.

This means that the repetitive cycle of wetting and drying may again be responsible for the losses to the seed population: the poor emergence in September, when environmental conditions were suitable, indicates there were few quiescent seeds still present in the plots. The only other possiblities are that the seeds had been removed by predators or had been washed into the soil and become buried, perhaps entering a seed bank. However, neither of these is a satisfactory explanation: with large numbers of native and often larger-seeded species all around on site D, it seems unlikely that any seed eaters would preferentially select the tiny buddleia seeds which were distributed on widely spaced plots. Similarly, there is no reason to suppose that a particularly high proportion of the seeds were washed into the soil in these 2 months when the rain was not so heavy as it had been on earlier occasions.

6:4:3 A comparison of the results from the 2 sites.

Figures 6.9(a-e) represent the data from site E, and show that the overall patterns of emergence and survival are often similar to those at site D, making it unnecessary to go through the results in the same detail. However, the differences between the 2 sites and the results from both are discussed, since they provide further

information on the environmental responses of the seeds and seedlings.

The sites differ in 3 major respects. Firstly, site D is on a slope while site E is level, but the main effect of this was to cause an increase in run-off and soil creep, which were minimised by using the plastic barriers and making sure loose sand was removed.

Secondly, the substrate at site E is much stonier than at site D, as shown in Figure 6.1. This means that the substrates at the 2 sites dry out at different rates and also present different microhabitats for seed germination and growth. The moisture characteristic curve for each substrate was determined, using a soil tensiometer with samples taken from each site, and following the same procedure as described for Molochite, in section 5.5. These curves are shown in Figures 6.10(a and b). There is a striking difference between these 2 curves: the sandy soil at site D holds much more water, a considerable amount of which is held at tensions that make it available for plants, compared with the stony soil at site E, which holds little water that can be used by plants.

Because of the stoniness, site E is likely to provide sheltered microhabitats for the seeds which facilitate germination, but the rapid drainage of the substrate, and the limited water holding capacity, even at field capacity, make it a poor site for seedling growth. Conversely, site D presents fewer suitable microhabitats for germination, since it has fewer stones and a more even texture, but the sandy soil can retain more moisture for the seedlings to use, and so is likely to support more of them over long time spans than at site E.

The third major difference in the sites is in their degree of exposure: site D is naturally quite sheltered by the surrounding

Figure 6.10(a) Moisture characteristic curve for soil from site D.

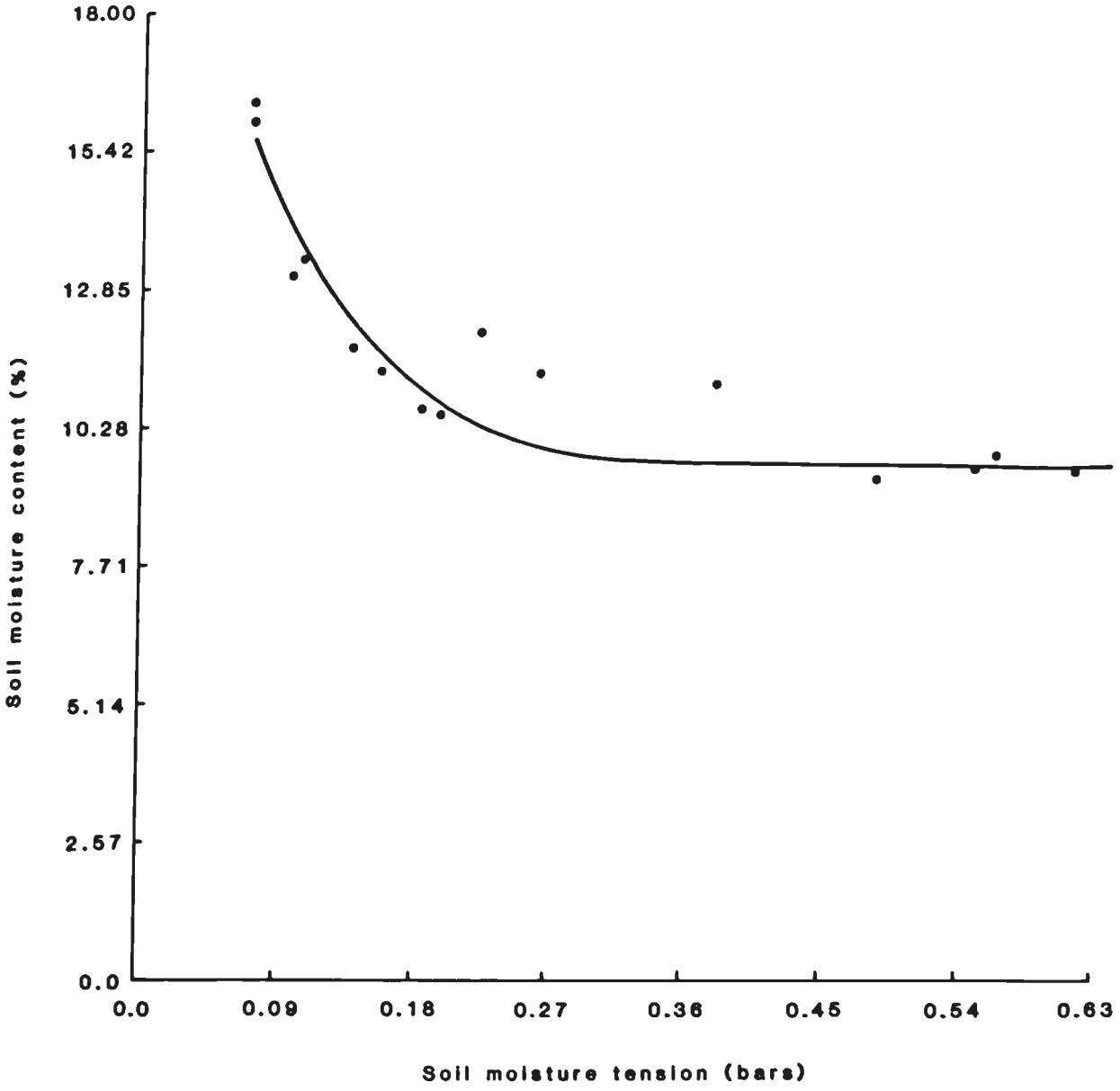
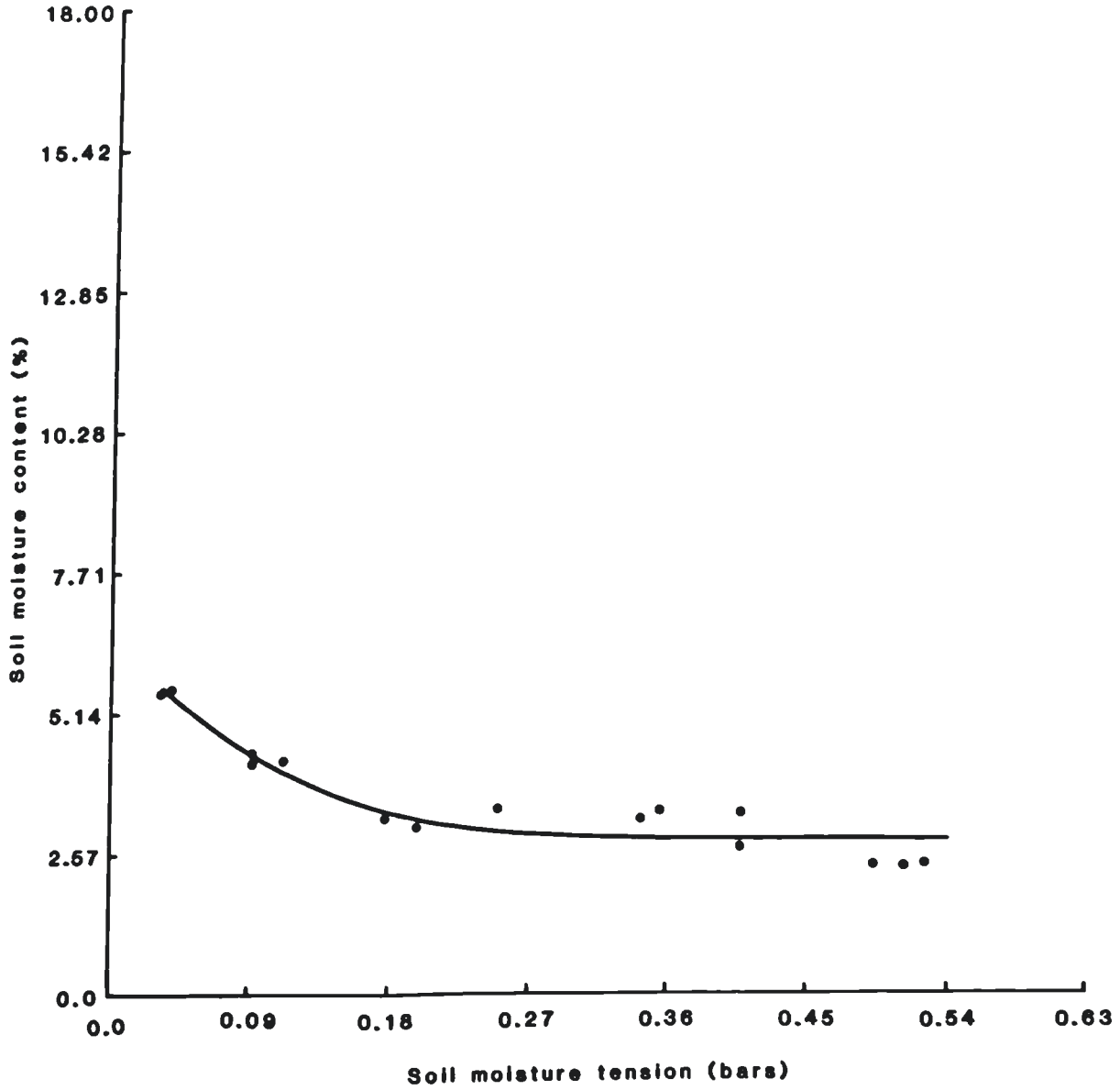


Figure 6.10(b) Moisture characteristic curve for soil from site E.



quarry walls and vegetation, both from high winds and from prolonged, intense sunlight. Site E is situated in the middle of an extensive plain, north of Oxford, and as there is no tall surrounding vegetation or topographical features to shelter it, the site is swept by winds and exposed to the full intensity of the sunlight during the day. This leads to damage, not only from the direct effects of the sun and wind, but also because they increase the evaporation of water from the surface of the site.

So it appears that site E is relatively inhospitable to young plants on account of the low water retention capacity of the soil, compounded by the increased evaporative losses from such an exposed site. However, site E may be expected to support the emergence of greater numbers of seedlings, except at times when the problems of water loss and exposure outweigh the advantages of the stones in providing local shelter, which is likely to be during summer.

As Figure 6.9(a) shows, there is a higher total germination in the autumn at site E: 33% of the seeds sown in September produced seedlings as opposed to 20% from site D, and Figure 6.9(e) shows that with seeds sown the following August, 17.9% produced seedlings, compared with 1.4% of the seeds sown at site D. In total, 1,950 seedlings were recorded at site E, compared with 1,607 from site D. However, most of this difference is accounted for in the autumn. In the spring there is little difference between the total numbers emerging at both sites, as can be seen from Figures 6.8(b) and 6.9(c), and in the summer more seedlings emerge at site D than at E, as shown in Figures 6.8(c) and 6.9(d). These results are consistent with those predicted from the physical characteristics of both sites.

The second difference in the 2 sets of results is in the longevity of the seedlings. All those which emerged before the winter in 1981 were dead by the spring of 1982, at both sites. However, many of the seedlings which emerged in the spring from the plots sown between November and February, were still alive at the end 1982 on site D, but at site E all had died by August 1982.

The difference becomes even more significant when considering the results in Table 6.2, which shows the number of plants which were still alive on the plots on 18 November 1983, including the number which had flowered and produced seeds. None of the seedlings at site E had survived to maturity, but at site D there were 26 plants, of which at least 18 were survivors of the recorded seedlings, including 5 individuals that produced seeds. These results confirm the hypothesis that site E was not a suitable site to support young plants. As Figures 6.8(a and b) and 6.9(c and d) show, when there was no rainfall to replenish the soil moisture, even those plants which were old enough to be drought tolerant died at site E, while the same aged plants stayed alive at site D, under similar weather conditions.

Finally, before concluding this comparison of the sites, the effects of the sloping site must be briefly mentioned. The problems were highlighted by the results of the August 1981 sowing at site D: the poor seedling emergence on these plots has been previously related to the slope (section 6:4:2). If the seeds were not firmly lodged in a suitable microsite then they were easily washed away, even though in all other respects the site was suitable for germination. The new site in which the seed lands may not be so suitable, particularly in the case of a small seeded species such as buddleia, which is likely to be buried and unable to penetrate to

Table 6.2 Seedlings and established plants still alive at sites D and E on 18.11.83

Date of sowing	Site D	Site E
31.8.81	1 seedling	0
30.9.81	0	0
30.10.81	0	0
30.11.81	2 seedlings 4 medium 1 established	0
28.12.81	3 medium 2 established	0
3.2.82	1 seedling 3 medium 2 established	0
5.3.82	0	0
2.4.82	1 seedling	0
4.5.82	0	0
2.6.82	3 seedlings 3 medium	0
7.7.82	0	0
5.8.82	0	0

Seedling = stem less than 5 cm tall, no lateral shoots.

Medium = stem 5-15 cm tall, with lateral shoots.

Established = stem over 15 cm tall, many lateral branches, had flowered and produced seed (average number of 5,000 per infructescence).

the surface of the soil. So it is vital that the seeds are lodged in some crevice or crack on a sloping surface, more so than on a flat site.

6:4:4 Conclusions.

The appearance of buddleia seedlings is highly synchronised in the field, despite the prolonged period over which seeds are shed and the possibility that some may persist in a soil seed bank. This demonstrates that there are remarkably few occasions in the year when environmental conditions are suitable for successful germination and seedling emergence. At the 2 sites used, both thought to be typical of the well-drained and stony habitats often colonised by buddleia, it appeared that although some germination probably occurred after a series of small but frequent showers, it required an input of at least 20 cm of rain over a short period to produce large numbers of seedlings. In addition, further rain was needed over the following weeks to maintain sufficient soil moisture for these seedlings to become established and to develop drought tolerance.

The maintenance of an adequate water supply becomes a real problem in the summer, when the warmer weather causes rapid evaporation, and the competition for water is considerable, due to the increased water demands of many other plants. In fact this competition for limited moisture may partly account for the absence of buddleia seedlings from sites with mature vegetation, even where there are gaps, such as under the adult shrubs.

In autumn and spring the water supply does not present such a problem since there is more rain and less evaporative or

transpirational loss. However, in winter freezing inhibits germination and kills many seedlings, particularly those which germinated in the second half of the year. This could be a direct effect of the low temperatures on the metabolism of the seeds and seedlings, or perhaps due to physiological drought imposed by freezing of soil water.

It is apparent from Table 6.2 that seedlings which become established, and can survive the water and temperature stresses of both summer and winter, are those which emerge in the spring, particularly in March and the first half of April. Some of the plants which appeared at site D at this time were able to produce seeds in the following year; the mean production from each flowering individual was 6,430 seeds: not many by comparison with a mature adult, but quite a large contribution to the seed population from an 18 month old shrub.

The other observation to be made from these results is that in predicting the fitness of an individual plant, the quantity of seed production is not so important as knowing what sort of habitats the majority of the seeds are likely to encounter and when they are dispersed. Since these results represent the survival of seeds which have been artificially introduced to a suitable site for colonisation it would be pointless to use them to construct a life table. How many seeds would be required to provide a similar density of seed rain on such a habitat depends on the location of the adult plant. The seed output from the young plants in this study may be sufficient to produce further individuals on this site, while a plant with a seed output of several million may not produce viable offspring if it happens to be in an unsuitable situation for the seeds to reach safe sites.

For a small-seeded colonising species the probability of some seeds dispersing over long distances and thus encountering suitable sites despite the original position of the parent, increases with greater numbers of offspring. But when the species is found in areas that are capable of supporting more individuals, which is obviously the case in many of the disturbed sites colonised by buddleia, then fitness is not so dependent on the spatial aspects of seed dispersal as on its timing.

The problems of fulfilling the environmental requirements over the late spring and summer when most of the seeds are dispersed are obvious in many of the sites where buddleia grows. It is probable that the correct combination of moisture and warmth will be infrequent: there may be no seedling establishment for many years at a particular site and then one season the conditions may be correct, leading to a flush of seedling emergence. This certainly fits with many anecdotal observations of the regeneration of the plant in Britain.

CHAPTER 7: DISCUSSION OF RESULTS IN RELATION TO THE ECOLOGY AND DISTRIBUTION OF BUDDLEIA IN BRITAIN.

7:1 The ecological role of external factors.

Buddleia has been shown to be capable of establishing itself on many different substrates: although not an obligate calcicole, it can grow in highly calcareous situations without suffering from obvious lime-induced iron chlorosis or lack of availability of other elements. It does not show a preference for either nutrient rich or poor sites, as shown by the cation exchange capacity and potassium content of soils collected from beneath naturalised plants.

The seedlings are generally found on well-drained sites that have a frequent supply of water, needed to support the initial growth of seedlings, since the established shrub is capable of withstanding prolonged drought. As reported in sections 4:8 and 4:9, the absence of self-established buddleia on poorly drained and acidic soils is not directly due to the pH of the substrate, or to the low oxygen tensions associated with such sites. However, further work may show that it is a consequence of toxic levels of aluminium or ferrous ions, associated with low pH or of poor seedling survival resulting from enhanced fungal attack under these conditions.

If the seed becomes buried then it is unlikely to produce a seedling from below 0.5 cm of soil, even when the water supply is plentiful. However, the seeds can become part of a seed bank, persisting for up to 40 months in the soil before they lose their viability (section 3:4), although the majority probably disappear over a much shorter period, their numbers depleted by soil fauna. Between March and September more than half the seeds from the

experimental seed bank were removed by animal activities. Invertebrate actions on the soil surface are probably equally important in the ecology of the seeds, and are bound to account for a proportion of the seeds entering the seed bank. This is not always advantageous: they might be buried when conditions at the surface are suitable for germination. However, in a frequently disturbed soil it may benefit the plant by extending the season over which there are viable seeds on the surface with a capacity for germination.

Many species with autumn ripening seeds develop a chilling requirement so that the seeds remain dormant until spring, avoiding the risks of overwintering as vulnerable seedlings. However, since buddleia seeds are not shed until spring, there is no need for a temperature sensitive trigger. Besides keeping the seed comparatively safe in the protected environment of a seed case high in the air, this over-wintering strategy has other advantages for the species. Seeds are distributed at a time when they are most likely to encounter suitable conditions for effective dispersal and germination. If they were shed in autumn on the river banks where they naturally occur, there would be a considerable risk of the seeds washing away before they germinated on a suitable site for establishment. Since the leaves are shed in autumn and often form a thick mat which persists for long periods on the surface, it is better that the seeds are not dispersed until spring. In this way there is no risk of them being buried beneath the litter, which they might not be able to penetrate.

The cost of seed retention on the plant comes from the increased losses due to bird predation. In the severe winter of 1981-1982 many of the infructescences on the plants at Dry Sandford were attacked, and the majority of the seeds removed. When food is scarce and snow covers the ground, such small seeds borne aloft are obviously more

attractive than is usually the case. In milder years the bird damage did not appear to be extensive at this or any of the other study sites.

Of the 4 major requirements for germination and seedling growth, buddleia naturally occurs in an environment in which sufficient light and air should be available for the seedling, and since the seed is not shed until spring, the ground temperatures should not present a problem (see sections 4:4 and 6:4). It is only water that normally limits seedling survival in this habitat and once this is adequately supplied there is no reason why germination should be restricted. Consequently, there are no environmental cues except for a prolonged imbibition requirement to achieve germination. It appears that buddleia has evolved this as a means of synchronising germination with the predictable seasonal increase in water supply, from rainfall or from rising river water levels. Once the levels have risen sufficiently for the seed to become fully imbibed for 4-6 days, then the probability of the water supply remaining plentiful is high in this environment.

The moisture availability is not necessarily so predictable in other habitats invaded by buddleia. Here the absence of environmental cues other then water supply may prove fatal for the seedlings. Bearing in mind that seeds can be "primed", they may finally germinate in conditions unfavourable for survival, for example, in mid-summer, when the water rapidly evaporates from the surface horizons of the soil, particularly in exposed and well-drained situations. The field data on seedling emergence and survival at sites D and E, reported in section 6:4, demonstrate this point. Only those plants which germinated in early spring were sufficiently advanced in their development to withstand the periodic summer droughts at both sites.

Those which emerged after a small amount of rainfall in the summer, died within a few days of emergence unless this was followed by rain soon afterwards.

Conversely, in urban situations buddleia can benefit from this lack of seasonality in germination. The sheltered street environment has a rather higher temperature than in the surrounding countryside, which can prolong the period over which seeds can germinate. Since emergence was still recorded on site E in December (section 6:4:3), in towns it can probably also occur earlier in spring. Early emergence is doubly advantageous, in enabling the plant to become established before other competitive species appear, and in allowing longer to develop an extensive root system and drought tolerance before it is subjected to summer water stress. The importance of this was particularly clear with the seedlings that appeared in March and early April on the experimental plots. They subsequently demonstrated high survival over the summer, but the periodic drought proved lethal for seedlings which had emerged later (section 6:4:2). Around drainpipes and gutterings and at the base of walls, not only is the supply of water frequent, but the volume is enhanced by the run-off from surrounding roofs and pavements, making the urban situation an even more attractive site for colonisation.

It might have been expected that buddleia would show some degree of photosensitivity, since a light requirement is often found in small-seeded species which form seed banks, presumably because it prevents them from germinating at depths from which they cannot emerge (Mayer and Poljakoff-Mayber 1982), but the seeds were capable of germinating equally well in dark or light conditions (section 4:5). In the absence of any other explanation of the persistence of buddleia seeds in the soil, it is likely that secondary dormancy is induced by

burial, either by darkness or by increased carbon dioxide levels in the soil. This has been demonstrated for many other species, including Senecio vulgaris (Compositae) and Capsella bursa-pastoris (Cruciferae) (Popay and Roberts 1970). Less than 1% of incident light penetrates beyond 2.2 mm in clay, loam or sand (Woolley and Stoller 1978), so dark induced dormancy could be imposed within the top few millimetres, and will become deeper as the seed drops further into the soil (Wesson and Waring 1968).

The depth of seed burial has been demonstrated as a critical factor in seedling emergence, together with the microtopography. Germination and initial survival were enhanced at site E by the shelter provided by the surface mulch of stones (section 6:4:3). This is linked to the observation that in gardens containing a mature buddleia, seedlings rarely appear except on stony ground such as that shown in Figures 7.1 and 7.2, and especially in cracks and crevices on pathways. Periodic soil disturbance of the kind carried out by gardeners produces a flush of seedlings of many weed species, but not of buddleia, unless the soil is particularly stony and well-drained. Possibly they are more susceptible to fungal attacks or to rapid deterioration in seed viability in the damper environment of a loam soil. The stony soil is not only better aerated and drained, but also provides locally sheltered microenvironments in which atmospheric humidity builds-up, preventing desiccation which is one of the chief causes of mortality. An irrigated gravel thus provides the long term imbibition period and, in surface crevices, does not demand much shoot extension without light. All of this is reminiscent of the river banks to which buddleia is native, and indicates the extent to which it has evolved to exploit this habitat.

In cultivated situations there are often extensive areas of



Figure 7.1 A dense population of buddleia seedlings growing in the shelter of stones on a patch of waste ground.



Figure 7.2 Stony soil, particularly at the base of a wall, provides a suitable site for large numbers of buddleia seedlings to become established.

bare soil which are free of competition for seedlings that emerge there. The absence of self-set buddleia suggests that it is not simply poor competitive ability that prevents the plant from colonising other habitats in Britain, but some prerequisite for germination or emergence that is not met in most of these areas. This argument is also supported by looking at the plant growing among other species: Figure 7.3 shows a number of buddleia seedlings growing in a tightly-packed community, on sandy soil. Figure 7.4 also shows some new seedlings, which had recently germinated on top of the moss in this community. The communities were actually maintained artificially by frequent watering, but this was the only alteration to the natural situation. This indicates once again that water is the limiting factor for successful buddleia establishment. There is no indication that buddleia is overwhelmed by competition for resources other than water. It is probably the severity of competition for water with established plants which prevents buddleia from continuing to colonise a site once the vegetation becomes mature and gaps start to close.

7:2 Suggested reasons for the patterns of growth observed in the field.

The absence of a clear range of age groups in natural populations has been referred to several times, but so far no adequate explanation of this phenomenon has been proposed. So this is a suitable point to consider other possible reasons for this observation.

The mechanical resistance provided by a clay soil may have been important in limiting the recolonisation of the cleared sites at Slindon, which were based on clay, as reported in section 2:3. The only vegetation on these sites was a regrowth of woody plants,



Figure 7.3 Buddleia seedlings (left side of picture) growing amongst dense vegetation, where the water supply was not limiting.



Figure 7.4 Newly emerged buddleia seedlings (bottom right of picture) growing on moss, in dense vegetation, where the water supply was not limiting.

including buddleia, from old roots. There were considerable tracts of bare ground, but the surface was typically massive, and no seedlings of any species were observed growing on it. The occurrence of seedling buddleia in the adjacent woodland, where it formed the dominant understorey species, indicated that surface compaction following clearance is a more likely explanation then root competition for the failure to colonise anew. The soil in the wood was also massive clay, but with large fissures in it, and it was in these the buddleia seeds had lodged and established seedlings.

There are also problems associated with lighter soils. Heavy rain on an open surface, clear of protective vegetation, causes the breakdown of the weak soil peds at the surface. The disturbed soil is then sedimented, creating a cap of closely-packed particles (Mayer and Poljakoff-Mayber 1983). This seals the surface making it difficult for fresh seeds to lodge on it and for seeds which were already in the soil to penetrate it. The deeper the seed is buried the less force it can exert without the hypocotyl buckling, and the more it has to invest in shoot growth. Having demonstrated in section 4:10 that the critical depth of burial is within the top cm of soil, cap formation will greatly increase the problems of emergence for a seed with such small food reserves, and few are likely to emerge in this situation.

The above comments suggest that the apparently even age structure of many stands of buddleia is a result of similar events to those described above rendering a surface impenetrable to seedlings, or creating a lack of suitable places for seeds to lodge. Initially, conditions for recruitment may have varied, so that in some years no seedlings became established, while in others colonisation was good, leading to age uniformity. In the longer term, physical weathering and the growth of lichens and mosses over sites which

previously supported germination, may have made the surface unsuitable for further colonisation.

A single population may also show the variation in density and survivorship which reflects changes in environmental conditions during seedling recruitment. Where conditions were good, the survivors formed a dense stand, but with smaller individuals than those which survived in a poor year for recruitment.

The other striking features of the populations at the Slindon gravel pit were the densities of young plants, and the failure to fit the mature plant data to Yoda's -3/2 Power Law, which implied that plants were not subjected to density-dependent mortality, despite the regularity of their pattern. This may be a function of the plasticity of these shrubs and of their stress-tolerant strategy, but there is another feature which may be important in explaining the apparent lack of self-thinning in such a dense population.

On inspecting the roots of a number of mature buddleia plants it was apparent that in several cases the roots of different seedlings had become grafted together. The root structure in these instances was conspicuously different from the regeneration which occurred from damaged old root stocks, as described in section 2:4. Grafting was particularly prevalent in instances where several small plants were growing in close proximity. Thus some of the large plants recorded as a single individual may have originally been several seedlings, which subsequently grew together and effectively became one plant. In this instance the Power Law should still fit the data, since it is immaterial whether several plants finally became reduced to one individual by the death of the rest or by their fusion. However, if a small plant became root-grafted to a large individual some distance away, they would have been recorded as separate plants,

although they may be sharing resouces and so function as one individual. Thus the smaller plants could stay alive, in contravention of the usual rules of self-thinning in dense stands, and disrupting the analysis of the data by the -3/2 Power Law.

The situation presents a similar problem to that found in plants with clonal growth: how to define what constitutes a functional individual. The regularity of the distribution pattern of mature shrubs still indicates that these plants are in competition for a limited resouce, probably water, but the "individuals" concerned in this competition may be single large plants, or a "cooperative" of smaller plants, grafted together by their roots. Without records of the extent to which this phenomenon actually occurs in the field or of the degree of resource sharing that it facilitates, this can be no more than a speculative explanation of the irregularities in the relationship between size and density in this mature stand.

7:3 A comparison with demographic studies of other shrub species.

Few demographic studies have been carried out on shrubs, because they are neither sufficiently short-lived to provide convenient material for the population ecologist, nor of enough economic importance to attract the attention of foresters or agronomists (Silvertown 1982). Additional deterrents are the problems of determining the age of a shrub, as discussed in the previous section, and of defining what constitutes an individual when the plant may be capable of clonal growth or of repeated regrowth from below ground.

When Harper published "The Population Biology of Plants" (1977), he noted only three detailed studies of the population dynamics of

shrubs: a study of Larrea tridentata (Zygophyllaceae), in Arizona (Chew and Chew 1965), Vaccinium myrtillus (Ericaceae), in Sweden (Flower-Ellis 1971), and Symphoricarpos occidentalis (Caprifoliaceae), in Minnesota (Pelton 1953). Since then there has been a small surge of shrub research. Silvertown (1982) records several further studies: Acacia burkitti (Leguminosae) (Crisp and Lange 1976), Artemisia tripartita (Compositae) (West, Rea and Harniss 1979) Sarothamnus scoparius(Leguminosae) (Waloff and Richards 1977). Most of the other recent studies have also been of arid land shrubs.

All of the populations of shrubs so far investigated have been shown to have complicated age structures, but fitting the Deevey Type III survivorship curve, indicating high initial seedling mortality which then levels out, as the probability of mortality decreases with age. Few of the established individuals die until they reach the end of their physiological life span. At first glance it may appear that most plants will fit this pattern, but if they are divided into annuals, monocarpic perennials and polycarpic perennials, then the difference becomes clearer. Annuals generally fit a Deevey Type I curve, monocarpic perennials are typically Deevey Type II, and polycarpic perennials, are usually Deevey Type III.

The problems encountered by a shrub growing in an arid environment are different from those faced by a shrub which has evolved in a situation where water is not normally limiting, at least in the early stages of development. West, Rea and Harniss (1979), worked on sagebrush-grass communities in Idaho and looked at the survivorship curve of Artemisia tripartita (Compositae), a shrub which recruits from seedlings, as buddleia does. They also investigated these populations to see if seedlings contributed

steadily to the population, or of they occurred in irregular "pulses". They found that surviorship of this shrub fitted a Deevey Type III curve; a similar curve was obtained for buddleia on the experimental site D, reported in section 6:3, with initially high losses, but seedling survival levelling out by the end of the second year, when seed production began.

The most interesting comparison comes from the occurrence of periodic "pulses" of seedling production, which had also been found by other workers for different species (Noy-Meir 1973, Chew and Chew 1965). These pulses were triggered by a combination of favourable weather conditions for seed formation, germination and seedling survival. Such pulses and their causes are comparable with the annual fluctuations in buddleia seedlings described in the preceding section.

Furthermore, there was no clear relationship between size of individuals and age in Artemesia tripartita: the plants in this community were highly plastic and showed considerable variation in both density and basal area of individuals recruited in different years. This is remarkably similar to the results of the surveys of buddleia at Slindon, in which the density and size of individuals in a newly recruited population were shown to vary from year to year, in response to the environmental conditions at each site that was colonised. The plasticity of the age-size relationship enabled plants in a single stand to grow to the same size and hence appear even-aged, although actually of different ages.

The conclusion from both of these studies has to be that simple age-based demographic analysis, as originally developed by animal ecologists, cannot be directly applied to shrubs. In addition to the age data, information about size classes is required, together with an accurate definition of what constitutes an "individual", to produce

a useful demographic model.

7:4 The growth strategy adopted by buddleia.

It has been proposed by Grime (1979) that flowering plants are divided into 3 groups, competitors, stress tolerators, and ruderals. He divides these on the basis of their response to stress and to disturbance, where stress is the restriction of photosynthetic production by shortages of physical factors, such as light and heat, and disturbance is the destruction of aerial plant material as a result of biotic and abiotic factors. The responses of the three groups are:

a). Competitors exploit conditions of low stress and low disturbance.

b). Stress-tolerators exploit conditions of high stress and low disturbance.

c). Ruderals exploit conditions of low stress and high disturbance.

It should be noted that ruderals are generally distinguished by a short life cycle: production of seed is soon followed by death of the parent, which is often not the case with the 2 other categories.

As a shrub, buddleia can be either a stress-tolerator or a competitor, and its shoot and leaf morphology do not enable any further distinction to be made: the leaves have the robust form typical of competitors during the summer, but they are small, leathery structures in the winter, which are typical of a stress tolerant species. The longevity of the leaves indicates that the plant is stress tolerant, since when theyare abscised they are immediately replaced by a smaller flush of leaves which remain on the shrub until the following autumn. In fact the new leaves are initiated before the old ones have been shed; although this is not the same as a total

evergreen, it indicates that nutrients are being retranslocated within the plant, an adaptation to conserve nutrients while reducing water loss over the winter. It is also more than simply maintaining some photosynthetic activity over winter, which other deciduous shrubs such as Vaccinium myrtillus and Euonymus europaeus (Celastraceae) do by the possession of evergreen stems (Perry 1971). On the other hand, buddleia has the competitive strategy of peak leaf production in the spring, which coincides with the maximum period of growth.

The flowering frequency and regenerative strategy of numerous small wind-dispersed seeds are typical of a competitive species, as is the ability to bank some seeds.

As a seedling the physiology of buddleia seems to fit with a stress tolerator, with slow growth rates, small responses to stress, and a well developed acclimation to seasonal changes in temperature and moisture supply, after the seedling is 4 weeks old. Figure 7.5 shows a seedling growing in an exposed situation on a sandstone cliff. At the time it was photographed it was at least 2 years old and growing very slowly, its roots forcing their way along lines of weakness in the rock. Nevertheless, it was capable of withstanding winter temperatures below -20°C, which were lethal to neighbouring mature buddleia plants.

As an adult the growth rate increases dramatically, the enhancement of photosynthesis in late spring coincides with peak productivity and the nutrients and photosynthate are incorporated rapidly into the structure. However, buddleia still remains strongly acclimated to seasonal changes in external conditions, and as mentioned above, the new flush of small leaves enables it to conserve its nutrient resources, during the stressful period of the winter.



Figure 7.5 Buddleia seedling growing on bare rock, on a cliff face at Dry Sandford, Oxon.

So it appears that in its established phase buddleia can be classified as a stress-tolerant competitor, although between the seedling stage and establishment it behaves more like stress-tolerator. This supports Grime's hypothesis, mentioned in section 1:8, that many trees and shrubs of unproductive habitats exhibit particular attributes which fall between the two extremes and so fit into the overall catagory of stress-tolerant competitor.

There are 5 major regenerative strategies proposed by Grime (1979), which are vegetative expansion, seasonal regeneration into vegetation gaps, possession of a persistent seed bank, the production of numerous wind-dispersed seeds and the formation of a bank of persistent seedlings. Many plants adopt several of these strategies to ensure successful regeneration in a variey of habitats, and which one is predominant depends on environmental circumstances. Each of these catagories can be considered in relation to the information collected about the regeneration of buddleia.

The ease with which gardeners propagate buddleia from cuttings indicates that it can reproduce vegetatively, and in the field there are occasional examples of broken boughs developing roots. However, this phenomenon is not sufficiently widespread to class it as vegetative expansion. This form of regeneration is characteristic of species found on undisturbed land, including several shrubs such as Hippophae rhamnoides and Prunus spinosa, but has little value for a species like buddleia which often colonises highly disturbed and heterogenous sites.

The riverine habitat in which buddleia naturally grows is subjected to seasonably predictable flooding and drought, but the adult plant is capable of withstanding these fluctuations and persisting perennially, so there are not necessarily seasonally

predictable gaps in the vegetation. The release and germination of seeds is cued by factors which are seasonally influenced, as shown in sections 3:5 and 6:5, but although this often leads to synchronous emergence, this may occur many times in one year, so it is not strictly seasonal, in the sense implied by Grime. The ecological consequence of this is that buddleia can colonise different areas at various times of the year, not just sites where the disturbance is seasonally predictable.

An interesting ecological comparison is provided by the regenerative strategy of Fraxinus excelsior, the ash. It also retains its seeds on the parent for a prolonged period, but germination is seasonally cued by a chilling requirement, so that masses of seedlings appear in early spring. This gives it a competitive advantage in herbaceous vegetation, that is increased by its large food reserve. Since buddleia does not grow in densely vegetated areas it has no need to invest in large seeds, channelling its resources instead into producing a multitude of small seeds which are released over a prolonged period, thus increasing its potential for dispersal.

Trees and shrubs which have large persistent seed banks are characteristic of sites that are subject to frequent disturbance, for example areas which are periodically swept by fire or cleared by man. However, in undisturbed forest areas tree species have banks of persistent seedlings instead of seeds. Presumably in these more stable and productive environments it is advantageous for new plants to have emerged, ready to grow as soon as a gap appears, since competition will be more intense.

Buddleia produces vast numbers of wind-dispersed seeds, which is typical of the fugitive species that occupy landscapes subject to

large scale and erratic disturbance, for example many species of the Compositae, and of the genera Asplenium, Epilobium and Salix. The propagules will be widely dispersed and are likely to encounter widely spaced and often innaccessible gaps. Another species which employs a similar regenerative strategy to buddleia is Tussilago farfara, coltsfoot. This perennial also releases its seeds in the spring, and they rapidly germinate under light or dark conditions, over a wide range of temperatures. It is another riverine species and this strategy results in rapid colonisation of gaps left after seasonal flooding in spring, but unlike buddleia the seeds rapidly lose their viability once shed. Despite this it is still a successful weed in Britain, while buddleia is not (Grime 1979).

In the introduction it was stated that buddleia is a pioneer species, and one plant invading a suitable site is capable of reproducing widely, forming dense thickets of evenly-sized plants. The absence of seedlings beneath the adults reflects the growth form of the plant: in a dense stand gaps will not appear as they would do among forest trees. It is unlikely that the whole shrub will die at once: if 1 or 2 stems break off it will regenerate from the base. Furthermore, since the shrubs can grow at high densities (see section 2:6), it is reasonable to expect that if 1 plant dies then the neighbouring individuals will rapidly expand laterally to fill the space, rather than leaving a gap in the canopy. Consequently, there is no advantage in buddleia seedlings forming a bank of persistant seedlings beneath the parents. It is better to disperse the seed as widely as possible, to increase the probability of locating fresh sites.

Those seeds which fall under the parent can persist in the soil for some time, so that in the event of some sudden catastrophe

overtaking the adult population, regeneration at the site is still possible. Since it is a long-lived perennial and releases large numbers of seeds each year this bank will be regularly replenished, so the seeds do not need to remain viable for many years. Similarly, as a pioneer plant it will ultimately be replaced by later successional species unless the habitat is severely disturbed, so there is no advantage of maintaining a long-term seed bank once the adults have disappeared.

Thus buddleia appears to possess 2 main regenerative strategies, which to some extent are contrasting. As Grime (1979) points out, the main function of a soil seed bank is to permit regeneration in situ, while production of many small seeds is an adaptation to increase dispersal from the origin. In Britain the former strategy is rarely required, except on sites which are frequently redisturbed, such as on a quarry. Most of the regeneration results from the colonisation of new sites by highly mobile seeds.

A further consequence of this is that the population of buddleia growing on a particular site will generally have arisen from a single source, except in situations where there are many cultivated buddleias in a small area, all acting as potential sources of seed, The vast production of seeds over a prolonged period indicates that few will ever become established, so the probability of seed from 2 or more shrubs arriving on safe sites in the same area is low. A plant which is mass flowering is unlikely to have much outcrossing, as discussed in section 3:2, so most of the seeds will be genetically similar. Thus from one original colonist a number of sibs frequently appear in the immediate vicinity, possessing very similar genes. Most of the apparent variation is a consequence of the parent being heterozygous for certain genes, and so giving rise to a proportion of

homozygotes, in addition to the spontaneous mutations which will arise among large numbers of seeds.

The long-term consequences of this small gene pool will be similar to those which apply to clonally reproducing individuals, that is, in the event of an attack by insects or disease, there is little variation in the range of plant defences to be overcome, and the outcome of a successful attack may be catastrophic for the population, as has recently been the case in Britain with Dutch elm disease.

7:5 Conclusions and predictions of future spread in Britain.

Until the end of the last century Chamaenerion angustifolium was regarded as a rare British plant (Fitter 1947). This would indicate that the habitat in which it grew was also rare, and consequently that few native species were specifically evolved to live there. The population explosion which followed the World War II occurred when suitable habitats suddenly became widespread, and in the absence of native colonists, introduced species which were often better adapted to this novel environment, were able to spread very rapidly. C. angustifolium, which is referred to in Clapham, Tutin and Warburg (1965) as a native, had evolved to grow in situations which had recently been burnt, so providing a flush of nutrients. Another species mentioned in Chapter 1, Senecio squalidus, which was introduced from the slopes of Mount Vesuvius, also evolved to take advantage of post-fire conditions and spread in a similar pattern. Buddleia, which is found on similar sites, is mainly associated with the areas that have a plentiful water supply rather than of nutrients, as in Figure 7.6, where they are pictured growing along the line of an old drainpipe. Thus direct competition for



Figure 7.6 A line of flourishing buddleias, growing along the guttering and drainpipe of an old Bristol building.

resources within sites is avoided.

An interesting comparison may be made with a fourth plant, Matricaria matricarioides (Compositae), another inhabitant of urban wasteland and pavements, which has also spread spectacularly since its introduction at the end of the last century. A robust, prostrate plant, it is tolerant of trampling and so able to exploit a different microhabitat on wasteland sites to the other 3 species. Unlike the others, it is not wind-dispersed, but its seeds are distributed in mud, clinging to feet and wheels, which accounts for its rapid and universal spread. However, the most notable difference between this plant and the others is that it is not confined to urban wasteland, but has invaded rural areas, appearing on footpaths, gateways and other highly trampled places on agricultural land.

It appears that plants which have been able to colonise the recently expanded habitats offered by urban dereliction are unlikely to spread much beyond these sites unless they have specific adaptations which allow them to grow in other situations, under conditions that few endemic species can tolerate.

Buddleia, with its remarkable tolerance of high calcium levels, and like many shrubs, its ability as an adult to withstand considerable water stress (contrary to its classification as a mesophyte by Segal (1965)), has been able to colonise old walls and coastal sites, as well as disused quarry and extraction sites, Figure 7.7 shows an example of a buddleia which has even managed to establish itself on top of an old pile of gravel, rather than at the base. It seems likely that this individual germinated elsewhere, but was accidentally relocated along with the gravel by the extractors, and has since flourished in this unlikely situation. The speed with which buddleia generally spreads locally when introduced



Figure 7.7 Self-established buddleia, growing on top of an old gravel heap, in Sussex.

to newly-created sites reflects its vast seed production and the efficacy of wind dispersal.

Returning to the comparison with elder first mentioned in Chapter 1, it appears that although the 2 species can occur together, most of the sites on which they do so are near the edge of the limits of one or other. Elder is essentially a woodland and hedgerow shrub, equipped with a bigger seed and a different dispersion pattern to grow in these more fertile, competitive situations. Buddleia, with its prodigious output of tiny seeds is not equipped for such competitive habitats, but instead is able to withstand the stresses imposed by life in urban and mural environments. Both species will occasionally invade the typical habitat of the other, but their niches do not overlap to the extent that might originally be expected from a survey of established plants in towns. Consequently, the competitive interactions are reduced and both plants can flourish in Britain.

The mechanisms which enable such a large plant to flourish on exposed situations are interesting. Mycorrhizal associations are probably responsible for its ability to obtain sufficient nutrients to support growth on the extreme substrate that the wall provides. However, the roots themselves must be very extensive and grow at many angles, to provide sufficient anchorage for the shoot in the windy conditions which frequently occur around the roofs and guttering. Further investigations are required to show exactly what peculiarities of root morphology and physiology are responsible for the plant's success in these unlikely situations.

Nevertheless, there is nothing in the ecology of the seed or seedling to suggest buddleia is capable of extensively colonising further different habitats in Britain. However, changes in its distribution are likely, as a result of further urban development,

more quarrying activities, and a consequence of its wider distribution increasing the probability of seeds encountering suitable old walls for establishment. There may even be periodic local population explosions as a result of local developments. Over the next few decades buddleia will probably follow the same pattern of progress that it has shown since 1940, continuing to move into those areas which have previously lacked a suitable source of seed innoculum.

By the end of this century the I.T.E. 10 km square map of buddleia in Britain will probably resemble that for another introduced mural plant, Cymbalaria muralis (Scrophulariaceae), which is so widespread, though largely confined to old walls, that it is often mistaken for a native species. Buddleia, which is already naturalised on rocks and cliff-faces, in woodland and on sand dunes, as well as on old walls, must have an even better chance of passing as a native of Britain in the next century!

The impact of this spectacular and long-lived plant should be considered by planners and conservationist alike. It is already used by quarry companies to provide cheap, fast growing and effective screening of their workings, and its potential in recolonising old sites and spoil heaps should not be neglected. In urban areas it is already widely regarded as an attractive addition to our flora, but it has further significance for the ecologist.

Since it presents such a large and obvious source of pollen and nectar, it is probable that buddleia is detracting pollinators from visiting other species in the vicinity. The short term effects of this for many species are that seed production and hence numbers of offspring that are successfully established will fall. In others the effects may not become obvious for some time, if they are capable of self-fertilisation, but ultimately the gene pool of these species will

be reduced and species of both groups may even become locally extinct. This can only be speculation at present, until further work is carried out to quantify the effects of this competition for pollinators. However, it does highlight the point that many more detailed life histories of urban species are required, to build up useful pictures of the urban ecosystem, and to predict what impact invasions of particular species will have on them.

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APPENDIX 1.

County floras reviewed for references to buddleia.

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