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Studies on the regeneration of perennial weeds in the glasshouse

I. Temperate species

I.E. Henson

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I. Temperate species

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1. SUMMARY

In a study of the effects of season on the regeneration of twelve species it was found that eight were relatively easy to propagate throughout most of the year. These eight were Agrostis gigantea Roth., Calystegia sepium (L.) R.Br., Cirsium arvense (L.) Scop., Mentha arvensis L., Rorippa sylvestris (L.) Bess., Rumex acetosella L., Sonchus arvensis L. and Tussilago farfara L. The remaining four: Achillea millefolium L., Aegopodium podagraria L., Cardaria draba (L.) Desv. and especially Senecio jacobaea L. - were poor regenerators. Several of the easier species had short periods when regeneration was poor; vis. Mentha arvensis in October, and Calystegia sepium and Sonchus arvensis in October and November. Fragments of the latter two species were dormant in those months. When cold storage was tested as a means of preserving material for planting it was found that many of these species survived storage at -2°C for several months without detriment. For example, Rorippa sylvestris still produced 95% regrowth after 12 months storage.

In experiments with Convolvulus arvensis L., the regeneration of underground stem fragments showed a marked seasonal fluctuation. Regeneration was best in the winter months from November to February. Fragments often produced shoots at other times but failed to produce roots and hence to sustain growth. Root fragments tested from April to October only did not give good establishment. Underground stem material withstood cold storage and fragments regenerated even after seven months at 0°C. Application of the hormone IBA (indolylbutyric acid) did not induce root formation, either in stem or in root fragments.

Rhizomes of Equisetum arvense L. taken from the field at intervals throughout the year failed to give good plant establishment. Pot-grown rhizome fragments tested in September regenerated well, both when planted in soil and laid on the surface of moist fibre-glass matting. Pot-grown tubers gave 100% regrowth on fibre-glass but only 47% regrew when planted in soil.

Experiments conducted with Agrostis stolonifera L. to determine the relative influence of the planting method and material on regeneration of stolon fragments showed that method of planting generally had the greatest influence. More of the 2-node fragments survived when planted at a 45° angle with the lower node buried and the upper node exposed than when buried horizontally or laid on the soil surface. However, fragments surviving surface planting displayed a tendency for both of the nodes to develop shoots. With buried fragments only one of the nodes generally developed its shoot. Single-node fragments survived best if the node was buried.

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2. INTRODUCTION

The ability to regrow from small fragments of root or rhizome makes many perennial weed species resistant to eradication by cultural measures. Indeed such measures very often lead to the spread of these plants. However, little is known of the factors which influence such regeneration, even for common species such as Convolvulus arvensis. It is not known to what extent regeneration is subject to seasonal variation, or what the critical environmental factors are, particularly endogenous ones, which determine the chances of establishment.

The significance of such information becomes particularly apparent when the experimenter is faced with the task of growing large numbers of different species with the object of using them as experimental material. He needs a measure of the "reproducibility" of the species, perhaps at different seasons of the year, on which to base his production schedule and experimental programme.

Many herbicides are now available for the control of annual weeds but few of these control perennials. There is thus a need to include perennial weeds in research programmes to an increasing extent.

For the early stages of herbicide evaluation work, container-grown plants provide the most convenient and reliable experimental material. With containers some control can be exercised over the size and degree of development of the plants. Clonal material can also be used. In addition, assessment of herbicide effects may be made easier, especially effects on the subterranean systems of the plant.

This report deals with some investigations of regeneration in several important perennial weeds and with their ease of propagation and growth in containers in the glasshouse. The first part of the report (Section 3) concerns the seasonal behaviour of a general range of species while the following three sections deal with individual species in more detail.

3. SEASONAL VARIATIONS IN THE REGENERATION OF SOME TEMPERATE PERENNIAL WEEDS

Seasonal variations in the regeneration of roots and rhizomes have been found to occur in several species. Parker (1966) found that creeping thistle (Cirsium arvense) was difficult to establish from small excised root or underground stem fragments in mid-summer but much easier in March. Hudson (1953) found that root cuttings of the raspberry (Rubus idaeus L.) exhibited a marked rhythmic seasonal response which was in part susceptible to modification by the environment. The cuttings produced more new plants in the winter months than at other times. Dore (1953) noted a similar response with horse radish (Armoracia rusticana L.). The periods of poor regeneration in both the raspberry and in horse radish were coincident with the main flowering periods of these species. The regeneration of leafy spurge (Euphorbia esula L.) has also been found to vary seasonally (Raju et al, 1964) with a depression in regenration again correlated with the period of maximum flowering. Such correlation has been ascribed to various factors such as changes in hormonal status and redistributions of metabolites.

Seasonal fluctuations of food reserves which may be associated with seasonal variations in regenerative capacity have been demonstrated in several species by Arny (1932). He found that reserves in the subterranean systems of C. arvense, E. esula, Nasturtium austriacum Crantz. and Sonchus arvensis were minimal during late April to early May, subsequently increasing during the summer. Conversely, little seasonal variation occurred in rhizome reserves of Agropyron repens (L.) Beauv.

Carbohydrate reserves tend to accumulate progressively during the growing season and to become depleted over winter. Barr (1940) and Frazier (1943) found carbohydrate reserves in the roots of Convolvulus arvensis, sampled between April and October, to be maximal in October. Total carbohydrates in the roots of Cardaria draba were found by Barr (1939) to increase to a maximum in July followed by a slight fall to a moderate level in December. Reserves in the roots of C. arvense and in the rhizomes of Tussilago farfara were found by Bakker (1960) to reach their lowest level during June and to rise to a maximum in September and October. Barr (1940) observed that a general shift from starch to sugar occurred in the roots of C. arvensis at the onset of cold weather which would serve to increase the cold tolerance of the plant; a process also suggested by Arny (1932). More recently Lipke et al, (1965) have found positive correlations between bud activity and total and protein nitrogen content of the rhizomes of Polygonum coccineum Muhl. Total and protein nitrogen content were low during the period August-October but increased following the onset of cold weather. A general scheme may be proposed in which (for example) climate affects food reserves which in turn govern regeneration.

In addition regeneration of fragments may be affected by innate dormancy mechanisms which may or may not be controlled by the state of food reserves. The dormancy of lateral buds on the rhizome of A. repens in the USA was found to be influenced by nitrate supply (Johnson and Buchholtz, 1958; 1962). Relatively few other plants regenerated purely by means of roots or rhizomes have been found to show innate dormancy, although this is a common phenomenon with certain bulbous species, e.g. Allium vineale (Håkansson, 1963). Chancellor (1967) could find no evidence of any innate dormant period for rhizome fragments of Polygonum amphibium L. However Monson and Davis (1964) report that both Vernonia baldwinni Torr and E. esula showed dormancy in late summer. In a more detailed investigation of dormancy Davis and McCarty (1966) also found that the innate dormancy of the fragments of V. baldwinni could be eliminated by exposure to 1-3°C in moist sand for 15-20 days. This suggests a vernalisation requirement for regrowth, a probable need also in the case of P. coccineum (Lipke et al, 1965).

The experiments described in this section were undertaken to determine the ease of propagation of several important temperate weed species at different seasons. It was also hoped that the experiments might indicate (by locating periods in the life cycle when regenerative ability is poor) when practical control measures could be undertaken with the best chances of success. As a further practical measure, the effects of cold storage, used as an aid to plant production in "poor" periods, were investigated.

Materials and Methods

Root or rhizome material was gathered either from natural plants in the field or from plants established in 25 cm diameter clay pots. Material of C. draba, Mentha arvensis and Rorippa sylvestris was clonal, as was all pot material. Material taken from the field was from undisturbed sites and was used only where pot-grown material had not been established. The origin and type of material used for the preparation of fragments are shown in Table I.

Fragments 3 cm in length were obtained from healthy mature roots or rhizomes. The rhizome fragments, because of their divisions into nodes allowed bud numbers to be controlled. Unless internode length was less than 3 cm (as with Achillea millefolium) fragments of rhizome with one centrally-placed node were obtained.

Table I

Species, source and type of material used

Species	Original location of material	Source of material	Reproductive parts used	Approx. range in fragment diameters (mm)
Achillea millefolium L.	Begbroke Oxon	Field/Pots * outside	Rhizome	2.0 - 4.0
Aegopodium podagraria L.	Kidlington "	Pots-outside	"	3.0 - 4.0
Agrostis gigantea Roth.	Wytham Oxon	" "	"	1.0 - 2.5
Calystegia sepium (L.) R.Br.	Begbroke Oxon	Field	"	4.0 - 5.0
Cardaria draba (L.) Desv.	Cambridge	Field	Root	3.0 - 4.0
Cirsium arvense (L.) Scop	Wytham Oxon	Pots-outdoors	"	4.0 - 6.0
Mentha arvensis L.	Wytham Oxon	Field	Rhizome	1.5 - 4.0
Rorippa sylvestris (L.) Bess	Cambridge	Field	Root	1.5 - 3.0
Rumex acetosella L.	Marlborough	Pots-glasshouse	"	1.0 - 1.5
Senecio jacobaea L.	Oxford	Field	"	1.0 - 1.5
Sonchus arvensis L.	Begbroke Oxon	Pots-outside	"	3.0 - 5.0
Tussilago farfara L.	Brackley Northants	" "	Rhizome	3.5 - 6.0

*from pots from May 1967 onwards

Material subjected to low temperature storage (-2.2°C ± 1°) consisted of intact lengths of clean rhizomes or roots placed in sealed polythene bags. For each species at each planting date 20 fragments were planted horizontally at 1.25 cm deep in a light sandy loam soil (with 2.5% organic matter and pH 7.3). Tin-plate containers 19.0 x 13.7 x 7.6 cm deep were used, and after planting were kept in a glasshouse with a mean air temperature ranging from 15° to 23.5°C.

The plants were maintained in the glasshouse for either 42, 56 or 70 days before assessment, depending on the relative speed of growth of the species (see Table II). The percentage of fragments producing emerging shoots within the allotted time was then recorded. In addition, the

percentage of fragments producing at least one shoot which had attained a stage of development indicated in Table II, was also recorded. This latter record is referred to in the results as the percentage of usable plants, as only plants which had reached such stages of development would normally be considered suitable experimental material.

Table II

The time allowed between planting and assessment and the growth stages used for assessing the vigour of the weed species

Species	Time allowed from planting to assessment	Stage of Growth ("usable")
A. millefolium	56 days	rosette with 3-4 expanded leaves
A. podagraria	70 "	3-4 expanded leaves
A. gigantea	42 "	3-4 expanded leaves with tillers appearing
C. sepium	42 "	6-9 expanded leaves
C. draba	56 "	6-9 " "
C. arvense	56 "	6-9 " "
M. arvensis	42 "	4-6 expanded leaf pairs
R. sylvestris	42 "	rosette with 3-4 expanded leaves
R. acetosella	70 "	" " 6-9 " "
S. jacobaea	70 "	3-4 expanded leaves
S. arvensis	42 days	5-6 " "
T. farfara	42 "	3-4 " "

Results

(a) Fresh material

The percentage emergence and the percentage of usable plants produced during 12 months is shown for each species in Table III. These results were obtained with freshly gathered material from field or pot.

Of the twelve species studied, eight of these, namely A. gigantea, C. sepium, C. arvense, M. arvensis, R. sylvestris, R. acetosella (clones of both sexes), S. arvensis and T. farfara, attained an emergence of 70% or more for 12 or more of the 16 months over which regeneration was studied. Of the remaining four species, C. draba attained 70% or more emergence for 11 months, A. millefolium and A. podagraria attained 70% or more for three and two months respectively while S. jacobaea attained 60% for one month only. When the 50% level of emergence is considered S. jacobaea is recorded as attaining this in only one of the months. A. millefolium and A. podagraria each attained 50% or more for six months while the remaining species attained this level for 14 or more months.

Emergence of all species was greatly depressed in October and November and again slightly in March and April (Fig. I). There was a close correlation with these bulked data between the level of emergence and the number of usable plants produced. Values for individual species were not always correlated. For example, none of the 95% of regenerating fragments of M. arvensis planted in November produced usable plants, nor did fragments of

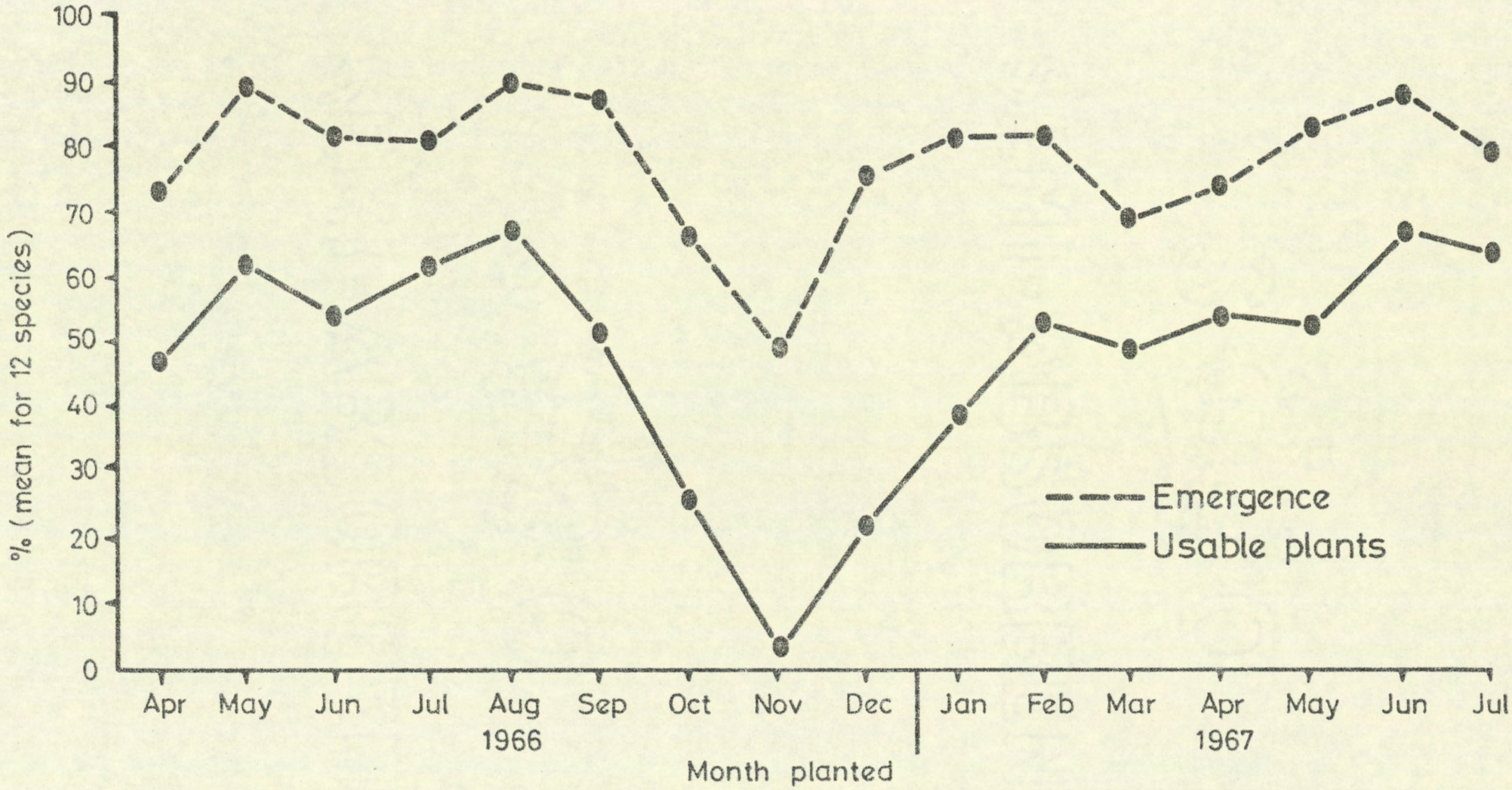

Fig. I. The seasonal variation in emergence and in usable plants produced from underground fragments of 12 species (as % of fragments planted)

Table III

The percentage emergence (i) and percentage of usable plants (ii) produced by freshly gathered material of 12 species of perennial weeds (data for 12 months)

Species	Response assessed	Month of planting											
		April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March
A. millefolium	i	35	40	45	45	60	45	15	45	15	50	60	20
	ii	20	20	15	40	45	20	5	0	0	25	35	15
A. podagraria	i	50	65	50	30	45	70	40	20	25	70	35	45
	ii	30	40	35	25	20	25	0	0	0	30	0	35
A. gigantea	i	60	90	100	95	80	90	95	60	80	95	100	65
	ii	55	85	55	60	75	85	85	40	40	50	90	60
C. sepium	i	100	100	100	75	100	60	35	0	95	100	95	100
	ii	100	70	95	65	100	45	15	0	60	85	95	90
C. draba	i	100	100	60	70	70	100	85	60	45	70	90	45
	ii	60	65	25	35	50	50	50	0	5	15	40	30
C. arvense	i	100	90	95	100	100	95	95	85	95	90	85	75
	ii	40	60	70	70	75	85	75	55	15	65	60	40
M. arvensis	i	100	100	100	100	100	80	45	95	100	90	100	90
	ii	80	85	95	100	75	10	10	0	20	70	70	85
R. sylvestris	i	100	100	100	95	100	95	90	60	100	30	95	90
	ii	75	80	90	65	75	70	0	0	5	0	40	60
R. acetosella - male	i	65	100	100	100	95	100	75	95	70	85	70	75
	ii	65	70	50	95	85	75	50	20	45	50	60	60
R. acetosella - female	i	60	100	80	100	95	95	85	50	65	65	80	70
	ii	35	75	45	80	90	50	60	15	35	35	75	60
S. jacobaea	i	0	0	0	10	35	35	25	5	45	60	0	15
	ii	0	0	0	10	15	25	0	0	0	0	0	0
S. arvensis	i	100	100	90	85	100	100	65	0	100	90	90	90
	ii	50	65	50	50	70	50	0	0	40	50	70	70
T. farfara	i	80	90	95	90	100	100	75	65	95	95	100	90
	ii	25	65	65	85	60	70	30	0	70	50	45	65

R. sylvestris planted October to January (except for 5% in December), those of S. arvensis planted in October, nor those of T. farfara planted in November.

The behaviour of several of the species studied showed interesting features:

1. Fragments of C. sepium and S. arvensis planted in early winter were dormant at the time of assessment, i.e. externally healthy but without growth. Even when these fragments were replaced in fresh soil they did not sprout until early summer the following year when by mid-July 90% of the fragments of C. sepium and 80% of those of S. arvensis had emerged and produced considerable shoot growth.

2. Few fragments of S. jacobaea established healthy plants. Regeneration was confined to two main periods: during August to October and December to January. Fragments planted in August and September gave rise to healthy plants but many shoots emerging from fragments planted in October, December and January turned yellow and subsequently died. No new roots were formed on these fragments.

   Throughout the tests root fragments were obtained from mature flowering plants. In addition, on a few occasions non-flowering or rosette plants were tested and regeneration was improved. It was found that fragments from rosettes planted in October gave 60% emergence whereas fragments from flowering plants gave only 20%. However, in the following April material from both sources failed to regenerate.

3. Both male and female clones of R. acetosella established easily. There was no statistically significant difference in the percentage emergence of the two clones. However, the male clone produced a statistically significant greater number of usable plants than the female clone, a difference of 6% in nineteen months.

(b) Cold-stored material

Ten species were subjected to varying periods of cold-storage at -2°C. following collection at different times of the year. No significant differences were found between fresh and cold-stored material of the following eight species; A. millefolium, C. sepium, C. arvense, M. arvensis, R. sylvestris, S. jacobaea, S. arvensis and T. farfara. There was a relatively small reduction of growth, even following 8-12 months continuous cold-storage, of several of these species. As an example, R. sylvestris still gave 95% emergence after 12 months storage. Conversely, there was some evidence (Table IV) that under some conditions increasing periods of storage reduced the vigour of fragments of A. podagraria.

C. draba material collected in October 1966 failed completely to survive one month or more in cold-storage although fresh material gave 85% survival. This may be attributable to internal decomposition of the roots due to the storage conditions. A. podagraria, C. arvense and S. arvensis also showed some degeneration during storage.

Table IV

The effects of cold-storage on regeneration of rhizome fragments of A. podagraria (mean results of 8 months of collecting of material)

Storage time in months	2	3	4
% emergence	41	26	16
% usable plants	21	10	3

Discussion

The species considered here can be divided into three groups characterised by the ease with which small fragments regrow and establish new plants. The first group which gave a high proportion of established plants over much of the year includes A. gigantea, C. sepium, C. arvense, M. arvensis, R. sylvestris R. acetosella, S. arvensis and T. farfara, although some members of this group displayed short periods of poor establishment. The second group comprised those that gave a lower proportion of established plants and exhibited greater seasonal variation. This group includes A. millefolium, A. podagraria and C. draba. The third group consists only of S. jacobaea which regenerated only to a limited extent. Species of the first group are thus readily propagated from fragments, whilst with those of the second group due allowance needs to be made by planting extra fragments to obtain the required number of plants. Finally, with S. jacobaea it is doubtful whether it is worthwhile pursuing vegetative propagation. Seed is easily obtainable and when fresh has a high viability (Harper and Woods, 1957). Seedlings do however, often produce plants of markedly variable forms which renders them less suitable experimental subjects.

Certain periods of the year, e.g. October - November, with M. arvensis, C. sepium and S. arvensis, are to be avoided for propagation. If plants are required at such times the unreliable species may be grown successfully if previously-gathered and cold-stored material is used.

Cold-storage did not appear to affect plant material apart from the occasional degeneration recorded with A. podagraria, C. arvense, C. draba and S. arvensis. Degeneration resulting from prolonged storage was found definitely to affect only one species - A. podagraria. The degeneration recorded for the other species was possibly due to a temporary rise in store temperature resulting from an electrical fault. The general absence of intrinsic effects of cold-storage on growth after planting permits seasonally variable species to be propagated t difficult times by using material gathered during more favourable periods. Such storage does not appear to have any great vernalising effect.

The causes of seasonal variations were not exhaustively examined. It is likely that both innate factors and the environment affected the behaviour of the plant material. Environmental factors may be expected to have much less effect on fragment regeneration than on subsequent aerial shoot growth. The rate of shoot growth was important in determining the percentage of usable plants produced.

An examination of the bulked data shows a variation in both emergence and growth coinciding with environmental changes (Fig.II). November is the month with the lowest figures for emergence and growth. Fragments planted in this month are subjected subsequently to the poorest light conditions of the year. The innate condition of the material also influenced growth at this time; for example, M. arvensis gave a lower percentage emergence and an even greater reduction of vigour in October, possibly associated with the type of fragment used. By September lateral buds on main rhizomes formed aerial shoots and this prevented their use for propagation. As a result less mature fragments were used which tended to grow very slowly.

The apparently dormant condition of C. sepium and S. arvensis fragments has been described. The dormant period was well defined. It was not coincident with probable periods of low food reserves but it was with the onset of short days, reduced temperatures, and senescence of top-growth. A further study of this condition is required, particularly to investigate whether dormancy can be alleviated by a period of moist cold storage, as shown by Davis and McCarty (1966) with V. baldwinni.

Results for S. jacobaea may have been affected by lack of suitable material. Harper and Wood (1957) report that 50% of root fragments (1.5 cm long) regenerated in a heated glasshouse, but do not mention the time of planting. Poole and Cairns (1940) first noted the superiority of rosette (non-flowering) plants over flowering plants in regenerating and attributed this to the lower food reserves in flowering plants. However, regeneration of roots from flowering plants was best in the present study during the flowering period, which suggests that antagonistic behaviour between flowering and vegetative growth does not occur. The low level of regeneration obtained with small fragments supports cultivation as a means of control (Great Britain, M.A.A.F., 1960).

Standard planting depths and size of fragments for all species were used in this experiment. It is probable that larger fragments would in some instances give improved establishment, e.g. C. draba (Scurfield, 1962). One-inch fragments were found to give better establishment than 0.5 inch fragments. Increased planting depth reduced the viability of C. draba fragments. Although such factors might affect emergence, they are possibly not so important as seasonal variations.

Clearly, an assessment of the phenology of vegetative reproduction in a wide range of species is essential, both in planning the production of plants for experimentation and in devising control measures. Periods of poor regenerative ability are evident for several species but to evaluate their true significance and usefulness, further work is required.

4. REGENERATION BY CONVOLVULUS ARVENSIS L.

Convolvulus arvensis, field bindweed, is an important perennial weed species of widespread distribution. The species presents a serious weed problem in a number of situations, particularly amongst perennial crops (Roach, 1966).

The growth habit and morphology of the subterranean system of C. arvensis has been described by Kennedy and Crafts (1931), Frazier (1943a), Alley (1962) and others (Great Britain, M.A.A.F., 1957), and its reproduction by seed has received some attention principally by Brown and Porter (1942).

Frazier (1943a) found that the root system of undisturbed plants growing in the absence of appreciable competition was composed of a primary vertical root which originated as the seedling tap root and lateral roots which spread horizontally and produced secondary vertical roots. These lateral roots eventually turned downwards but at the point of the bend secondary lateral roots and shoot buds arose. Lateral spread was rapid and 29 weeks after seedling emergence the plants had achieved a radial spread of over 10 ft.

The vegetative spread of the plant was also investigated by Best (1963) who found a plant, originally produced from a two-inch root fragment, to spread laterally up to 52 in. during a period of 4 months growth. A similar rate of spread was reported by Frazier (1943a) for plants raised from seed.

C. arvensis is not susceptible to eradication by cultural methods unless these are extreme. Zhukov (1958) reported that all roots and "suckers" from below the depth of cultivation regenerated shoots regardless of depth or the type of cultivation. Alley (1962) reported that control can be achieved by cultivation to 4 in. depth every 14 days throughout the growing season if this is continued for 3 years. Survival after cultivation would appear to depend on regeneration from the root and shoot system which is left intact below the level of soil disturbance. In Russia, Zhukov (1958) found that cultivation largely prevented regeneration of cut-off parts of roots and suckers (time of year unspecified). However, in Great Britain it is reported that cultivations spread C. arvensis, and that regeneration from small fragments readily occurs (Great Britain, M.A.A.F., 1957).

Soil and climatic factors evidently affect regenerative behaviour. Smirnov (1956) refers to a dormant period induced by either drought or absence of cultivation. He suggests that (under certain edaphic conditions peculiar to the area) enforced bud dormancy is broken either by fragmentation or by an increase in available soil moisture.

There is little information based on experimental evidence regarding the regenerative behaviour of C. arvensis under British conditions. The aim of the preliminary studies described here was to determine in the glasshouse (i) the ease with which small fragments of root or underground shoot regenerated and (ii) which factors might influence the results. An attempt is made to extrapolate certain results and to predict possible behaviour in the field.

Materials and Methods

Roots and underground stems of C. arvensis were obtained from a natural population growing in an arable field at Begbroke and tested after fragmentation for regenerative ability in a glasshouse.

Fragments of various lengths (3 - 12 cm) were planted horizontally 1.25 cm deep in a light sandy loam soil of pH 7.0 and with a low organic matter content. They were contained in either pots 9.0 cm diameter, or tin-plate boxes 19.0 x 13.7 x 7.6 cm deep. Twenty fragments were planted per box and 1 - 3 fragments per pot. The containers after planting were placed in a glasshouse with a maintained minimum temperature of 10°C.

Where seasonal differences in regeneration were being studied the fragments were planted at monthly intervals throughout the year beginning October 1966. Twenty fragments were tested each month and growth was assessed 70 days after planting. Shoot growth was designated as either 'healthy' or 'unhealthy' and the presence or absence of root growth on each fragment was recorded. Unhealthy shoots were those which displayed tip dieback, necrosis of lower leaves, or chlorosis.

Results

(a) Seasonal variations in regeneration of 3.0 cm stem fragments

The response of the fragments (Table V) varied considerably according to the time of year at which they were gathered, prepared and planted. More fragments (55 - 75%) produced both healthy shoots and roots during January and February than in the rest of the year when less than 30% did so. From November until February there was maximum 'healthy' shoot growth and minimum 'unhealthy' shoot growth while from March until October the opposite occurred. Healthy shoot growth and root growth were closely correlated (Fig. II).

Although poor shoot growth was invariably accompanied by the absence of roots, lack of roots alone did not inhibit shoot growth which continued for as long as rhizome food reserves lasted. The presence of roots did not always ensure healthy shoot growth, but in no instance did root growth occur in the absence of shoots.

Table V

Seasonal variations in regeneration of underground stem fragments of *C. arvensis*

Month planted	percentage of total fragment number			
	Healthy shoots		Unhealthy shoots	
	with roots	no roots	with roots	no roots
1966 Oct.	15	5	25	30
Nov.	20	30	5	40
Dec.	25	35	0	15
1967 Jan.	75	15	5	0
Feb.	55	20	0	20
March	15	0	5	10
April	10	5	10	40
May	10	0	0	45
June	5	0	0	15
July	0	0	5	0
Aug.	15	0	0	25
Sept.	0	0	0	0
Oct.	0	0	10	20

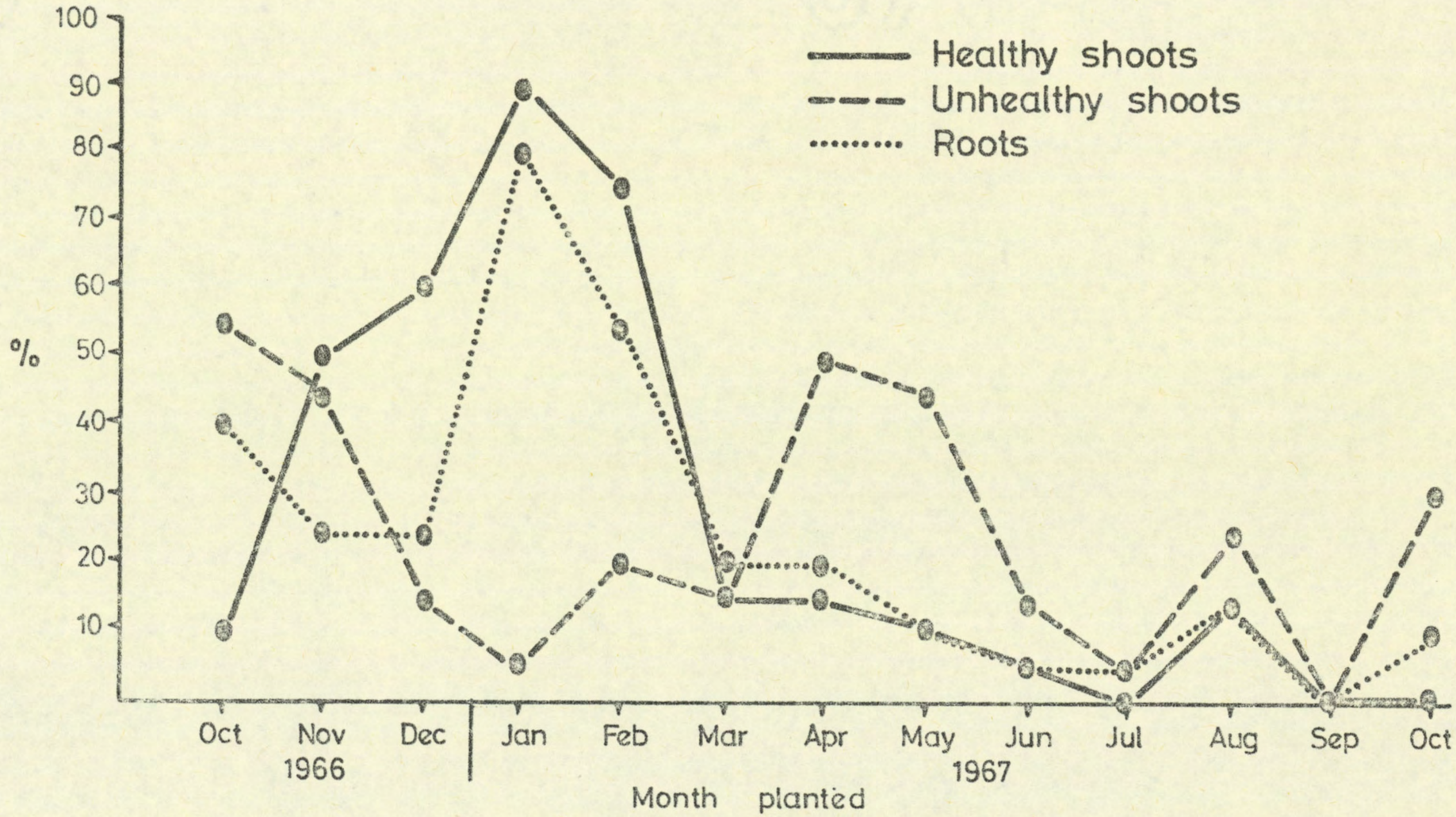

Fig II. The proportion of stem fragments of C.arvensis producing healthy shoots, unhealthy shoots and healthy roots when planted at monthly intervals throughout the year (as % of fragments planted).

(b) Seasonal variations in the regeneration of 3.0 cm root fragments

Root fragments were only tested during part of the year from April to October 1967. During this period the fragments displayed a greater tendency to produce new roots than did stem fragments, and few root fragments produced healthy shoots in the absence of new roots (Table VI). New lateral roots were produced in the absence of shoots by a few of these fragments planted in April, September and October.

Plant establishment was still poor, although somewhat better than that achieved by rhizome fragments during the same period.

Table VI

Seasonal variations in regeneration of root fragments of C. arvensis

Month planted	percentage of total fragment number			
	Healthy shoots		Unhealthy shoots	
	with roots	no roots	with roots	no roots
1967 April	50	0	30	0
May	10	0	0	5
June	30	0	0	0
July	20	0	5	5
Aug.	30	5	0	5
Sept.	5	0	40	15
Oct.	0	0	10	10

(c) Regeneration of stem fragments following cold-storage

Underground stems were gathered in November 1966 and January and February 1967, and stored in sealed polythene bags at 0°C (± 2°C) for periods of 1 - 7 months. The results are shown in Table VII.

Table VII

The regeneration of underground stem fragments of C. arvensis after cold-storage

(results are means of three planting dates)

Number of months storage	percentage of total fragment number			
	Healthy shoots		Unhealthy shoots	
	with roots	no roots	with roots	no roots
1	65	8	8	7
2	63	5	5	17
3	59	3	6	7
4	60	2	4	13
5	62	3	5	15
6	52	5	13	15
7	27	5	5	10

The stems withstood cold storage and fragments regenerated even after seven months continuous storage. The time at which the material was lifted slightly modified regeneration and the response to such storage in that material gathered in November continued to grow well and there was little difference in regeneration with increasing duration of storage, while material in January and February showed a somewhat decreased capacity for regeneration as storage time increased.

(d) Effects of fragment type, size and planting media on regeneration

Root and stem fragments of two lengths and ages were planted in soil or a 50/50 peat/sand mixture to gauge the effects of these various factors on regeneration. The experiment, the results of which are shown in Table VIII, was carried out in June-September 1966.

These results can be considered to give only a rough indication of fragment behaviour owing to the limited number of fragments per treatment. The number of fragments sustaining shoot growth decreased during the duration of the experiments following an initial assessment approximately 1 month after planting. Lack of root production was associated with death of shoots and rotting of the fragments subsequently occurred.

Table VIII

Effects of fragment type, size and planting media on regeneration of C. arvensis

Fragment type	Fragment	Number of fragments surviving (out of five)				
		5 weeks after planting		12 weeks after planting		
		Soil	Peat/sand	Soil	Peat/sand	Total
Roots 1 year old	3 cm	2	3	0	3	8
	6 cm	5	5	4	5	19
Stems 1 year old	3 cm	1	2	0	1	4
	6 cm	1	4	1	3	9
Stems 2 years old	3 cm	5	4	3	3	15
	6 cm	4	5	3	4	16
Total		18	23	11	19	71

The peat/sand medium was more favourable to survival than soil by a margin of 20%. Survival of root and younger stem fragments was increased when 6.0 cm fragments were used. There was no difference due to length of fragment with the maturer stems. There was also little difference in performance between roots and maturer stems but younger stem fragments were poor.

(e) Effects of auxin on regeneration of mature fragments

A limited trial using indolyb turic acid (IBA) did not provide any positive results. IBA was applied in a dust carrier ("Seradix B") to cut surfaces of fragments. In a first experiment underground stem fragments, 3.0 and 6.0 cm long were tested for root production following auxin application

both in soil in pots in the glasshouse and on filter paper in petri dishes in an incubator at 25°C. The auxin was applied to either proximal or distal ends of the fragment. There were no observable differences resulting from the treatments. In a second experiment root fragments also failed to show any benefits.

Discussion

In view of the importance of this weed it is surprising how little is known of the patterns of regeneration of C. arvensis under arable conditions in the British Isles. The results presented here do not agree with the many assumptions relating difficulty of controlling the weed by cultivation to a high regenerative capacity.

These results indicate that fragments, whether root or stem, do not readily establish healthy plants at all times. Their pre-formed shoot buds may develop initially only to die due to the failure or slowness of root production. There is however a marked influence of season on root production and regeneration. Regeneration in the glasshouse after gathering of material in the field is maximal in mid-winter (Fig. II) when external conditions most inhibit development in the field. During mid-winter both root and shoot growth are maximal. Regeneration is markedly seasonal and essentially similar to the variations in regeneration recorded by Hudson (1953) for raspberry, Dore (1953) for horseradish and by Raju et al (1964) for leafy spurge. Such regeneration follows a period during which food reserves have been accumulated and so are probably at a peak. Barr (1940) found total available sugars in the root system of C. arvensis to be at a maximum in late October, having increased progressively through the summer. Frazier (1943b) also studied seasonal fluctuations in food reserves and found that the maximum peak of "readily available carbohydrates" occurred in the lateral (horizontal) roots in October. Food reserves of underground stem material were not included in these studies but it is probable that stem reserves vary in the same way as root reserves. The two types of roots studied by Frazier showed similar fluctuations in their organic reserves with time although there were some statistically significant differences with root type in levels of the various organic fractions considered.

In applying results of the present study to the field it might be assumed that conditions in the field during the summer are frequently as suitable for the regeneration of small fragments as conditions in the glasshouse. As fragment regeneration in the glasshouse during the summer months is poor it may be expected that regeneration of fragments in the field would be poor also, as found, for instance, by Zhukov (1958). Therefore spread of C. arvensis by fragmentation following cultivation should not present a problem. Reappearance of the weed can be presumed due to regrowth from the intact root/stem system remaining undisturbed below the level of cultivation, which is merely decapitated by cultivation operations. The importance of regrowth from fragments under field conditions in Britain still needs further investigation.

In conclusion, these results are of value in demonstrating the limitations involved in propagating C. arvensis in the glasshouse. For success, gathering of material in November to January appears to be necessary. Since the material is unaffected by prolonged cold storage then such storage can be used to enable propagation to be achieved satisfactorily for a major part of the year.

5. REGENERATION BY EQUISETUM ARVENSE L.

The perennial, spore-bearing common horsetail, Equisetum arvense, is renowned for its persistent regeneration in the field from rhizome fragments. The ability to regenerate plus the fragility and depth of penetration of the rhizome renders control by cultivation very ineffective. Trials of regeneration were undertaken in the glasshouse to determine the ease with which new plants could be propagated for experimental use. The results of two experiments are described in this section.

Materials and Methods

In Experiment 1 rhizomes of E. arvense were dug up in the field at Begbroke at monthly intervals throughout the year. One-node fragments 3 cm long were cut up and planted horizontally 1.25 cm deep in a light sandy loam soil in tin-plate containers 19.0 x 13.7 x 7.6 cm deep. Twenty fragments were planted each month. The tins were kept in a glasshouse with a maintained minimum air temperature of 10°C. After 70 days the number of fragments which regenerated was recorded.

In Experiment 2, tubers and different sizes of fragment were compared, taken from pot-grown plants kept in the glasshouse. The plants were from the same source as those in Experiment 1. Experiment 2 was conducted during September - November 1967. Three sizes of rhizome fragment and dormant tubers of E. arvense were used. Two contrasting environments were compared. The material was either planted as in Experiment 1 or laid on the surface of moist fibre-glass matting exposed to light. For each treatment there were five replicates. Each replicate contained 12 nodes (i.e. 12 one-node, or 6 two-node or 4 three-node fragments), or 12 tubers. A final record of regeneration was taken 70 days after the start of the experiment.

Results

(a) Experiment 1

The results (Table IX) show that regeneration of one-node fragments was poor and that in July, August and February no fragments survived at all. There were two periods of regeneration: the first in September - December and a second in April - June. Aerial shoot growth of the surviving fragments was very slow, and such growth was almost exclusively diageotropic.

Table IX

Regeneration of one-node rhizome fragments of E. arvense taken from the field at monthly intervals (Assessed 70 days after planting)

Month planted	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July
% of fragments regenerating	0	25	35	30	25	15	0	5	55	20	20	0

(b) Experiment 2

The results of this experiment are shown in Tables X and XI. It can be seen from Table X that a high proportion of all fragments regenerated both when planted in soil and when laid on fibre-glass. Tubers were an

exception to this; all tubers sprouted and produced roots on fibre-glass but only 47% did so when planted in soil.

The regeneration of individual nodes was also recorded. When placed on fibre-glass all nodes regenerated equally well and there was no tendency for any node to become dominant. In soil however the majority of two-node fragments produced shoots from one node only. The percentage of nodes regenerating on three-node fragments was also lower in soil (40%) than on fibre-glass (91%).

In addition to producing aerial shoots and roots, fragments also produced new underground rhizome (Table XI). With three-node fragments rhizome production was favoured more at the distal than at the proximal end.

Table X

The percentage of regenerating fragments and nodes of E. arvense

(Means of five replicates)

			Fragment type			
			Rhizomes			Tubers
			One-node	Two-node	Three-node	
Fibre-glass	% of fragments regenerating		97	93	100	100
	% of nodes regenerating	Distal*	97	83	95	-
		Central	-	-	100	-
		Proximal*	-	83	80	-
Soil	% of fragments regenerating		91	87	85	47
	% of nodes regenerating	Distal*	91	80	40	-
		Central	-	-	35	-
		Proximal*	-	17	45	-

* with respect to the base of the rhizome

Table XI

The production of new rhizomes by fragments of E. arvense in soil

(% of nodes producing rhizomes within 75 days)

	Rhizomes			Tubers
	One-node	Two-node	Three-node	
Distal node	70	53	50	35
Central node	-	-	45	
Proximal node	-	47	30	

Discussion

Field-harvested rhizome material of E. arvense was much inferior to pot-grown rhizome of similar constitution in its capacity to regenerate. Pot-grown material regenerated freely and produced aerial shoots with a diageotropic habit.

It is not entirely apparent why the field material did so poorly. The rhizome was obtained from plants growing in a light sandy soil in uncultivated land near a stream. They suffered heavy competition from grasses and other plants and this possibly reduced the level of food reserves in the rhizome. The rhizome was black with a large central cavity which contrasted to the younger, brown, relatively solid rhizomes from pot-grown plants. It is probable that these particular results would not be obtained using field material from more favourable sites.

With pot-grown material all types of fragment used regenerated equally well whether placed in soil or on fibre-glass. Only tubers were affected by the conditions of sprouting with over 50% rotting or remaining dormant when planted in soil. The planting method was therefore a factor of importance only with tubers.

In conclusion, suitable subjects for herbicide experimental work can thus be produced using various types of material obtained from pot-grown plants kept in the glasshouse. To what extent field material is of use may depend on the site in question.

6. REGENERATION BY AGROSTIS STOLONIFERA L. FROM STOLON FRAGMENTS

Agrostis stolonifera is an important grass weed in British agriculture and as such is frequently included in both pre- and post-emergence herbicide selectivity tests at the WRO. In pre-emergence experiments in particular a reliable technique is required which allows for maximum plant establishment.

A. stolonifera produces a slender branching system of above-ground stolons which under suitable conditions root adventitiously at the nodes and serve to spread the plant. Two-node stolon fragments have been used in the past as experimental planting material, but they have occasionally given unsatisfactory results. The investigations described here aimed at determining the relative importance of the method of planting and type of material used on plant establishment.

Materials and Methods

Stolon fragments were taken from healthy pot-grown plants of clonal stock. Fragments containing either one or two nodes were cut and graded according to the state of extension of the lateral bud or buds. The buds were designated as (i) undeveloped, if they were visible only on close inspection, (ii) developing or extending buds <5.0 mm in length and (iii) shoots, 5.0 - 40.0 mm in length. With two-node fragments both buds were of approximately equal extension.

Fragments were planted in 9.0 cm diameter pots using a light sandy loam soil. The pots were kept in a glasshouse maintained at 10° - 18°C and watered overhead.

Two-node fragments were planted at (i) 1.25 cm deep, (ii) 0.6 cm deep, (iii) on the soil surface and (iv) at an angle of 45° with one (the proximal) bud or shoot buried and with the other (distal) bud or shoot exposed.

One-node fragments were inserted vertically into the soil with the bud either at the surface or 0.6 cm below.

Treatments were replicated five times with three fragments per replicate pot. After 34 days the number of surviving fragments and the number and positions of nodes of the original fragments which had produced shoots were recorded. In addition freshweights of shoots were determined.

Results

In the first experiment the effects of planting method and degree of initial shoot extension were determined using two-node fragments. The main results are shown in Table XII.

The degree of shoot extension had no effect on the number of fragments surviving or on the number of nodes forming shoots. Effects on shoot freshweights were variable, greater weights being recorded for fragments with undeveloped buds and for those with shoots >5.0 mm long than for those with shoots <5.0 mm.

Table XII

The effects of shoot extension and planting method on the establishment of two-node fragments of A. stolonifera

Condition of lateral shoots	Planting method	Number of pots with surviving fragments	% of fragments surviving	Mean number of primary* shoots per surviving fragment	Mean shoot freshweight per pot (g)
Buds undeveloped	1.25 cm deep	5	54	1.00	1.05
	0.6 cm deep	5	40	1.00	0.95
	Surface	4	54	1.38	0.78
	45° angle	5	74	1.36	1.57
Shoots extended < 5.0 mm	1.25 cm deep	5	54	1.00	0.66
	0.6 cm deep	5	74	1.27	1.11
	Surface	1	14	2.00	0.19
	45° angle	5	94	1.14	1.40
Shoots extended 5.0 - 40.0 mm	1.25 cm deep	4	54	1.00	1.53
	0.6 cm deep	5	60	1.00	1.15
	Surface	2	20	1.67	0.52
	45° angle	5	87	1.07	1.50
L.S.D. (P = 0.05)		-	36	-	0.46

* Shoot arising directly from a node on the fragment

The method of planting had the greatest influence. Surface planting decreased the number of fragments surviving, the total shoot freshweight and the total node number producing shoots, but increased the number of primary shoots per fragment. Those fragments surviving surface planting displayed an increased tendency for both nodes to produce shoots in contrast to buried fragments in which usually only one shoot continued development.

Planting at a 45° angle (with the proximal node buried) led to the greatest number of fragments surviving, the greatest fresh weights per pot and to the greatest number of nodes producing shoots. The upper exposed bud (shoot) had a greater tendency to develop than the buried one (Table XIII).

Table XIII

Shoot development from 2-node fragments of
A. stolonifera planted at a 45° angle

	Number of nodes sustaining shoot growth per surviving fragment (mean of 5 replicates)		
	Above soil	Below soil	Total
Buds undeveloped	0.73	0.63	1.36
Shoots <5.0 mm	0.93	0.21	1.14
Shoots >5.0 mm	1.00	0.07	1.07

As the initial development of the shoot when planted increased, its capacity to survive below ground decreased.

Table XIV shows the results for the second experiment using one-node fragments. Here the fragments were at two stages of bud development. The greatest survival and growth occurred when the node was buried, a behaviour opposite to that of the two-node fragments.

Table XIV

The effect of shoot extension and node burial on the establishment of one-node fragments of A. stolonifera

Condition of lateral shoots	Planting method	Number of pots with surviving fragments	% of surviving fragments/	Mean shoot Freshweight per pot*
Buds undeveloped	Node placed at soil surface	4	40	0.43
	Node placed 0.6 cm deep	5	80	0.98
Shoot extended <5.0 mm long	Node placed at soil surface	4	40	0.56
	Node placed 0.6 cm deep	5	67	0.74

/ L.S.D. = 33% (P = 0.05)

* not significant

Discussion

Of the two main factors influencing plant establishment from vegetative parts, namely the plant material itself and the external environment, the latter has been of most importance in this investigation. The method of planting affects several environmental factors the most important of which are the moisture/aeration relationship and possibly light.

Light does not appear to be an important factor in regeneration of A. stolonifera as many buried fragments survive. However, light may have some influence since buried two-node fragments had a distinct tendency to produce one shoot only while fragments exposed to light commonly produced two. In addition, when two-node fragments were planted at an angle, the above-ground node usually gave rise to a shoot. This effect may be one of apical dominance since the exposed nodes were always distal. Apical dominance could also be assumed to be operating in the buried two-node fragments but the absence of any such effect with surface planting contrasts with this and deserves further study.

With the one-node fragments such apical dominance effects and influences of light are precluded. In this instance survival probably had a simple dependence on root formation, which in the absence of any completely buried node was retarded. Furthermore, tissue turgor was likely to decline more rapidly for the degree of exposure was increased.

This study has indicated the factors of importance in establishing A. stolonifera from stolon fragments but several questions remain that require elucidation. The effects of polarity on bud growth of several-noded fragments were not considered. It is evident that single node fragments can give a reasonable establishment but that multi-node fragments can fail to develop all buds. A study of the extent of interference between buds on the same fragment would be of interest. Type of plant material and its propensity for root formation is another factor. It may be noted here that the maximum survival of surface-planted fragments occurred with fragments having undeveloped buds.

Finally, differences in season as they affect both the parent plant material and the environment are of particular consequence. The relationships reported here thus require verifying under other conditions.

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