STUDIES ON BIOLOGY AND CONTROL

OF *OXALIS LATIFOLIA* H.B.K.

A Thesis

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by

Josiah Njiru Gitari

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ABSTRACT

Gitari, J.N., M.Sc., The University of Manitoba, April, 1986.

Studies on the biology and control of Oxalis latifolia H.B.K. Major Professor: Dr. G. Marshall, Department of Plant Science.

Growth analysis studies of Oxalis latifolia H.B.K. were conducted in outdoor-grown plants over a fourteen week period. Three main phases of growth which appeared to coincide with major physiological developments of both above and below-ground plant organs were noted. The first or vegetative phase lasted until the sixth week after planting. Dry matter (DM) gain during the first phase was slow but relative growth rate (RGR) was high in all plant organs. Net assimilation rate (NAR) rose sharply and was highly correlated ($r = 0.91$) with the leaf area ratio (LAR) throughout the first phase of growth. The second phase (weeks 7 to 12) was mainly reproductive in which bulbils were formed at the apices of the stolons. Some bulbils formed leaves of their own while still attached to the parent bulb. The RGR, NAR and LAR all declined during most of the second phase whereas DM accumulation was in the order highest to lowest: bulbils > peduncles > leaves > petioles > stolons = roots > parent bulb. The third or senscence phase (weeks 13 and 14) was marked by a sharp drop in DM gain in all organs except the bulbils. The RGR, NAR and LAR all declined throughout this last phase of growth in the life cycle of O. latifolia. Very limited development of the contractile roots occurred in this study.

Examination of the leaf surface using a scanning electron microscope revealed that a platelet type of crystalline wax ultrastructure occurs on both the upper and lower surfaces of leaves at different ages. A thick epicuticular wax deposit (59.3 to 88.8 $\mu g\ cm^{-2}$ of leaf area) dominated by hydrophobic wax components, particularly the hydrocarbons, was formed in both indoor and outdoor-grown plants.

Retention studies were carried out using a water-soluable dye solution to imitate a foliage-applied herbicide. The results showed that inclusion of a surfactant (0.25% v/v, Tween-20) significantly increased the amount of spray retained per cm^2 of leaf area. Retention increased with increase in spray output (137,250 and 327 l/ha), in presence or absence of a surfactant. The drooping or folding of the leaves reduced the amount of spray retained by between 49 and 74%.

Post-emergence treatments applied to two months old O. latifolia plants revealed that glyphosate (1.068 kg a.i/ha) killed 95% of the developing bulbils. By contrast, paraquat (0.625 kg. a.i/ha) and defoliation treatments did not affect the viability of the bulbils but fresh weight and number of bulbils per plant were reduced significantly when compared with untreated controls. All assessments were made 4 weeks after application of the treatments.

INTRODUCTION

Oxalis latifolia is a perennial weed which belongs to the family Oxalidaceae. It occurs in New Zealand, Australia, India, Sri Lanka, Britain, France, the Mediterranean region, East and South Africa as well as central and equatorial America from where it originated (Young, 1958). In Kenya, the weed is found in cultivated highlands above 1,000 m (Ivens, 1967).

The above-ground structures of a mature *O. latifolia* plant consist of cylindrical petioles terminating in three clover-like leaflets as well as the peduncles bearing inflorescences. The flowers are conspicuous due to their brightly coloured purple petals. The peduncles are usually taller than petioles (Robb, 1962). Below-ground structures include the bulbils which are formed at the apices of the stolons. It is the presence of these subterranean, perennating and regenerative structures, namely the bulbils which makes *O. latifolia* a difficult weed to control (Chawdhry, 1974). Presently, no selective soil or foliage-applied herbicides have been recommended for control of this weed in various crops.

The main objectives of the studies reported herein were: 1) to study the correlated growth of various plant organs and characterize the main growth stages with particular reference to the potential susceptibility of *O. latifolia* to control measures, 2) to determine the characteristics of leaf surface and general plant morphology as they relate to control via voliage-applied herbicides, and 3) to examine the effect of glyphosate, paraquat and defoliation on 2 month old pot-raised *O. latifolia*.

1. REVIEW OF LITERATURE

1.1 Ecology

The genus Oxalis has about 800 species occurring all over the world. All the species fall into two main groups, those that reproduce mainly sexually by seed and those that reproduce primarily asexually by bulbils. The species that reproduce by seed have not been reported as serious weeds although they may be a nuisance particularly in glass-houses. It is the bulbous species of Oxalis that have become difficult weeds to control in agricultural situations. The three species which have been listed as serious weeds include Oxalis latifolia, O. corymbosa and O. pes-caprae (Young, 1958).

The name Oxalis is derived from the Greek word 'Oxus' which means sour in reference to the taste of the foliage. The sour taste is due to the presence of oxalic acid which accumulates in aerial parts of the plants (Michael, 1965; Parsons 1973).

Young (1958) attempted a revision of the British list of Oxalis species. According to this review, the two forms of O. latifolia which occur in the British Isles are the 'typical' (H.B.K.) and the "Devon and Cornwall" form (Kunth) which he regarded as two different clones rather than distinct species. The Kunth form differs from the 'typical' form in that it has numerous almost sessile bulbils and has leaflets with narrower sinus with curved edges (Robb, 1962). Further, the 'typical' form has a few hairs at the edges of the leaves, whereas the Kunth form has none (Young, 1958).

In Kenya, O. latifolia H.B.K. is most commonly reported. Three

other bulbous Oxalis species which have been reported in East Africa include Oxalis semiloba, O. anthelmintica and O. obliquifolia. All three species can, however, be easily distinguished from O. latifolia (Ivens 1967; Chawdhry, 1974).

1.1.1 Distribution and Habitat

Oxalis latifolia H.B.K. is indigenous to central and equitorial South America. It has been naturalized as a weed of cultivated ground in both temperate and tropical climates. The weed has been reported to occur in the West Indies, South Africa, Sri Lanka, New Zealand, the Mediterranean region, as well as the British Isles (Young, 1958). In New Zealand, O. latifolia has proved to be the most established and aggressive of the various weedy Oxalis species (Jackson 1961). In India O. latifolia H.B.K. is regarded as the most common and predominant weed in apple orchards of Uttar Pradesh hills at the slopes of the Himalaya mountains (Ram and Tewari, 1979). In Kenya and the rest of Eastern Africa, O. latifolia is most notably widespread in high altitude cultivated highlands mainly above 1,000 m (Ivens, 1967), but the weed is believed to be rapidly invading new areas and is increasing in number where it is already established (Chawdhry, 1974).

Jackson (1960) noted that bulbs and bulbils are killed by temperatures below freezing point. He proposed that O. latifolia cannot be a problem as a weed where frost penetrates deeper than 5 cm in the soil. This was supported by Chawdhry and Sagar (1974) who found that freezing temperatures below -15°C were sufficient to kill the bulbs exposed for only 30 minutes. Fryer and Makepeace (1977) state that O. latifolia is

restricted to frost-free areas in the British Isles.

1.1.2 Economic Importance

Economic losses incurred due to *Oxalis latifolia* as a weed have not been properly documented. In New Zealand the species has become established as a major weed of gardens, nurseries and small holdings. In Uttar Pradesh region of India, *O. latifolia* is regarded as the most predominant weed in apple orchards, consisting of up to ninety percent of the total weed population in certain farms.

There appears to be no quantitative assessment of the economic importance of this weed in Kenya and the rest of East Africa. The weed seems to have been introduced deliberately as a plantation cover plant, especially in coffee. It was therefore not regarded to be strongly competitive with coffee, but remained a serious problem in small-seeded annual crops (Stephens, 1961). According to Church and Henson (1969), *O. latifolia* is most competitive during the early growth stages of the crop due to the weeds' ability to regenerate rapidly from the underground storage organs, and may decrease the stand and yield of the crop (Green *et al.*, 1974). The species has also become a serious weed in arable land, pasture, orchards, market gardens, horticulture and in nurseries (Chawdhry, 1974). *O. latifolia* is also an alternate host of *Puccinia sorghi* Schw. (corn rust) in different parts of the world (Versluys, 1977; Estelita-Teixeira, 1982).

1.2 Biology

1.2.1 Morphology

The underground structures of O. latifolia consist of a bulb as the central point. A dormant bulb consists of a stem and some scales. The true stem is reduced to a cone of tissue in the centre of the bulb. Two main types of scales originate from the stem: (1) outer membranous scales which bear, at the tip, the rudiments of petioles and normally extend to form fully developed leaves. The scales are closely appressed and some may extend completely around the bulb. Membranous scales are moderately thick at the centre and thinner towards the membranous hairy edges, (2) inner true scales which are glabrous and succulent. Fleshy scales are incapable of producing leaves. Some scales are transitional between the two types which makes it difficult to find where one type finishes and the other one begins. All scales are believed to be undeveloped leaves whose origin is the apical meristem. The scales are arranged on the stem in a spiral manner (Jackson, 1960; Estelita-Teixeira, 1982).

A sprouted bulb of O. latifolia may form stolons in addition to the scales and stem. The stolons are thin underground stems with nodes and internodes. Some scale leaves are found at the nodal sections of the stolon (Jackson, 1960). He noted that stolon production, from the axils of the true scales, progresses in an acropetal pattern.

The tip of the membranous scales gives rise to the petiole and the trifoliate leaves. Petioles may be 10-30 cm in height. Leaflets, usually broader than long, are glabrous and may at times, appear purplish (Young 1958). The petiole ends bluntly, three leaflets arising

laterally from it with pulvini at the junction of leaflets and petiole. The pulvinus is responsible for reaction of this species to stimuli which cause leaf movements (Robb, 1962). Such nastic movements occur in different species and are commonly due to turgor changes in the cells of the pulvini, situated at the base of apex petiole (Ball, 1969). In O. latifolia, nyctinasty or 'sleep-movement' is exhibited by the leaflets whereby the leaflets droop at night or when weather conditions are hot and dry (Robb, 1962) or when disturbed (Chawdhry, 1974).

Purple coloured flowers are borne at the ends of erect peduncles arising from the axils of the membranous scales (Jackson, 1960; Robb 1962). Flowers of the genus Oxalis contain five stigmas and ten stamens, the latter being arranged in two whorls of five. Lengths of the styles vary relative to the two whorls of stamens, a characteristic referred to as trimorphism. Three flower forms in Oxalis are: (1) short-styled, where style length is shorter than the shorter series of stamens, (2) mid-styled where style length falls between the two series of stamens, and (3) long-styled where style length is greater than the longer series of stamens (Pierce, 1973).

1.2.2 Growth and Development

Under temperate climate, O. latifolia grows in summer and the bulbs remain dormant below the soil in winter (Jackson, 1960). In a tropical climate, some leaves tend to remain green throughout the year except in severe drought (Chawdhry, 1974). Jackson (1960) observed that germination of bulbs of O. latifolia in New Zealand commences in spring when the

soil temperatures reach about 60°F (16°C).

Chawdhry (1974) studied the growth pattern of O. latifolia under glasshouse conditions where temperatures ranged between 15 and 30°C. He observed that the life cycle of O. latifolia may be divided into three phases; the first phase was represented by germination and emergence of shoots from the parent bulb, the second phase corresponded to the tuberisation of one (usually) of the roots at the base of the bulb. During this phase, formation of bulbils occurred, the third and last phase was represented by senescence which was marked by death of above-ground parts as well as contraction of the tuber.

Chawdhry and Sagar (1973) studied the movement of ^{14}C-labelled assimilates at various growth stages during the development of this plant. Ten stages which were thought to coincide with important physiological changes in the underground organs were studied. According to this study, distribution of assimilates in O. latifolia follows a definite pattern which may be divided into three main phases.

The first phase is represented by lack of import or very little import of labelled assimilates into the root system. This phase was observed to last until the five-leaf stage. The authors argue that the developing adventitious root system is supported by food reserves present in the parent bulb. The second phase, which lasted until the 25-leaf stage, was marked by transport of ^{14}C-labelled assimilates mainly to the developing bulbils. Although ^{14}C was sometimes detected in stolons and adventitious roots, the authors appear to indicate that the two organs were not a sink for carbon products, but rather served as transit or

temporary storage organs. The authors also caution that only a time series study would resolve the question of the function of carbon products observed in the stolons and adventitious roots. Further interpretation of the radiography was complicated by the fact that the isotope was detected in the parent bulb. They attributed such labelling to the presence of labelled stolons and petioles among the scales of the parent bulb. The third and last phase, which began from the 25-leaf stage and lasted until advanced senescence, was marked by substantial movement of ^{14}C-labelled assimilates to the well-developed new bulbils. In conclusion, Chawdhry and Sagar (1973) noted that new bulbils of O. latifolia mainly depend on the aerial parts for assimilates during early stages of development. The requirement of assimilates after senescence of aerial parts is met by the reserves in the tuber.

1.2.3 Sexual Reproduction

Sexual reproduction through seed has not been observed by most researchers. Flower formation occurs but no seed is set (Young, 1958; Chawdhry, 1974). Although most authors seem to believe that the flowers are self-incompartible, Robb (1962) reported production of some seed which he suggested were non-viable since no germination occurred immediately or after some period following maturation. Length of photoperiod has no effect on either the number or the time of flower formation in O. latifolia (Jackson, 1960).

1.2.4 Asexual Reproduction

The main method of propagation in O. latifolia is through bulbs.

The bulbs are produced at the apices of the stolons. A stolon arising from the parent bulb is referred to as a primary stolon and may branch to form a secondary stolon. Apices of primary and secondary stolons form primary and secondary bulbils, respectively. Young bulbs are called bulbils. As the bulbils mature, a rapid increase in size takes place. The outer membranous scales of the bulbils, formed at the base of the bulbil, extend to form leaves under favourable growth conditions. In absence of moisture, favourable temperature and light, the meristem at the apex of the membranous scales dies and they serve only as a protective cover for the developing true scales (Jackson, 1960). The outer scales are brown, papery and overlap to form a protective sheath enclosing the inner scales (Robb, 1962). Bulbils become detached from the mother bulb at the end of the growing season through decay of stolons. The unattached bulbils, having lost their connection with the mother bulb are referred to as bulbs. Detached bulbils may be dormant (Jackson, 1960). Downward contraction of the tuber pulls the bulbils downwards, thus adjusting their depth. This contraction aids in vertical dispersal, though over very small distances. The stolons are mainly responsible for diageotropic dispersal of new bulbils away from the parent bulb (Jackson, 1960; Chawdhry, 1974).

1.2.5 Dormancy in Bulbs

The length of time that bulbs of O. latifolia can remain dormant has not been determined but Chawdhry (1974) suggested eighteen months. He further noted that though the majority of bulbs sprout during the following growing season, a proportion usually remain dormant even under

favourable conditions. According to Jackson (1960), a certain period of time, ranging between four to six months, has to elapse before dormancy of bulbs can break. Whatever length of time it takes for dormancy to break naturally, laboratory methods for terminating dormancy have met with limited success. Parker (1966) reported that dormancy of O. latifolia bulbs can be broken almost completely by peeling off the outer scales. These observations do not, however, fully agree with the findings of Chawdhry and Sagar (1974a) who found that about half of the peeled bulbs could not germinate. Neither peeling of the scales nor heating at 45°C for four hours, or even a combination of peeling and heating, could fully terminate the dormancy of the bulbs. Heating treatment however, increased the proportion of bulbs which sprouted. High levels of sprouting were achieved when the heated bulbs were previously pre-chilled for about three weeks.

1.3 Chemical and Cultural Control

1.3.1 Chemical Control

Attempts have been made to determine the most suitable and effective control measure for the bulbous species Oxalis latifolia. In chemical control, various herbicides have been screened in different parts of the world, but information in the literature on the performance of the herbicides is often conflicting.

Soil-applied herbicides: Parker (1966) found that EPTC retarded emergence and caused distortion of the first few leaves, but once emerged, O. latifolia plants recovered quickly. He also observed that trifluralin applied at 4.0 lb/acre (4.5 kg/ha) completely inhibited

emergence of the bulbs but a lower rate of 1.0 lb/acre (1.1 kg/ha) only delayed emergence for several weeks. Chawdhry (1974) found that a concentration of more than (0.5 x 104) parts per million (ppm), that is, 2.5 kg in 500L water per ha EPTC or trifluralin, could prevent the emergence of bulbs of O. latifolia for at least three months, in growth chambers maintained at 23°C. Neither of the two herbicides killed the dormant bulbs irrespective of whether the bulbs were planted in treated soil or the herbicide was applied directly on the bulbs before covering them with the soil. Church and Henson (1969) concluded that trifluralin suppressed the development of O. latifolia bulbs, but the herbicide had no effect on their viability.

Foliage-applied herbicides: Parker (1966) studied the effect of paraquat, a foliage applied contact herbicide, on pot grown O. latifolia plants. He concluded that paraquat (applied at 0.35 kg/ha) had great potential value for eradication of the weed, but timing of application was critical for best results. Only at the 2-to 4-leaf stage could the parent bulb be killed without leaving behind any viable daughter bulbs. Clearly, the success of paraquat as a control measure would depend on the degree of synchronization of the emergence of the weed. Chawdhry and Sagar (1974) reported that chemical defoliation of O. latifolia with paraquat could check the increase in number and dry weight of new bulbils. When sprayed at the intervals of 2, 4, 6 and 8 weeks, there was 87, 81, 55 and 29 percent reduction in the number of new bulbils, respectively. To achieve this level of control, pot-raised O. latifolia plants had to be treated four times each with 0.25 kg ha^{-1} of paraquat before the assessments were made.

Cox (1978) investigated the effectiveness of glyphosate, a foliage-applied translocated herbicide, applied at various stages over a period of four consecutive seasons under field conditions. Sixteen regularly spaced bulbs of O. latifolia were planted in 1 m x 1 m size plots which had previously been sterilized to provide a weed free situation. Glyphosate was applied, at the rate of 1.5 kg ha^{-1} in a spray volume of 700 l ha^{-1}, approximately 1 1/2, 2 1/2, 3 1/2 and 4 1/2 months after planting. Assessments at the end of the first season (seven months since planting) revealed that there were 23, 36, 46 and 106 new bulbils per plant respectively compared with 239 in untreated control.

The plots were re-sprayed with equivalent treatments in the following 2nd and 3rd seasons or years, but only foliage re-growth was assessed. The results in the fourth and final season showed that the treatment applied 2 1/2 months after planting was the most effective with 185-fold increase in the number of bulbils per originally planted bulb compared with 1280-fold in control.

In conclusion, Cox (1978) noted that glyphosate was probably the most active foliar herbicide against O. latifolia but did not eliminate bulb production even when leaf growth was greatly reduced.

Cox and Kerr (1981) studied the efficacy of glyphosate and oxadiazon [3-(2.4-dichloro-5-iso-propoxyphenyl)-5-t-butyl-1,3,4-oxadiazolin-2-one) each applied at 2.0 kg. ha^{-1} at different intervals over two consecutive growing seasons under field conditions. The most effective control programme was oxadiazon applied four times at approximately two-monthly intervals. Excavation of below-ground plant organs, in this treatment, at the end of the second growing season revealed that there was an

average of about 78 bulbs per m^2. Three other treatments resulted in a significant reduction of bulbs and bulbils from about 14,000 for untreated control to about 600 bulbs and bulbils per m^2. These three treatments were (1) three applications of oxadiazon at 2 1/2 monthly intervals, (2) four applications of glyphosate at intervals of nine, six and five weeks, and (3) an early application of oxadiazon followed by glyphosate after four months.

Further work on the efficacy of glyphosate as a means of control of O. latifolia under field conditions has been reported (Sakira, 1981). The herbicide was applied at 1.6, 1.78 and 1.96 kg ha^{-1} using a knapsack sprayer. A repeat application was made after five weeks but unfortunately a heavy shower is reported to have occurred half an hour after spraying. The results showed that only the applications of 1.78 and 1.96 kg ha^{-1} were effective enough to cause a slight reduction in the dry matter yield of the bulbils, but the reduction was not statistically significant. Sakira (1981) suggested that new foliage arising from re-growth as well as the root tuber, was contributory to the increased number and dry weight of the bulbils after spraying. He concluded that it would not be advisable to attempt control of the weed after it had tuberised because the bulbils would have been formed.

1.3.2 Cultural Control

Cultural means of control have been considered for eradication of Oxalis latifolia. Crop rotation, where applicable, can be of great value. It is possible to choke out the weed plants by turning the infested land to a grassland for a period of three years or more, until

all the dormant bulbs have died. The main drawback in such a method is that it would be uneconomic to most farmers. Furthermore, a vigorous sward would be necessary to prevent any growth of the weed and it would not be certain whether some bulbs may persist over the entire period of the sward (Young, 1958; Ivens, 1967).

A simple and inexpensive cultural control method for controlling O. latifolia would be mechanical defoliation of the plant at the appropriate growth stage(s). Esler (1962) investigated the long-term effect of this control method. O. latifolia bulbs were planted in 6 1/4 inches (16 cm) diameter pots at a depth of three inches. Two bulbs were planted per pot. Defoliation was carried out at weekly, bi-weekly as well as monthly intervals during the eight months growing period. The experiment was terminated after three consecutive growing seasons. Esler (1962) observed that nine of the 20 plants survived the weekly and bi-weekly defoliation, but none of the plants, which were defoliated monthly, died. An assessment of the plant yield showed that the average number of bulbs or bulbils per plant was 37 and 197 for the weekly, bi-weekly and monthly defoliated plants, respectively. The control pots had an average of 231 bulbs per plant. Esler (1962) concluded that O. latifolia does not withstand frequent and regular defoliation, but cautioned that defoliation cannot be recommended as a practical means of control for the weed.

Chawdhry and Sagar (1974b) studied the susceptibility of O. latifolia to repeated defoliation at various growth stages under glass-house conditions. The plants were clipped at 4-, 10-, 20- and 30-leaf stages as well as a combination of 4- and 10-leaf stage. Final harvest-

ing was carried out when no regeneration occurred for at least three or four subsequent months. The results showed that all frequencies of clipping strongly suppressed dry matter increase of the plants. At the second clipping, for instance, bulbils of the clipped plants had attained only one-third of the dry weight of the bulbils in undefoliated plants. Clipping at the 20- and 30-leaf stages was less effective in reducing the dry weight of the plants. The authors concluded that regular and relentless defoliation before the 20-leaf stage would result in a useful degree of control of O. latifolia. However, clipped plants still form new bulbils whose development is checked as soon as they are formed. These results are not in agreement with those of Esler (1962) who found that monthly defoliation does not influence the yield of new bulbs of Oxalis latifolia.

2. MATERIALS AND METHODS

2.1 Developmental and Structural Studies

2.1.1 Growth Analysis

Oxalis latifolia plants were raised outdoors in 25 cm diameter plastic pots with a capacity of 6.75 litres. The pots were filled with unmodified clay soil and 0.5 g per pot (100 kg/ha) of ammonium phosphate (16-20-0) was applied by shallow incorporation on the soil surface. A soil test previously carried out showed that the soil contained 48.6 and 24.8 kg/ha of nitrogen and phosphorus, respectively.

The bulbs of *O. latifolia* which were selected had a uniform size of about 0.5 g (fresh weight) each. All the bulbs showed an appearance of roots at the base of the stem - an indication that dormancy had terminated and that all the bulbs were at a similar stage of physiological maturity. The bulbs were first germinated in a sand/clay soil mixture and transplanted when emergence of the leaf stalks was observed. Planting was carried out on 21 May 1985 at a depth of 3 cm. The pots were watered from above as necessary during the dry periods. Emergence occurred between June 3 and 10 and so pots were blocked to effect groups of uniform emergence. The experiment was arranged as a randomized complete block design.

Starting June 13, harvesting was carried out on a weekly basis until August 1 thereafter the harvesting interval was increased to two weeks until August 29 when the last harvest was taken. At each harvest, eight pots were removed. The underground parts of each plant were carefully washed free of soil and the plant dismembered into roots including where

present tubers i.e. the contractile roots, parent bulb, stolons, new bulbils, leaflets, petioles and the peduncles bearing the inflorescence. Assessments made at each harvest period included petiole and peduncle height, leaf area, stolon length as well as the numbers and dry weights of various organs per plant. Leaf area was measured using a leaf area meter.[1] Dry weights were determined after the plant parts were dried at 80°C for at least 48 hours. The basic data collected were used for growth analysis purposes following (Radford, 1967). Calculations were made as described in the table below:

Parameter Calculated	Formulae Used
Relative Growth Rate (RGR)	$\frac{\ln W_2 - \ln W_1}{t_2 - t_1}$
Net Assimilation Ratio (NAR)	$\frac{(W_2 - W_1)(\ln A_2 - \ln A_1)}{(t_2 - t_1)(A_2 - A_1)}$
Leaf Area Ratio (LAR)	$\frac{(A_2 - A_1)(\ln W_2 - \ln W_1)}{(\ln A_2 - \ln A_1)(W_2 - W_1)}$

Where W_1, A_1, W_2, A_2 represent dry weights (of the whole plant) and leaf areas at time t_1 and t_2.

In addition, the underground/aerial weights ratio was calculated. Where appropriate, statistical analysis was carried out for each harvest to calculate the mean number and dry weights of various plant organs, as well as their respective standard errors.

2.1.2 Scanning Electron Microscopy (SEM)

Leaves of *O. latifolia* (ranging between 1 and 10 weeks old) were removed from outdoor grown plants and examined under a scanning electron

[1] Delta-T. A product of Burnwell, Cambridge, England.

microscope (SEM) on 13 September 1984 and 10 September 1985.

To prepare the leaves for SEM, the leaflets were frozen by immersing them in liquid nitrogen and subsequently freeze dried. Each sample was mounted on stubs with Electrodag, a conductive adhesive,[2] before coating with approximately 200A° gold on a Sputter coater.[3] The leaves were viewed under a stereoscan[4] and the images recorded on Kodak Panatomic film.[5]

2.1.3 Quantitative and Qualitative Assessment of Epicuticular Wax in O. latifolia

Leaves of O. latifolia for use in these studies were harvested from plants grown outside during summers of 1984 and 1985. In each of the years, leaves were detached from the petioles, divided into two portions and the leaf area of each portion measured using a leaf area meter. Each portion was subsequently treated similarly but separately throughout.

To remove the epicuticular wax, the leaves were washed in chloroform for 30 seconds (Martin, 1960) at room temperature. The wax/chloroform mixture was passed through glass wool to remove any plant debris. Chloroform was then removed in vacuum using a rotary apparatus under a water-bath maintained at 35°C. The wax, from each of the two leaf portions, was finally weighed and a mean value recorded in $\mu g/cm^2$ leaf area. Cabbage wax was also extracted in a similar manner and used as a

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2 Electrodag 416., IMANCO, Cambridge, England.
3 Balzer Sputter coater, Balzer Union, Liechtenstein, West Germany.
4 Model MK II A, IMANCO, Cambridge, England.
5 Kodak Company, New York.

standard. The Oxalis and cabbage wax samples were subsequently used in chromatography studies.

In order to determine the components of epicuticular wax of O. latifolia, a qualitative assessment was made using thin-layer chromatography (t.l.c.) plates.

Epicuticular wax (20 mg) was dissolved in 1.0 ml of chloroform. Silica Gel G plates, which were activated by drying at 80°C, were spotted with 10 ul of Oxalis and cabbage wax samples. The samples were chromatographed in 100% benzene solvent. The solvent was allowed to run to a distance of 12.5 cm from the sample origin. After evaporation of benzene in the fume hood, the plates were sprayed with concentrated sulfuric acid (50% aqueous) before charring in an oven at 110-120°C (Touchstone and Dobbins, 1983). Permanent recording of the chromatograms were done photographically using a black and white film (Plate 3).

This experiment was repeated six times and the mean Rf values of the wax components.

2.2 Retention Studies

2.2.1 The Effect of Surfactant and Carrier Volume on Spray Retention by O. latifolia

A uniform group of previously sprouted bulbs of O. latifolia were planted on 23 November 1984 at an average depth of 4 cm in 15 cm plastic pots containing 2:1:1 clay, sand and peat soil mixture. The pots were watered every two to three days from above. The walk-in growth room in

which the plants were grown was set at 25/15°C day/night temperatures. Lighting, at 16-hour photoperiod, was provided by Sylvania Gro-Lux flourescent lamps[6] which supplied about 207 μEs^{-1} m^{-1} photosynthetic photon flux density (PPFD) as determined by a quantum sensor.[7]

The dye[8] used in all experiments was water soluable while the surfactant[9] was non-ionic, obtained as a viscous liquid.

Spray solution was made up of 0.5% w/v dye dissolved in distilled water where no surfactant was present and with 0.25% v/v Tween20 where the surfactant was included.

Uniform plants were selected from the batch for the spraying experiments which were carried out between 15 January and 5 February 1985.

Prior to spraying, a calibration line of the dye was established for estimating the concentration of the dye retained by the plants. The calibration line was prepared as follows:

1. A stock solution of the dye was made by dissolving 0.1 g of dye in distilled water with 0.25% Tween-20. This solution had a concentration of 100 parts per million (ppm) w/v of the dye in water.
2. A series of 10 standard solutions ranging between 1.0 and 10.0 ppm was prepared from the stock solution.
3. A blank consisting of water without dye was set aside.
4. Absorbance values for each of the standard solution were determined using a spectrophotometer[10] at 630 nm, which was shown to

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6 VHO/WS Sylvania, New England.
7 Model L1-190 SB, L1-COR inc., Lincoln, Nebraska.
8 Niagara Sky Blue 6B. A product of the Allied Chemical Industries.
9 Polyoxyethylene sorbitan monolaurate (Tween-20). A product of Atlas Chemical Company.
10 Model PM QII, Carl Zeiss, Wuerttemberg, West Germany.

be the absorption peak of the dye.

5. Absorbance values of three different replications were determined and a mean value calculated for the final plot.
6. A linear regression equation between the amount of dye (ppm) and absorbance was determined (Figure 1).

The plants were sprayed in a cabinet sprayer at three different carrier volumes with and without a surfactant. Teejet flat spray nozzle tips 8002, 8004 and 8006 delivering 137, 250 and 327 l ha^{-1}, respectively, at 275 kPa of pressure were used. Each pot, which represented one treatment, was sprayed singly. The experiment was replicated four times.

Sprayed plants were excised 2 cm above the soil surface after the spray had dried. The spray was washed off by immersing the plant parts in distilled water containing 0.25% v/v Tween-20 used as a wetting agent. The washings from any one replicate were filtered through Whatman No. 1 (quantitative) filter paper and the solution brought to a volume of 125 or 250 mls in a measuring cylinder.

After the rinsing water had evaporated, fresh weight of plant parts per replicate was determined. The surface area of the leaves was measured using leaf area meter.[11] Dry weight of the leaves, alone or together with the rest of the aerial parts, were determined after drying at 80°C for at least 48 hours. The concentration of dye in each washing was estimated by measuring the absorbances in a spectrophotometer at 630 nm. From the initial concentration of the dye in each spray solution, the volume of washings (adjusted for dilution where necessary) and the

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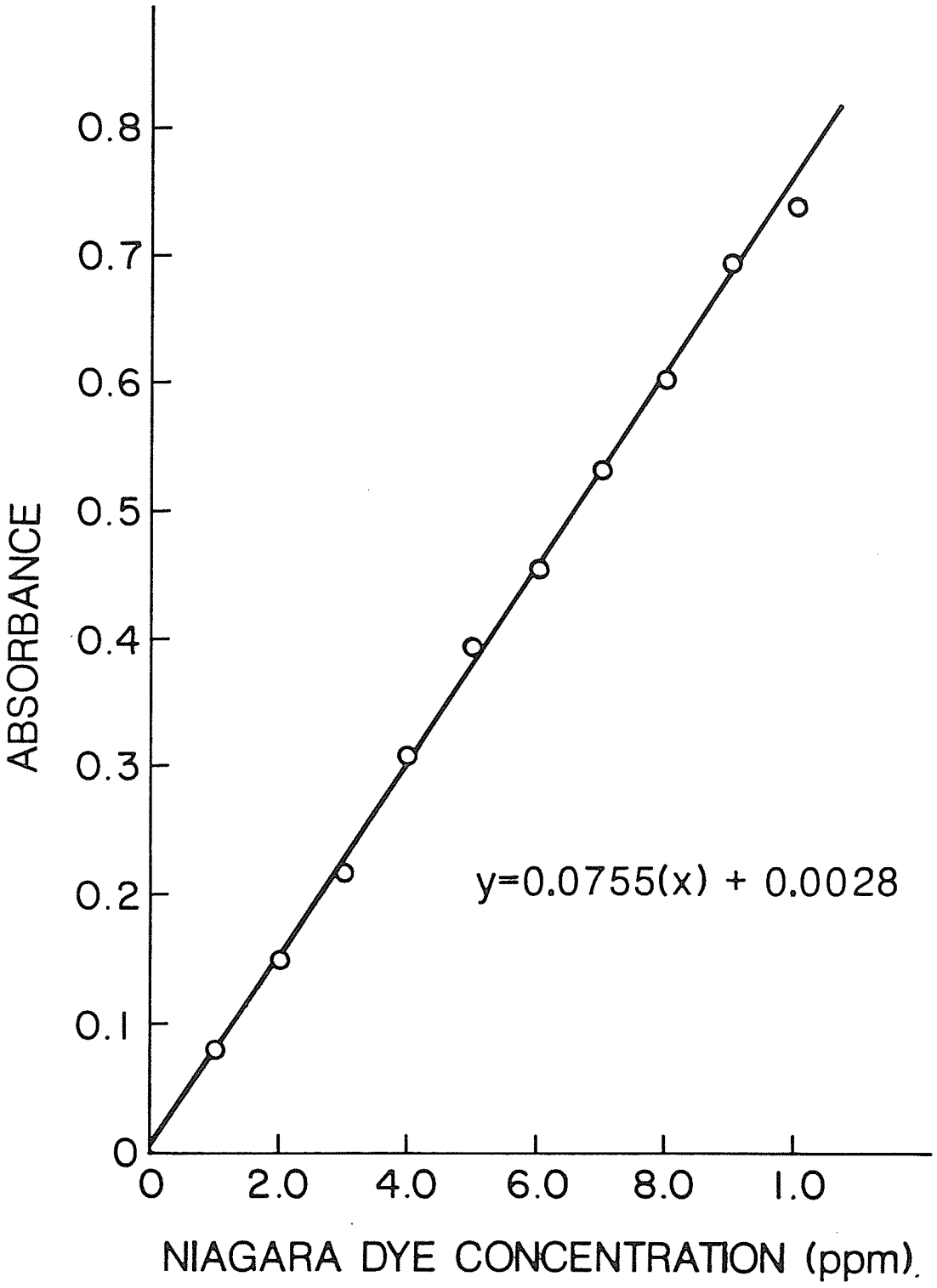
[11] Model L1-3000, Lambda Instruments Corp., Lincoln, Nebraska.

Figure 1. Calibration line for Niagara Sky blue solution.


ABSORBANCE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2.0
4.0
6.0
8.0
1.0
NIAGARA DYE CONCENTRATION (ppm)
y=0.0755(x) + 0.0028

estimated concentration of dye in the filtrate, the amount of dye retained per replicate was calculated using a formulae modified from Hibbitt (1969).

$$\text{Spray retained per replicate } (\mu l) = \frac{\text{Concentration (ppm)} \times \text{Volume of wash solution} \times 10}{\text{Concentration of dye in spray solution (w/v\%)}}$$

Final retention values were expressed as µl of spray solution retained per cm^2 of leaf area.

The experiment was repeated three times using plants raised from the same batch. The experimental design was a 3x2 factorial. The results are presented as a means of four replicates per treatment. The data were analyzed statistically and the least significance difference (LSD) test was used as the test of significance. Only differences at 5% level of probability were considered meaningful.

2.2.2 The Effect of Leaf Orientation on Spray Retention of *O. latifolia*

Oxalis latifolia plants used in this study were raised in a similar manner to the description in Section 2.2.1. The bulbs were planted on February 4 and spraying was carried out on April 3 and 25 of 1985, using a water-soluble dye (0.5% w/v) with inclusion of 0.25% v/v Tween-20.

Leaves of *O. latifolia* exhibit nyctinastic leaf movements (Ball, 1969) in which leaf orientation may change from a normal horizontal to a vertical or near vertical position. Folding in this experiment was initiated by withholding the watering about a week prior to spraying. The plants in which leaves were unfolded were watered regularly every two days.

A photographic method was used to compare the area of folded and

unfolded plants. A few days prior to the spraying date, representative plants were randomly taken from the folded and unfolded group for photography. This method proved difficult in that some folding was initiated by disturbance of the plants during movement. To overcome this problem and probably ensure more accuracy, both sets of folded and unfolded plants were removed from the growth room and each photographed at both folded and unfolded position. The two sets of photographs were in close agreement and thus subsequent photography was carried out from a single plant at both folded and unfolded positions. Two different plants were used at each occasion. The plants (based on single leaf images) were photographed from above to determine the plan view or the vertically projected area for both folded and unfolded plants.

A camera fitted with a 50 mm macrolens was used. In order to avoid extra heat (which could initiate leaf folding), a fast black and white film, Kodak Tri-x pan with ASA No. 400 was used. When the prints were made, the leaf images were cut out for determination of weights and surface areas. The change in leaf area due to folding was recorded as a ratio of the weight or surface area of the cut out image.

Spraying was done using flat-fan nozzle delivering 250 l ha^{-1} at 275 kPa of pressure. The two treatments (folded and unfolded) were alternated in the spraying operation. After spraying, steps similar to those of Section 2.2.1 were followed in determining the amount of spray retained in µl per cm^2 of leaf area.

The experiment was arranged as a completely randomized design with two treatments, i.e. folded and unfolded. Each treatment was replicated three times and two pots or plants were used per treatment. A repeat of

the experiment was carried out on 25 April.

A third repeat was done using outdoor raised plants sown on August 26 and sprayed on 11 October 1985. Leaf area measurements were done using a leaf area meter. A total of eight replicates consisting of two plants per treatment were sprayed after the photographic details were taken.

2.3 Control Studies

Plants used in this experiment were generated from sprouted bulbs of *O. latifolia* planted outdoors on 28 June 1985 at the Department of Plant Science, University of Manitoba. The bulbs were planted 4 cm deep in 25 cm diameter plastic pots containing a 2:1:1 mixture of clay, sand and peat. About 1.0 g ammonium phosphate (16-20-0) was broadcast and shallowly incorporated by hand in each pot. Watering was provided every two-three days during the dry periods of the month of July, but none was required during the rainy month of August.

On August 28, two months after planting, the following treatments were applied:

1. Control
2. Defoliation
3. Paraquat (0.625 kg a.i/ha)
4. Glyphosate (1.068 kg a.i/ha)

The rates of paraquat and glyphosate used were based on some preliminary tests which showed that the rates controlled foliar growth.

Before the treatments were applied, six plants were selected at random for an assessment of the numbers as well as fresh and dry weights

of the new bulbils formed. The remaining pots were divided equally between each of the 2 experiments consisting of 24 pots each. In each experiment the plants were randomly allocated to each of the 4 treatments. Each treatment consisted of 6 replicates, which were harvested two weeks and the remaining ones four weeks after application of the treatments in the first and second experiments respectively.

Defoliation was carried out twice (with an interval of two weeks) about 2 cm from the ground level using a laboratory scalpel.

Paraquat and glyphosate were applied to the foliage in a cabinet sprayer using an 8002 Teejet nozzle with an output of 154 l ha^{-1} at 275 kPa of pressure.

The arrangement of the pots after treatments followed a complete randomized design in which each pot represented a plot.

Plants were harvested two weeks after treatment and assessed for numbers and fresh weights of the new bulbils as well as fresh and dry weights of the total top growth where applicable. Ten or fewer bulbils per pot were preserved for viability tests while the remaining ones were subjected to germination tests. Viability tests were conducted as follows:

Bulbs of O. latifolia (previously stored in moist conditions) were cut longitudinally to expose all the parts to the tetrazolium solution. The test solution was prepared by dissolving 5% w/v of the tetrazolium chloride salt in a neutral (pH 7.0) phosphate buffer. The split bulbs of each treatment were immersed in the test solution contained in 50 ml conical flasks. Bulbs from each replicate were submerged in a separate flask. The flasks were transferred to an

incubator maintained at 25°C in darkness. The bulbs were removed from the solution and viewed under a dissecting microscope after one hour and two hours. Bulb symptoms were recorded. Photographs of the viewed bulbs were taken on a color film.

Germination assessment was done by placing the bulbils in soil of the same type as was used to grow the plants. The germination pots were transferred to a growth room at 25/15°C day/night temperatures and 16-hour day length. Germination counts were taken a month after planting.

Similar assessments were made of the plants harvested four weeks after application of treatments except that no germination tests were conducted for the glyphosate treated plants.

3. RESULTS

3.1 Developmental and Structural Studies of O. latifolia H.B.K.

3.1.1 Growth Analysis

The life cycle of Oxalis latifolia H.B.K. was studied over a period of fourteen weeks in outdoor-grown plants as described. Three major phases of growth were distinguished during the fourteen week study period. These were: the first or early vegetative phase, the second or reproductive phase, and the third or senescence phase. The first and second phases lasted until weeks 5 and 12, respectively, while senescence was evident starting the 13th week.

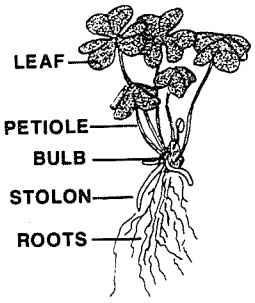
Emergence of O. latifolia bulbs started in the second week and was complete by the third week after planting, when harvesting commenced. The underground portions of these young O. latifolia plants consisted of the parent bulb, adventitious roots and stolons. The above-ground parts consisted of leaves and their petioles (Plate 1, Week 3).

The mean weekly temperatures between the third and fifth week as shown in Figure 2 were relatively low, ranging between 16°C and 15°C.

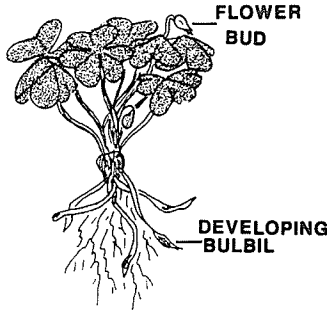
During the first phase of the life cycle of O. latifolia, the numbers of various plant organs did not increase significantly. Total dry weight per plant increased slowly from initial planting to the fifth week (Figure 3). At the end of this phase, the plants had ten fully expanded leaves and three young peduncles bearing flower buds (Plate 1, Week 5). At week 5, the partitioning of the total plant dry weight was 44% for leaves and their petioles, 37% for parent bulb and the remainder

Plate 1. Some important growth stages in the life cycle of *O. latifolia*.

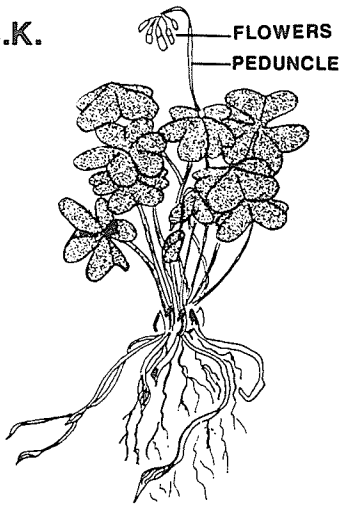
GROWTH STAGES OF OXALIS LATIFOLIA H.B.K.



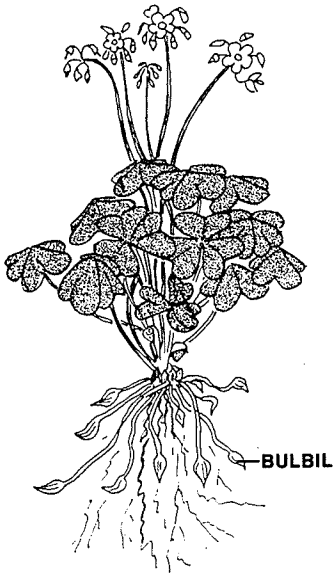
3 WEEKS



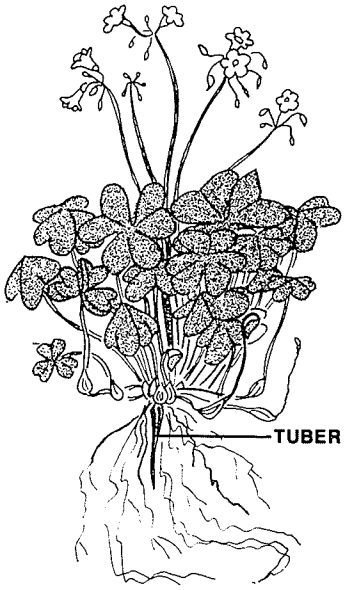
5 WEEKS



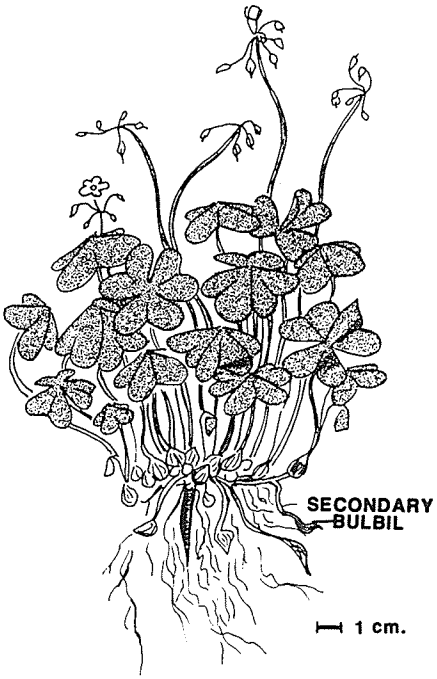
6 WEEKS



7 WEEKS

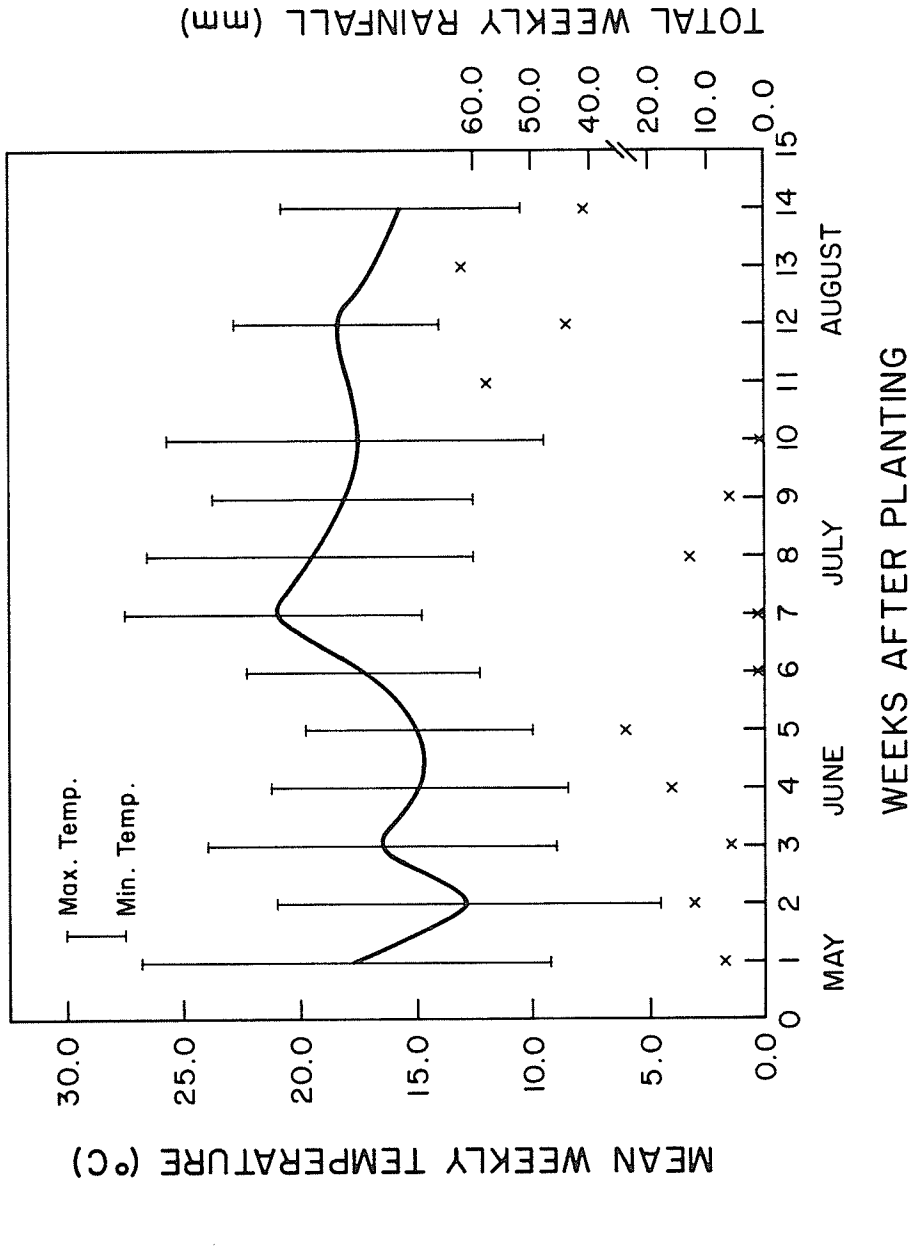


8 WEEKS



14 WEEKS

Figure 2. Mean weekly temperature (I) and total weekly rainfall (x) recorded between May 21 and August 29 of summer 1985.


MEAN WEEKLY TEMPERATURE (°C)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Max. Temp.
Min. Temp.
TOTAL WEEKLY RAINFALL (mm)
60.0
50.0
40.0
20.0
10.0
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
MAY
JUNE
JULY
AUGUST
WEEKS AFTER PLANTING

was shared between the roots, stolons and the young peduncles where they had been formed. The underground/aerial ratio (Table 3) declined during the first phase of the life cycle of this species. The presence of the parent bulb was responsible for the maintenance of this ratio above 1.0.

Relative growth rate (RGR) of the whole plant showed a net increase, although there was a decline between weeks 4 and 5. This trend of decline in RGR at weeks 4 and 5 was observed in all the plant organs except in the parent bulb and the peduncles (Figure 4).

A small increase in leaf area occurred between weeks 4 and 5, but leaf area ratio (LAR) and the corresponding net assimilation rate (NAR) increased steadily (Figures 5 and 6) throughout the first phase. There was a very high positive correlation ($r = 0.91$) between the LAR and NAR during this early or vegetative phase of the life cycle of O. latifolia.

The sixth week (after planting) appeared critical in the life cycle of this species because it was during this period that the plants seemed to enter into the reproductive phase. Between weeks 5 and 6, there was a sharp increase (1.4 times) in the number of leaves. The leaf area and the weight of the stolons doubled. Most of the plants during this week were in 20-leaf stage. The longest stolon was noted to have attained 5.4 cm compared with 2.7 cm for the first harvest. There was an average of three bulbils per plant and 84% of the bulbils had formed some developing 'petiole-like' structures (Plate 1, Week 6). From the time of their formation, the bulbils showed the highest RGR (Figure 4) which remained consistently higher than that of any other plant organ for the remainder of the growing season except during week 9 when the RGR of leaves and peduncles exceed that of the bulbils. The sixth week was also

TABLE 1. Leaf and peduncle production in Oxalis latifolia.

Weeks After Planting	PARENT BULB		PRIMARY BULBIL		
	Leaf Number Per Plant	Total Leaf Area (cm^2)	Leaf Number Per Plant	Total Leaf Area (cm^2)	Peduncle Number
3	7 (± 0.4)	26 (± 1.7)	0	0	1 (± 0.3)
4	10 (± 0.6)	47 (± 3.1)	0	0	1 (± 0.3)
5	10 (± 0.7)	58 (± 4.2)	0	0	3 (± 0.3)
6	14 (± 0.7)	117 (± 8.8)	6 (± 1.1)	2 (± 1.1)	7 (± 0.4)
7	13 (± 0.9)	159 (± 11.7)	7 (± 1.1)	20 (± 6.3)	10 (± 0.7)
8	16 (± 1.2)	227 (± 15.5)	8 (± 1.0)	41 (± 6.7)	17 (± 1.4)
9	15 (± 1.0)	217 (± 14.1)	10 (± 1.1)	80 (± 9.3)	16 (± 0.5)
10	17 (± 1.1)	268 (± 25.2)	10 (± 2.6)	80 (± 24.1)	18 (± 2.2)
12	24 (± 1.3)	405 (± 28.0)	20 (± 2.9)	187 (± 23.3)	23 (± 1.8)

(±) Standard error

TABLE 2. Stolon and bulbil production in Oxalis latifolia.

Weeks After Planting	Number of Stolons	Number of Bulbils	Percent Bulbils With Leaves
3	5 (± 0.4)	0	-
4	7 (± 0.9)	0	-
5	8 (± 0.9)	0	-
6	15 (± 1.9)	3 (± 0.7)	84
7	25 (± 2.4)	9 (± 1.2)	56
8	23 (± 1.4)	14 (± 1.4)	36
9	29 (± 1.9)	23 (± 1.7)	23
10	30 (± 3.3)	24 (± 2.3)	28
12	38 (± 2.5)	33 (± 2.2)	35
14	46 (± 2.5)	42 (± 2.2)	37

(±) Standard error

TABLE 3. Dry weight of the underground and aerial organs of O. latifolia and their ratio at various harvests.

Weeks After Planting	Total Underground Dry Weight[1] (g)	Total Aerial Dry Weight[1] (g)	Underground/ Aerial Ratio
3	0.235 (± 0.02)[2]	0.155 (± 0.02)[2]	1.516
4	0.269 (± 0.02)	0.222 (± 0.01)	1.212
5	0.349 (± 0.03)	0.292 (± 0.03)	1.195
6	0.443 (± 0.03)	0.612 (± 0.04)	0.723
7	0.926 (± 0.07)	1.203 (± 0.09)	0.770
8	1.670 (± 0.08)	1.779 (± 0.11)	0.939
9	2.513 (± 0.16)	2.147 (± 0.10)	1.170
10	3.081 (± 0.30)	2.692 (± 0.25)	1.145
12	5.267 (± 0.35)	4.064 (± 0.27)	1.296
14	6.704 (± 0.81)	3.739 (± 0.32)	1.793

[1]Mean of eight replicates per harvest.

[2](±) Standard error per harvest.

Figure 3. Dry weight accumulation in various organs of *Oxalis latifolia*.

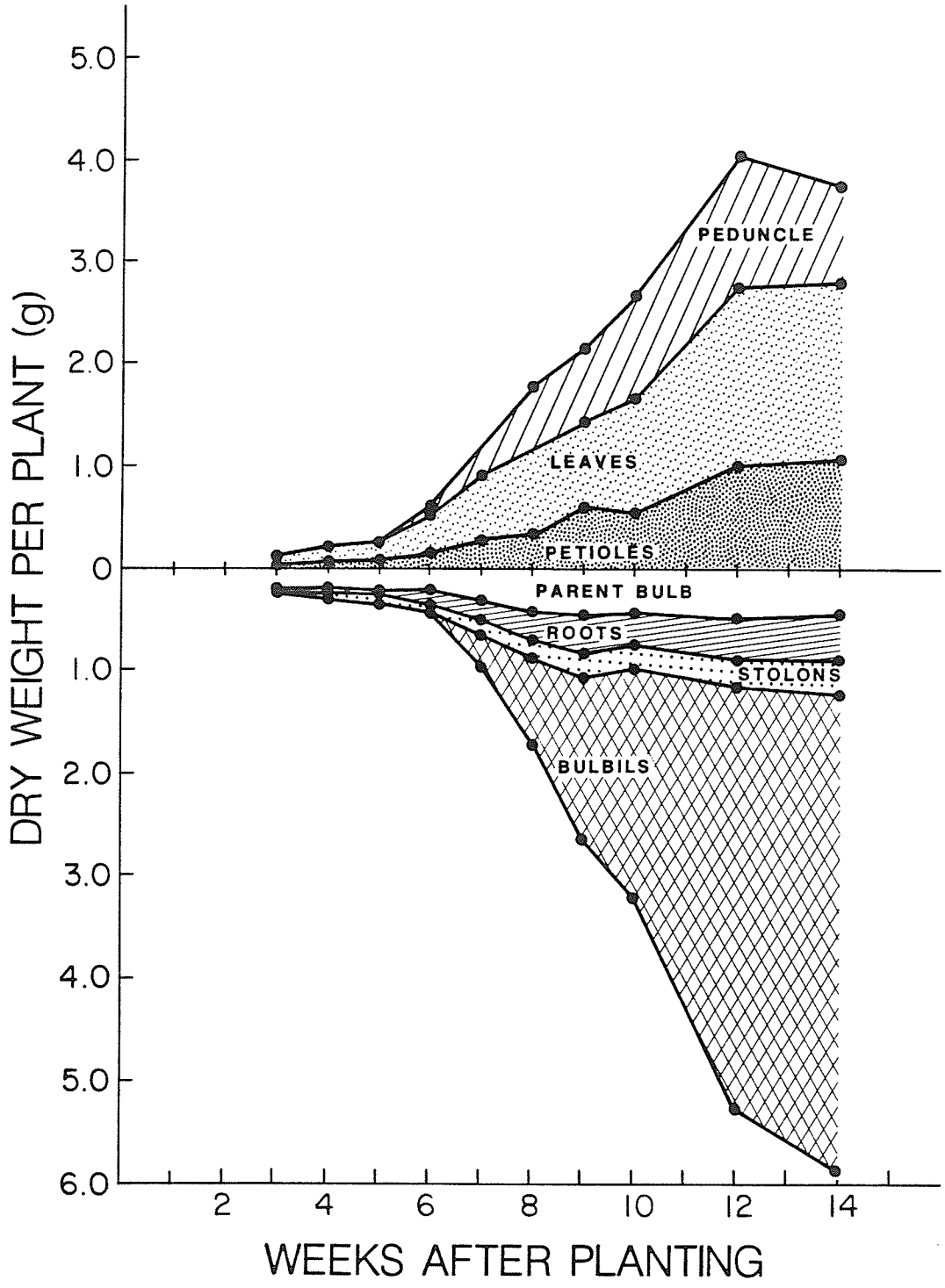

5.0
4.0
3.0
2.0
1.0
0
1.0
2.0
3.0
4.0
5.0
6.0
DRY WEIGHT PER PLANT (g)
PEDUNCLE
LEAVES
PETIOLES
PARENT BULB
ROOTS
STOLONS
BULBILS
2
4
6
8
10
12
14
WEEKS AFTER PLANTING

Figure 4. The relative growth rate of various organs of *O. latifolia*.

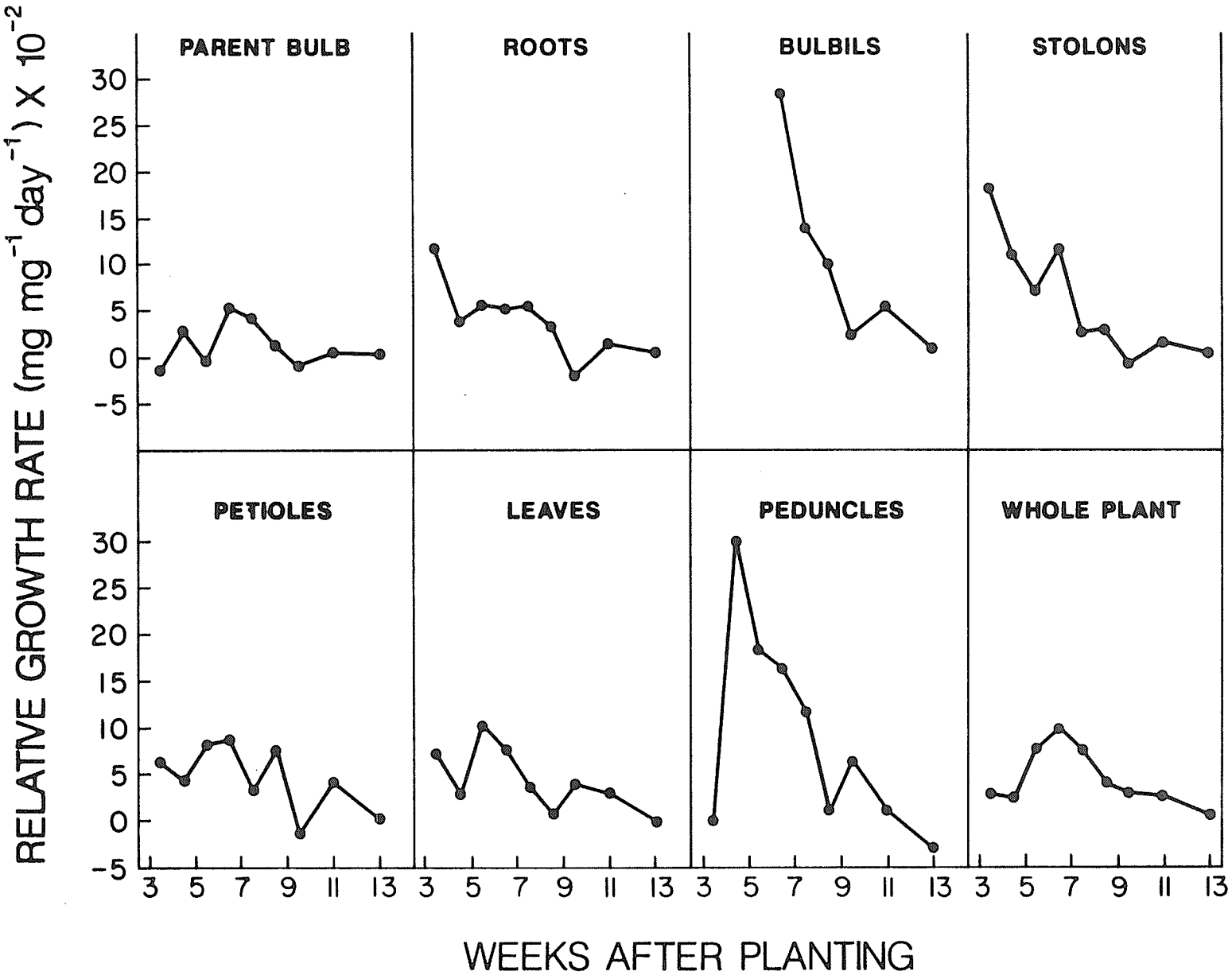
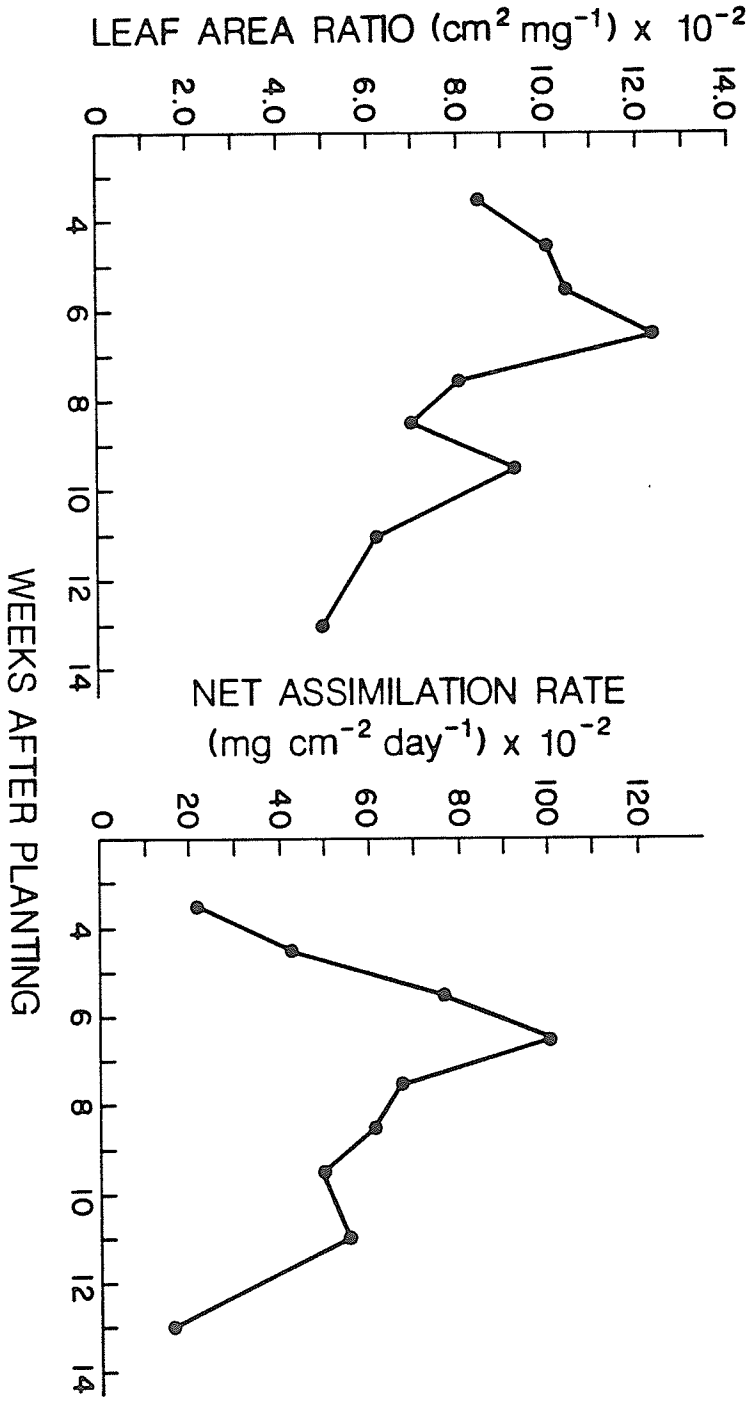

PARENT BULB
ROOTS
BULBILS
STOLONS
PETIOLES
LEAVES
PEDUNCLES
WHOLE PLANT
RELATIVE GROWTH RATE (mg mg^{-1} day^{-1}) X 10^{-2}
30
25
20
15
10
5
0
-5
3
5
7
9
11
13
WEEKS AFTER PLANTING

Figure 5. Leaf area ratio of Oxalis latifolia at various harvest intervals.

Figure 6. Net assimilation rate of O. latifolia at various harvest intervals.


LEAF AREA RATIO (cm² mg⁻¹) x 10⁻²
0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
4
6
8
10
12
14
NET ASSIMILATION RATE
(mg cm⁻² day⁻¹) x 10⁻²
0
20
40
60
80
100
120
4
6
8
10
12
14
WEEKS AFTER PLANTING

critical for the underground/aerial ratio because it declined to the lowest value of 0.723 (Table 3), subsequently rising for the rest of the growing season. LAR and NAR values were highest between weeks 6 and 7, decreasing thereafter (Figure 5 and 6).

The second or reproduction phase of the life cycle of O. latifolia commenced between weeks 6 and 7, but was more evident at the seventh week (Plate 1, Week 7). This phase was characterized by an initial sharp increase in the number of the reproductive structures, namely, the bulbils. There was a less sharp increase in the number of plant organs associated with the mother plant while those associated with the bulbils, particularly the leaf area, steadily increased (Tables 1 and 2).

The dry weight of various organs (Figure 3) rose slowly in the parent bulb, the roots and the stolons. In the remaining organs, the rate of dry matter accumulation was most rapid in bulbils > peduncles > leaves > petioles. The dry weight of the leaves remained higher than that of any other plant organ during the second phase of growth until week 8, after which the bulbils took over for the rest of the growing season.

During the second or reproductive phase of the life cycle, the RGR of the whole plant steadily declined (Figure 4) until senescence. The decline was most rapid in the bulbils and peduncles. All the plant organs showed a sharp but short-lived rise in RGR between weeks 10 and 12, except in the case of leaves and peduncles where the rise occurred a week earlier.

The LAR and NAR (Figures 5 and 6) show a declining pattern similar to that of RGR described above, except for the occurrence of a temporary

rise which was observed between weeks 9 and 10 for the LAR and a week later for NAR. The correlation between LAR and NAR for the second phase of the life cycle was 0.61 compared with 0.91 for the first phase.

The third and last phase of the life cycle of O. latifolia was evident in the fourteenth week (Plate 1, week 14). Prior to onset of senescence, there was a second 'flush' of new leaves which were produced mainly by the bulbils (Table 1). Mean weekly temperatures fell from about 18°C to 16°C during weeks 12 and 14. A heavy rainfall (109.6 mm) was recorded during this two-week period (Figure 2).

During this phase, dry weight (Figure 3) of each of the plant organs either remained constant or showed a very small increase. Only the bulbils showed a higher dry weight gain. By contrast, the dry weight of the peduncles declined. Although Table 1 shows a large value of leaf area, it was noted that most of the leaves were yellow, but more bulbils (37%) as shown in Table 2, had produced new leaves. The parent bulb had developed some scale-like structures on the upper side which were not conspicuous in the previous harvests. This structure contributed to the increased underground/aerial ratio (Table 3) which was at the highest value since harvesting began.

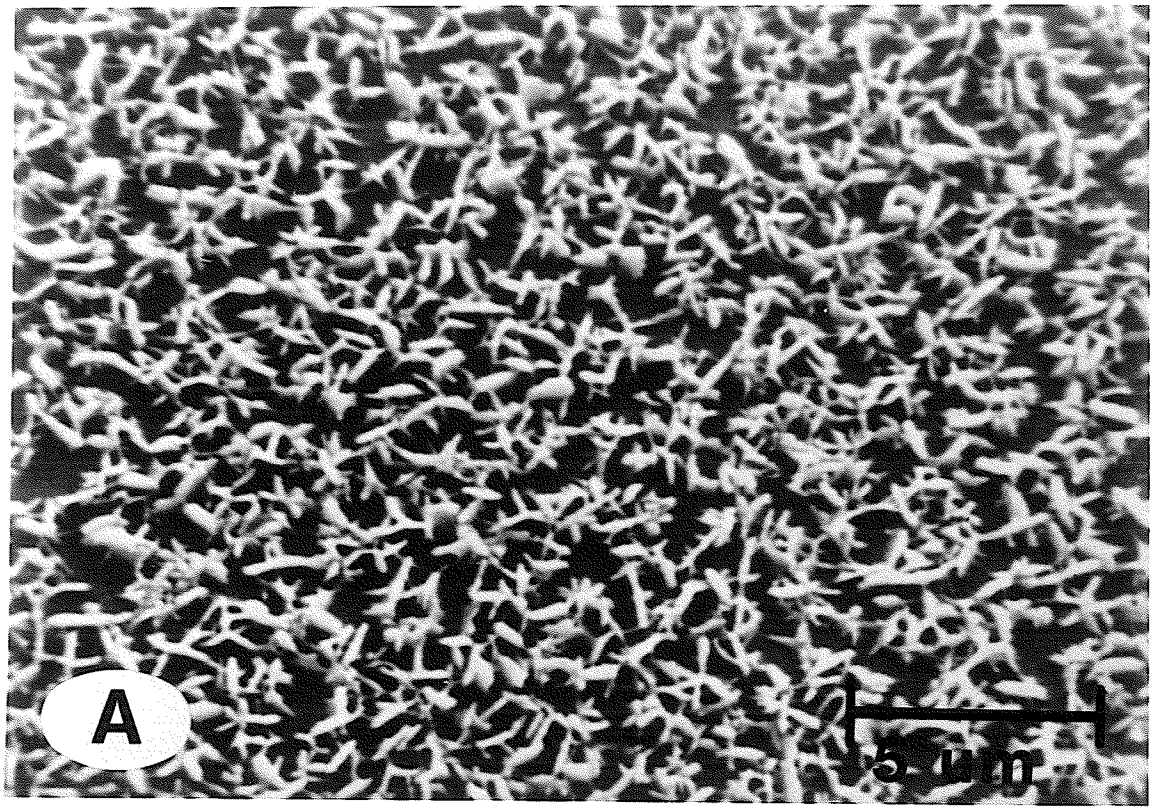
The RGR, LAR and NAR (Figures 4, 5 and 6, respectively) all declined consistently between weeks 12 and 14. The correlation between LAR and NAR for the entire growing period (14 weeks) was 0.71.

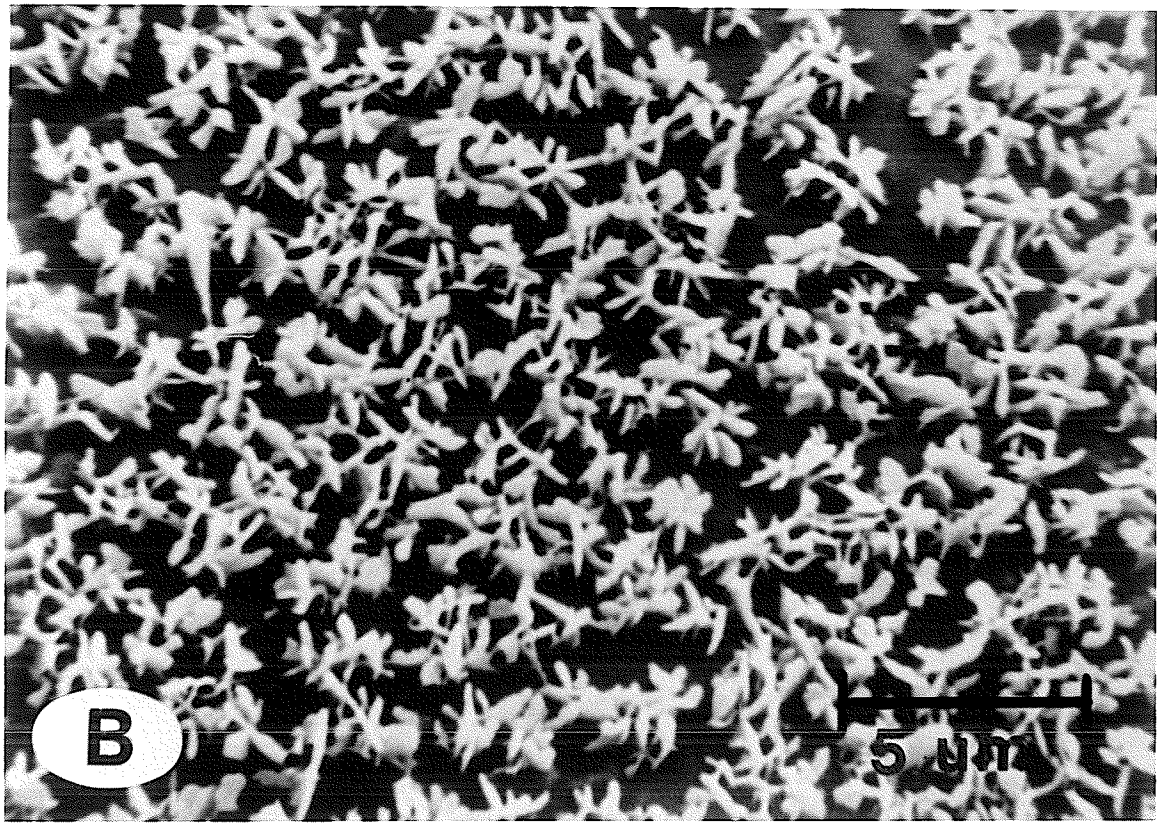
3.1.2 Scanning Electron Microscopy (SEM)

All details of the scanning electron microscopy (SEM) presented here are based on visual assessment. There were no major differences between the appearance of *O. latifolia* leaves of different ages (1 to 10 weeks). However, the micrographs showed that younger leaves had a heavier wax deposit than the older leaves. This judgement was enhanced by the fact that even at the highest magnification (x 10,000) the waxes of the younger leaves were still clustered together, leaving few or no zones of smooth wax.

A comparison of micrographs of young, middle-aged and old leaves showed that middle-aged (4 weeks) leaves exhibited a more uniform and elaborate wax coverage and were thus chosen for illustration (Plate 2). Further examination of this plate shows that leaves of *Oxalis latifolia* display a platelet type of crystalline wax ultrastructure. Waxes of the older leaves displayed a less well-defined ultrastructure, commonly appearing melted at high magnification. The melting created many artifacts which made the interpretation of the micrographs more difficult. Except in the case of younger leaves, the adaxial surface (Plate 2A) seemed to contain a heavier wax deposit than the abaxial surface (Plate 2B). There was no difference in visual appearance between micrographs of *Oxalis* leaves grown during the 1984 and 1985 summer periods.

Plate 2. Scanning electron micrographs of the adaxial (A) and abaxial (B) leaf surfaces of *O. latifolia*


A
5 μm


B
5 μm

3.1.3 Quantitative and Qualitative Assessment of Epicuticular Wax in *O. latifolia*

The quantities of epicuticular wax from the leaf samples taken during the 1984 and 1985 end of summer periods are shown in Table 4. The two years were characterized by different weather patterns with regard to rainfall and temperature. The total rainfall during the months of July and August (when the plants were grown) were 37.9 mm and 25.3 mm for 1984, compared with 21.2 mm and 205.5 mm respectively for 1985. The mean monthly temperatures for July and August 1984 were 21.0°C and 22.2°C, compared with 19.2°C and 17.0°C, respectively, for similar months during 1985.

The results (Table 4) show that *O. latifolia* leaves taken from plants of an equivalent age had 1.5 times more wax during 1984 compared to those in 1985. Wax yields from indoor plants was intermediate by comparison with that of the two outdoor samples.

Qualitative assessment: Plate 3 shows a thin layer chromatogram of *O. latifolia* epicuticular wax photographed from one of the TLC plates. The Rf values of the main epicuticular wax components are presented in Table 5. Compared to the cabbage wax standard, the main components of *Oxalis* showed similarity in primary and secondary alcohols as well as the esters. In terms of Rf values, the hydrocarbon components of epicuticular wax differed between the two species with *O. latifolia* displaying a higher Rf value. A weak unidentified spot (R_f=50) was noted between the secondary alcohols and the esters.

TABLE 4. Quantity of epicuticular wax from indoor and outdoor-grown *O. latifolia* plants.

Date of Sampling		Mean Wax Yield ($\mu g\ cm^{-2}$)	SE (±)
Late August (Indoor)	1984	71.6	(6.9)
Late August (Outdoor)	1984	88.8	(21.4)
Early August (Outdoor)	1985	59.3	(11.7)

Plate 3. Thin layer chromatography of *O. latifolia* foliage epicuticular wax.

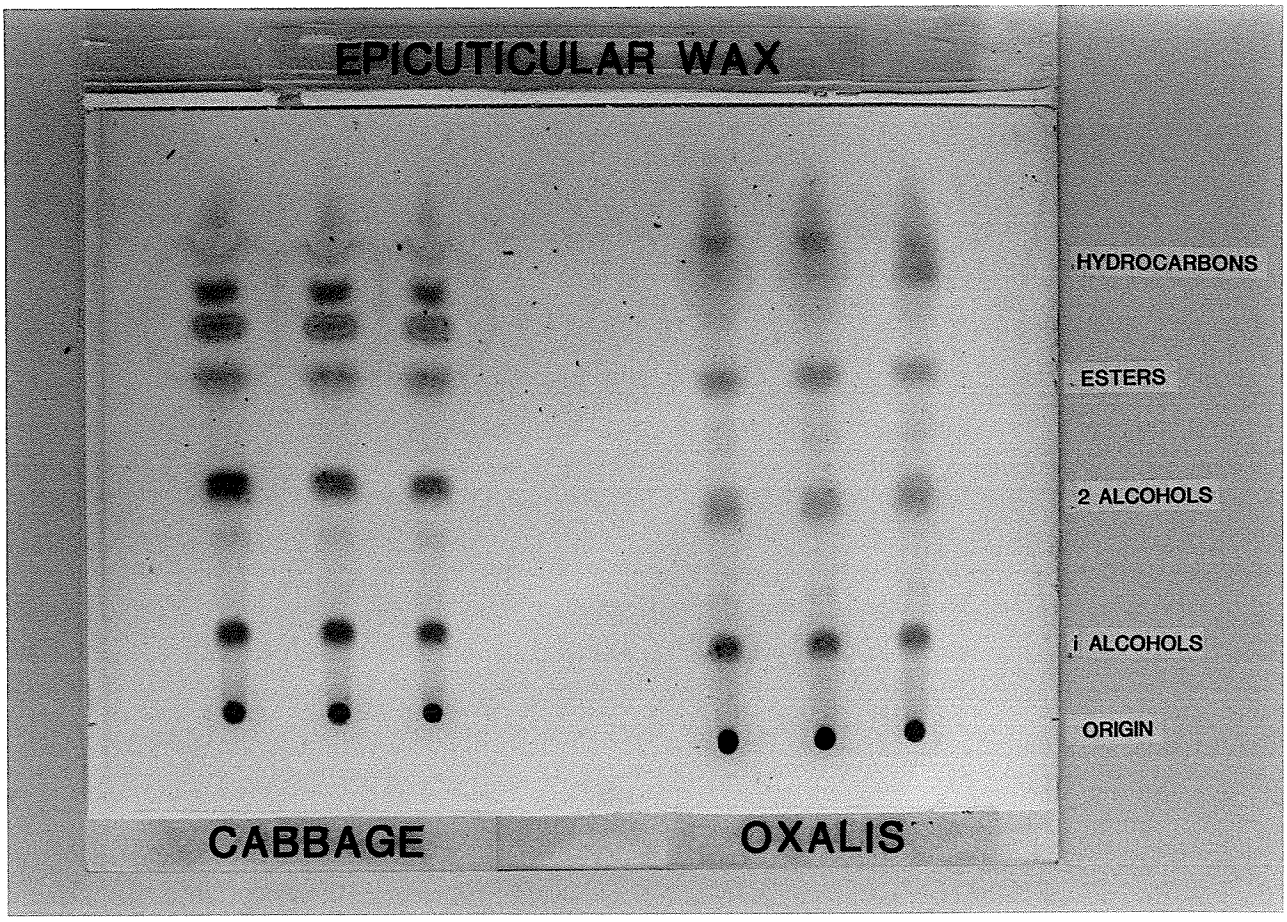

EPICUTICULAR WAX
HYDROCARBONS
ESTERS
2 ALCOHOLS
1 ALCOHOLS
ORIGIN
CABBAGE
OXALIS

TABLE 5. Thin layer chromatography of O. latifolia epicuticular wax.

Wax Component	R_f VALUES[1] x 100		
	Standard (Cabbage Wax)	Oxalis	SE[2] (±)
Primary Alcohols	(13.8)	14.2	0.17
Secondary Alcohols	(37.2)	36.7	0.49
Esters	(54.3)	56.5	0.56
Hydrocarbons	(70.2)	77.5	0.99

[1]Mean of six replicates.

[2]Standard error of the mean for each wax component.

3.2 Retention Studies

3.2.1 The Effects of Surfactant and Carrier Volume on Spray Retention of *O. latifolia*

Plants used in all the three trials were mature and large (about 500-1200 cm^2 of leaf area per plant), having few or no floral structures. After spraying, most of the spray droplets were retained on the large trifoliate leaves of this species. There were a few spray droplets on the inflorescence (where present), but no appreciable spray fell on the petioles or the peduncles. Spray retention, expressed as μl per g dry or fresh weight, was not found to be consistent in any of the three trials and, therefore, all data is presented as μl per cm^2 of leaf area.

The effect of carrier volume (137, 250 and 327 l/ha) and the presence or absence of a surfactant (0.25% v/v Tween-20) on spray retention are presented in Table 6. The retention values show that there was increased retention with increases in spray output with or without a surfactant. This observation was consistent in all instances except in the case of carrier volume of 327 l/ha in Trial 2.

Inclusion of the non-ionic surfactant, Tween-20, resulted in increased spray retention at all carrier volumes. The results in all three experiments showed that maximum retention occurred when spray output was high (327 l/ha) and the surfactant was included. There was a significant interaction between spray volume and surfactant.

TABLE 6. Effect of carrier volume and surfactant on the retention of Niagara sky blue solution in *Oxalis latifolia*.

(a)

Carrier Volume (l ha^{-1})	SPRAY RETENTION ($\mu l\ cm^{-2}$)[1]	
	No Surf.	Surf
137	0.117	0.180
250	0.177	0.367
327	0.291	0.466
Average	0.195	0.338

[1]Means of four replications per treatment.
$LSD_{0.05}$ for surfactant x carrier volume = 0.086 $\mu l\ cm^{-2}$.
$LSD_{0.05}$ for carrier volume = 0.061 $\mu l\ cm^{-2}$.
CV = 11.1%

(b)

Carrier Volume (l ha^{-1})	SPRAY RETENTION ($\mu l\ cm^{-2}$)[1]	
	No Surf.	Surf
137	0.138	0.224
250	0.256	0.417
327	0.190	0.428
Average	0.195	0.356

[1]Means of four replications per treatment.
$LSD_{0.05}$ for surfactant x carrier volume = 0.084 $\mu l\ cm^{-2}$.
$LSD_{0.05}$ for carrier volume = 0.028 $\mu l\ cm^{-2}$.
CV = 10.6%

(c)

Carrier Volume (l ha^{-1})	SPRAY RETENTION ($\mu l\ cm^{-2}$)[1]	
	No Surf.	Surf
137	0.169	0.302
250	0.275	0.349
327	0.314	0.601
Average	0.253	0.417

[1]Means of four replications per treatment.
$LSD_{0.05}$ for surfactant x carrier volume = 0.090 $\mu l\ cm^{-2}$.
$LSD_{0.05}$ for carrier volume = 0.064 $\mu l\ cm^{-2}$.
CV = 10.3%

3.2.2 Effect of Leaf Folding on Spray Retention

A photographic method was developed for use in estimating the amount of potential leaf area that would be exposed to an overtop spray as well as the various reductions in leaf area during the folding movements exhibited by this species. Plate 4 shows one of the photographs taken to determine the projected (plan view) area when the leaves were in horizontal or unfolded position (A), in folded or vertical position (D) and in the intermediate positions (B) and (C) for each of the two extremes.

The results for this folding experiment are presented in Table 7. The outdoor-grown plants showed a greater variability in the growth-stages of individual plants, but this was compensated for by using more (eight) replicates for treatment.

The projected leaf area (% folded to unfolded) showed that folding reduced the leaf area available for overtop spray by 82.4 to 75% in different plants. Spray retention of both indoor and outdoor-grown plants was similar in the unfolded plants, whereas, folding reduced the amount of spray retained by 74%, 55% and 49% in indoor trial 1, indoor trial 2 and outdoor plants, respectively. More spray (μl per cm^2) was retained when the leaves were in the unfolded or horizontal position than when in the vertical or folded position (Table 7).

Plate 4. Photographs of an O. latifolia leaf at four different positions.

A = unfolded position

D = folded position

B and C = intermediate positions

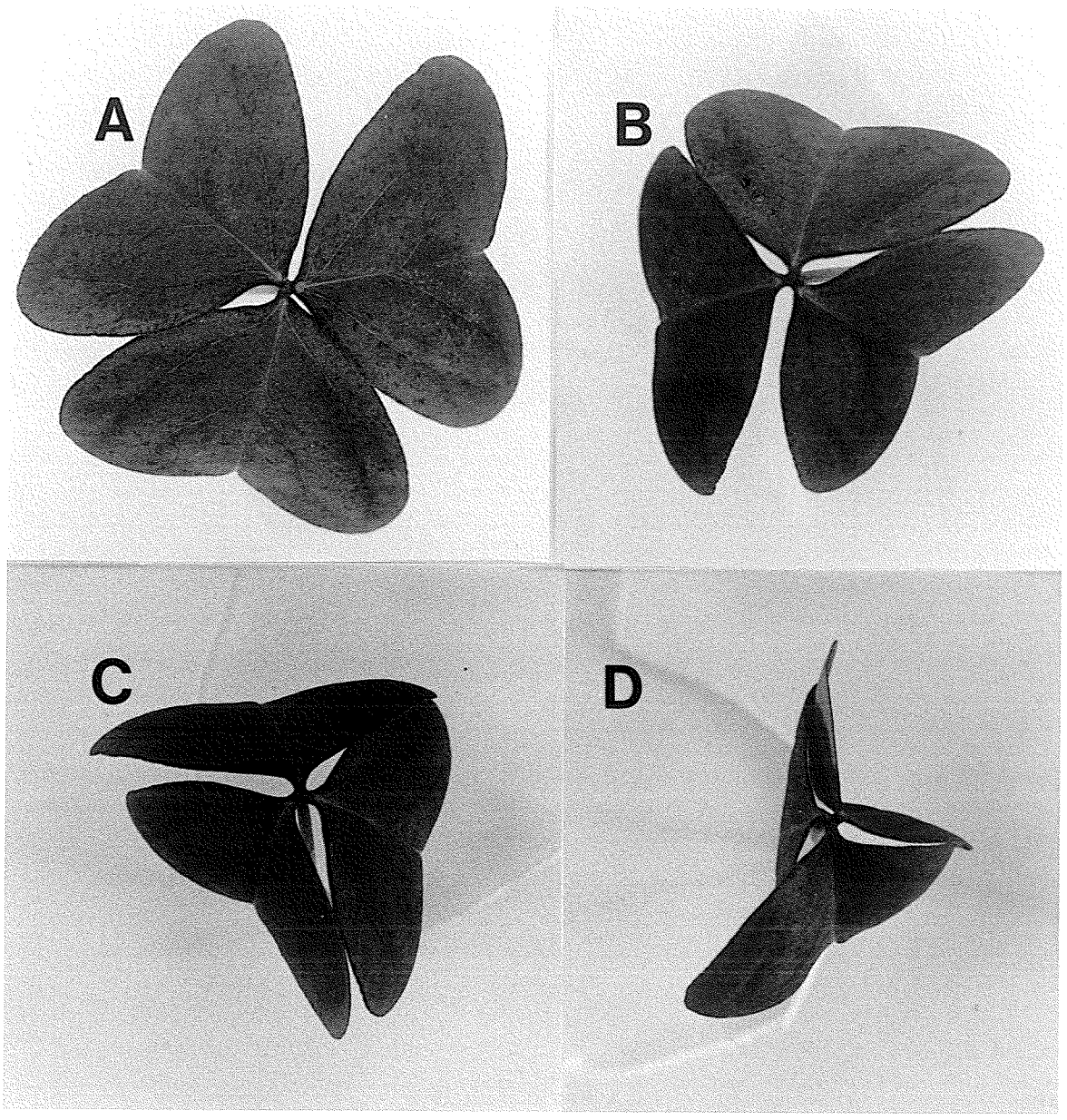

A
B
C
D

TABLE 7. The effect of leaf movements on spray retention of *O. latifolia* plants grown indoors and outdoors.

PARAMETER ASSESSED	INDOOR-GROWN PLANTS[1]				OUTDOOR-GROWN PLANTS[2]	
	Trial 1		Trial 2			
	Unfolded	Folded	Unfolded	Folded	Unfolded	Folded
Potentially exposed leaf area (cm^2)	504	502	812	678	419	357
% leaf area exposed (plan view)	100	23	100	25	100	17.6
Spray solution retained ($\mu l\ cm^{-2}$)	0.467	0.123	0.426	0.192	0.512	0.262
Retention (as % of unfolded)	100	26	100	45	100	51

[1]Mean of three replicates per treatment.

[2]Mean of eight replicates per treatment.

3.3 Control Studies

All the treated plants appeared healthy and vigorous in growth. Excavated below-ground plant organs consisted of relatively large contractile roots, roots and white primary bulbils attached to the parent bulb by stolons. Six plants which were harvested on the same day but prior to the application of the treatments, showed that the plants had produced an average of 34 bulbils per plant with 10.6 g and 5.8 g fresh and dry weights respectively. A summary of assessments made two and four weeks after the application of the treatments, is presented in Table 8.

Assessment After Two Weeks: Examination of the various plant parts for each treatment showed the following. In the control pots, vegetative plant parts continued in growth. Bulbils appeared white and all were still attached to the parent bulb by stolons. Defoliation treatment plants had some new foliage regrowth. Bulbils were white with some slight browning of the outer scales and all were attached to the parent bulb. Paraquat treatment bulbils appeared brown and some were severed from the parent bulb. There was no foliage regrowth in the glyphosate treatment. Bulbils had a brownish to black appearance and all were separated from the parent bulb due to decay of the stolons. The parent bulb and contractile roots had also started to decay.

Details of the assessment made show that on a dry weight basis (Table 8), the defoliated plants had 14.6 times more foliage regrowth than the paraquat treated plants. Bulbils recovered from all three treatments and those of control were fleshy and intact. There was no significant reduction in either the number or fresh weight of bulbils

TABLE 8. Effect of glyphosate, paraquat and defoliation on bulbil numbers and fresh weights of Oxalis latifolia, assessed 2 and 4 weeks after application of the treatments.

TREATMENT	ASSESSMENT AFTER 2 WEEKS			ASSESSMENT AFTER 4 WEEKS		
	Total Shoot Dry Weight (Regrowth) (g)	Bulbils Per Plant[1]	Fresh Weight of Bulbils Per Plant[1] (g)	Total Shoot Dry Weight (Regrowth) (g)	Bulbils Per Plant[1]	Fresh Weight of Bulbils Per Plant[1] (g)
Glyphosate (1.068 kg/ha)	0	31 a	14.78 a	0	18.3 a	6.24 a
Paraquat (0.625 kg/ha)	0.11	35.8 ab	14.53 a	0.14	41.5 b	13.34 b
Defoliation	1.61	34.5 ab	13.21 a	1.23	56.7 c	16.88 b
Untreated Control	N/A	44.2 b	23.19 b	N/A	72.2 d	35.75 c

[1]Means in a column followed by the same letter do not differ significantly at 5% level using Duncan's multiple range test.

per plant in the glyphosate, paraquat and defoliation treatments. Only the glyphosate treatment significantly reduced the number of bulbils per plant than control, whilst all the three treatments reduced the fresh weight of the bulbils per plant significantly compared to the control.

Viability tests conducted using tetrazolium chloride solution revealed that bulbils from the paraquat and defoliated pots had a reaction similar to that of control bulbils in the solution. The symptoms observed and recorded on coloured photographs showed that development of red stain (positive for viability) occurred more intensely around the stem and periphery of individual scales. By contrast, bulbils from the glyphosate treatment remained white (negative for viability) with conspicuous black areas scattered throughout the longitudinal section of each bulb.

Germination tests, terminated one month after planting, showed that there was 54% and 49% level of germination in bulbs from the defoliated and control treatments, respectively. By contrast, bulbs from the paraquat and glyphosate treatments did not germinate. Furthermore, only 7/10 or 85 out of 120 bulbs which were planted in the glyphosate treatment were recovered and 95%, that is, 6 out of 120 bulbs, were soft and decayed.

Assessment After Four Weeks: Control plants had similar characteristics to those of the two-week assessment plants in the preceding section. However, secondary bulbil formation was noted. In the defoliation treatment, half of the bulbils were still attached to the parent bulb while in the paraquat treatment, all stolons had decayed.

Brown colouration of the external protective scales was more prominent in both treatments. Glyphosate treatment bulbils were black in appearance and all were picked individually from the soil. The bulbils were soft and partly disintegrated. Out of 110 bulbils recovered from all the six pots, only 4 (or 3.6%) were hard and fleshy.

No vegetative regrowth was observed in the glyphosate treatment. Although regrown foliage had been removed two weeks prior to this assessment, the defoliated plants still showed a further regrowth which was 8.8 times higher (on a dry weight basis) than that of the paraquat treatment which had been left to grow for the four-week period. The number of bulbils were two-, three- and four-fold in the paraquat, defoliated and control plots, respectively, compared to the glyphosate plot. Fresh weight of the bulbils per plant significantly differed in all treatments except between the paraquat and the defoliated plants.

Viability tests with tetrazolium chloride solution were similar to those of the two-week assessment mentioned in the foregoing section.

The germination tests carried out for this assessment indicated that 58% and 53% of bulbils from the control and defoliation treatments, respectively, germinated whilst no bulbils from the paraquat treatment germinated.

4. DISCUSSION

4.1 Developmental and Structural Studies of O. latifolia H.B.K.

4.1.1 Growth Analysis

The present study has revealed that there are three main phases of growth in the life cycle of O. latifolia H.B.K. There were close similarities between the physiological processes observed in this study, assessed using mathematical growth analysis techniques, with those noted by Chawdhry and Sagar (1973) using $^{14}CO_2$ fixation studies.

At emergence, three weeks after planting, the parent bulb was the main plant organ accounting for 52% of the total dry weight of the plant. After emergence, young O. latifolia plants established very rapidly due to the presence of thick, fleshy scales (Jackson, 1960) which store large food reserves (Chawdhry, 1974). According to Chawdhry and Sagar (1973), the carbohydrate reserves are more important in the support of initial development of the adventitious roots but the scales never become completely depleted.

The first phase of growth of O. latifolia was predominantly vegetative with respect to organs arising from the new plant, that is, parent bulb excluded. During establishment, which lasts for most of the first phase, the increase in dry weight of various plant organs was slow (Figure 3). Production of reproductive structures (stolons) was initiated before or soon after the appearance of the leaves (Plate 1, week 3) whilst flowers appeared one or two weeks after emergence of the leaves (Plate 1, week 5). During the first phase, O. latifolia plants became fully established producing roots, stolons, petioles and leaves. At comparable growth stages, Chawdhry and Sagar (1973) suggested that the

leaves were net exporters of photoassimilates. The floral structures appear to have been the main sink for the assimilates during this phase. This is deduced from the occurrence of a very high RGR in the peduncles between weeks 4 and 5 (Figure 4). Low temperatures (15°C) between weeks 4 and 5 (Figure 2) may have been responsible for the lack of increase in the number of leaves as well as the slow rise in LAR (Figure 5). By comparison to the LAR, the values of NAR rose sharply between weeks 4 and 5 (Figure 6). This rise appears to have resulted from decrease in temperature. Since for a given species and stage of growth NAR has been found to increase with increase in ambient temperature, the rise in NAR between weeks 4 and 5 could not have resulted from decreased temperatures. An alternative explanation, therefore, would be that the rise in NAR was due to the presence of many mature leaves. This is evident from Table 1 which shows that the small increase in leaf area (11.0 cm^2) resulted from expansion of growing older leaves. In general, however, NAR was highly correlated with LAR throughout the first phase showing that the assimilatory ability per plant increased linearly during the first phase of growth.

Transformation of young plants from a vegetative phase to a reproductive phase occurred early in the life cycle, during the sixth week after planting (Plate 1, week 6). Seven out of all eight replicates (plants) harvested showed some swelling of the stolon apices which is an indication of bulbil formation. This early initiation of the formation of the bulbils is in close agreement with that observed by Chawdhry (1974). Working in North Wales, Britain, he found that bulbils were produced six weeks after planting. In the present study, most of the

stolons formed by the sixth week had not terminated into bulbils (Table 2) and thus continued to increase in length. Although individual stolon length is not reported in this study, it was observed that the stolons formed during the early phase of growth were comparatively longer than those formed in the latter growth stages. Thus, stolons fulfilled a role of spreading the bulbils which was achieved during the early stages of growth. Among the species of Oxalis which have been listed as serious weeds, O. latifolia Kunth, O. corymbosa and O. pes-caprae (Young, 1958), only O. latifolia H.B.K. does not form sessile bulbils. Formation of stolons is advantageous in two main ways. First, it enables a more rapid spread and colonization of new areas. Since most bulbils formed during the early phase produce leaves of their own (Table 2), such longer stolons allow these leaves to be located away from the mother plant. Spacial separation of shoot systems derived from mother and daughter plants is desirable since this avoids the mutual shading of the foliage and overcrowding of the bulbils which grow sessile to the parent bulb. Secondly, additional stolons develop from the axils of scale leaves present on the primary stolons and these terminate as secondary bulbils. This adds to the reproductive capacity of a species or clone such as O. latifolia H.B.K. in which long stolons are formed.

The second phase of the life cycle of O. latifolia was mainly reproductive. The highest rate of productive efficiency or NAR (Figure 6) was observed during the initial stages of this phase (week 7). This high NAR resulted from an increased LAR (Figure 5). At the seventh week, 44% of the total plant biomass was partitioned into leaves and their petioles. Bulbils present during the initial stages of the second phase (weeks 7

and 8) may still have been immature although the age at which bulbils mature still remains unknown. Young bulbils showed the highest RGR rivalled only by that of the peduncles, thus indicating that these floral structures were also an important sink for the assimilates. The RGR and NAR declined for all the latter stages of the second phase primarily due to the aging of the leaves. The rate of decline of these two parameters was probably influenced by the interaction of inevitable senescence and the weather conditions prevailing during that time. In a hot dry season, the decline may be very rapid, whereas, under more moist and warm conditions, there is a tendency for the mature plants to be rejuvenated. In this study, rejuvenation was observed between weeks 9 and 10 for the leaves and the peduncles. The flush of new leaves indicated by increased LAR (Figure 5) stimulated the RGR of all plant organs a week or two later (Figure 4) due to the "new" assimilatory leaves (Figure 6). Cox and Kerr (1981) working in New Zealand found that the second flush of leaves which occurred in autumn, (March) had a higher leaf density compared with those densities recorded in summer (January). Rejuvenation period in this study was very brief probably due to cool weather conditions which prevailed towards the end of the growing season (Figure 2). Another factor which affected the RGR and NAR within the second phase was the presence of new leaves produced by some bulbils (Table 1). These leaves probably export assimilates to rapidly developing organs (bulbils) via the parent bulb. The occurrence of aging and younger leaves lowers the correlation between LAR and NAR during the second phase of growth. This phenomenon is often referred to as ontogenetic drift in growth analysis (Hunt, 1978).

Chawdhry and Sagar (1973) found that during the second phase (which

lasted until 25-leaf stage), ^{14}C-labelled assimilates moved primarily to the bulbils and tubers. A similar pattern in movement of the assimilates in this study was indicated by a high RGR in the bulbils throughout the second phase. Tubers or contractile roots, however, though formed in the beginning of this phase did not show any significant gain in dry weight and thus their weights were considered together with the adventitious roots (Figure 3). Cool growth conditions were probably responsible for this phenomenon. Floral structures (peduncles bearing inflorescence) may have been an important sink for photosynthate since their allocation of biomass (expressed as percentage of total dry matter) rose from 13.5% to 19% between weeks 7 and 10, respectively. Jackson (1960) noted that plants growing in sunny conditions generally produce more inflorescences than those growing in shade.

The third or senescence phase which occurred thirteen or fourteen weeks after planting (Plate 1, week 14) was marked by yellowing and browning of most leaves. The increased number of bulbils with leaves (Table 2) resulted from excessive soil moisture caused by heavy rainfall (Figure 2) suggesting that the onset of dormancy in bulbils is partly determined by the moisture level of the soil. The occurrence of a scaly structure on the upper half of the parent bulb provides further evidence that the parent bulb has the ability to produce a new set of true and membranous scales (Jackson, 1960). However, at the end of the growing season, the parent bulb did not appear to have any ability to regenerate since no buds were observed on the scaly structure. It is also apparent that the true or fleshy scales of O. latifolia do not become depleted throughout the entire growing season. Starting in week 8, the dry

weight of these scales was determined separately. Although the results (of dry weight of scales) are not reported separately in the study, it was observed that the scales were fleshy in all ten harvests and the dry weight between weeks 8 and 14 remained fairly constant at 0.30g per plant.

At the onset of senescence, the reproductive capacity of O. latifolia had greatly increased due to branching of the primary stolons. The maximum number of stolons recorded in a single plant was 54, observed at the fourteenth week. During this week, 2 primary bulbils were observed to have produced stolons of their own which ultimately terminated in tertiary bulbils whose dry weight and numbers were considered with the primary and secondary bulbils (Table 2). Three primary bulbils had also produced flowers. Two generations of bulbils were thus observed at the conclusion of this study. This proves that several generations of bulbils may be produced even under field conditions if favourable growth conditions (temperature and rainfall) are prevalent.

4.1.2 Scanning Electron Microscopy

The scanning electron micrographs showed that the wax morphology of O. latifolia leaves display a platelet type of ultrastructure (Plates 2A and B). The morphology was similar in both young and old leaves, indicating that in the present study the general wax morphology in this species did not vary throughout the ontogeny of the leaves. The younger leaves, however, appeared to have a heavier wax deposit than the middle-aged and old leaves which probably means that most of the wax is secreted and formed when the leaves are still young and thus increas-

ing in surface area. A similar pattern of wax development has been observed in the foliage of certain plants including Trifolium species (Baker, 1982). By contrast, the size and distribution of wax platelets in Triticum vulgare seedlings were found to increase with increasing age of the leaves. In the present study, damage on waxes of mature leaves may have resulted from physical abrasion or melting caused by the radiant energy of the sun. Such damage may have been responsible for the ultrastructure observed in old leaves.

The adaxial (upper) surface of O. latifolia leaves (Plate 2A) displayed a higher density of wax platelets than the abaxial (lower) surface (Plate 2B). This observation is consistent with the findings of Holloway (1969) who concluded that the adaxial leaf surface of most plants has more wax deposits than the abaxial surface.

4.1.3 Quantitative and Qualitative Assessment of Epicuticular Wax in O. latifolia

The quantity of O. latifolia epicuticular wax was influenced by the environment under which the plants were grown. The weather conditions during the month of August in both years was more important in the formation of waxes since most of the leaves emerged and developed during that month. The results (Table 4) showed that plants assessed at similar stages of development but grown in a hot and dry environment (August, 1984) yielded more wax than those raised under more humid and cooler environment (August, 1985). The higher yields, however, may be more apparent than real when the standard errors (Table 4) are taken into consideration. Hull (1958) found that the total wax content of the mesquite (Prosopis juliflora) leaflets was highest in the plants grown

outdoors at the higher day and night temperatures. Higher temperatures and less humid conditions may enhance the development of a thicker wax deposit in O. latifolia foliage but final wax yields may be determined by an interaction of several environmental factors rather than any single factor independently. Temperature, relative humidity and light intensity are the main environmental factors influencing the thickness of wax deposits in most plants (Price, 1982).

The wax yields of O. latifolia plants grown in different environments ranged between 59.3 and 88.8 μg per cm^2 of leaf area. The degree of waxiness of foliage from different species is difficult to compare due to the differences in environmental conditions under which the plants grow. Baker (1982) has attempted to compare the mean wax yields of waxes obtained by different researchers working in different parts of the world with various species. According to this review, the four major categories of wax deposits are the sparse, thin, thick and exceptionally thick which yield 5-10, 10-25, 30-60 and 60-300 μg cm^{-2} of leaf area, respectively. When compared against this classification, it is evident that O. latifolia may be considered a plant with exceptionally thick epicuticular wax deposits, irrespective of the environment under which the plants were grown in the present study.

Qualitative Assessment: The unidentified component between the secondary alcohols and esters occupied a position in the chromatogram similar to that of ketones.

4.2 Retention Studies

4.2.1 The Effect of Carrier Volume on Spray Retention by O. latifolia

The use of dye solutions to estimate the amount of herbicide retained on plant foliage is common among researchers. Harper and Appleby (1984) found that the retention values obtained with dye (acid red) could be used for estimating the actual herbicide (asulam) retained on two different plant species. According to Hibbitt (1969), a dye suitable for use in retention studies should have the following characteristics; (a) does not affect the surface tension of water (b) light stable (c) easy to recover from foliage of the species used. In the present study, Niagara Sky Blue dye solution was used to imitate a herbicide solution (Hawton and Stobbe, 1971).

The amount of spray retained, expressed in μl per cm^2 of leaf area, increased with increase in spray output from 137 to 327 l/ha. This increase was independent of the presence or absence of a surfactant. Inclusion of a non-ionic surfactant (Tween-20) resulted in an increase in the amount of spray retained by O. latifolia plants at all carrier volumes and in all three repetitions (Table 6 a,b and c). The present study indicates that the peak level of spray retention by O. latifolia was not reached even at the highest carrier output (327 l/ha) in presence or absence of a surfactant. This conclusion is drawn from the fact that retention significantly increased between the medium (250 l/ha) and the highest (327 l/ha) volumes used in the study. The highest volume used was delivered by an 8006 Teejet nozzle tip which is considered to have a

relatively high output in conventional herbicide spraying for agricultural purposes. Based on retention studies, Blackman et al. (1958) classified the plant species which do not exhibit maximum retentivity as difficult-to-wet. The garden pea (Pisum sativum) was found to possess these characteristics whilst sunflower (Helianthus annuus) was classified as an easy-to-wet species. Two factors were observed in the present study which appear to confirm the classification of O. latifolia as a difficult-to-wet species. First, the inclusion of a surfactant greatly enhanced the amount of spray retained at all carrier volumes. Secondly, the peak level of spray retention was not reached even when the spray output was increased considerably. This low wettability exhibited by O. latifolia leaves may be ascribed to the nature of the leaf surface observed in the previous study. The leaves have a thick wax deposit. The low level of spray retention by O. latifolia leaves, in absence of a surfactant certainly confirms the importance of the inclusion of surfactant where O. latifolia is to be controlled by foliage-applied herbicides. The general properties which the inclusion of surfactant may have conferred include the improved coverage of the leaf surface and reduced inter-facial tension between water and the leaf cuticle. Further, surfactant may have minimized the rebounding of spray droplets similar to that noted in the waxy leaves of rape (Brassica napus L.) by Schafer and Stobbe (1973).

4.2.2 Effect of Leaf Folding on Spray Retention by O. latifolia

The gross morphology of O. latifolia plants was found to be of primary importance in the determination of the amount of spray retained. The total surface area of the aerial parts of O. latifolia is mainly determined by the available leaf area since formation of aerial stems does not occur. The total projected leaf area per plant in O. latifolia varied greatly due to the occurrence of leaf movements which are under control of internal (circadian) or external (environmental) factors. Internal factors cause the drooping or folding of leaves and petals to occur during the night (Ball, 1969) and may thus not be of great importance in retention of herbicide sprays which are normally applied during the daytime. In the present study, leaf movements resulting from external stimuli were observed during daytime and were found to affect the angle of leaves (from horizontal) as demonstrated in Plate 4. In growth room studies, the degree of folding was mainly determined by the moisture level of the soil. Thus, this factor could be manipulated for the purpose of experimentation. In outdoor plants however, a complex of factors including moisture, temperature and relative humidity might have been involved, since the initiation of leaf folding was not easily predictable.

Retention of the spray solution in plants with unfolded leaves was similar in both outdoor and indoor grown plants (Table 7). This similarity implies that leaves in both environments possess similar physicochemical properties which determine the level of spray retention. This conclusion is also consistent with the previous wax yield determinations.

Leaf folding reduced the total leaf surface area exposed by between 75 and 82.4% while reduction in retention (as % of unfolded) ranged between 74 and 49 per cent (Table 7). These results in Table 7 show that reduction in the retention of a foliage-applied spray was due to the folding of leaves, suggesting that availability of leaf area to intercept and retain the spray was the most important factor in determining the level of spray retention. Although the volume of spray retained ranged between 0.123 to 0.262 µl per cm^2 in the three experiments, it can be seen that retention in folded plants never rose above 51% of the amount retained by unfolded plants.

Other researchers have also demonstrated the importance of leaf angle in spray retention. Hibbitt (1969) found that the amount of spray retained by wild oat leaf strips held between 15 and 45 degrees (from horizontal) fell considerably whereas strips held 45, 60 and 75 degrees retained similar amounts of spray. Davies et al., (1967) found that retention on undetached barley leaves held at between 0° and 45° from horizontal declined marginally whilst retention declined sharply when the leaves were held 40° to 80° from horizontal. The reports by Hibbitt (1969) and Davies et al. (1967) do not seem to agree on the angle at which retention drops most rapidly but both reports point out that retention declines sharply when the leaf angle is large (45 to 80 degrees) from horizontal. In the present study, determination of leaf angles was not carried out but it was observed that folded leaves of O. latifolia may attain an angle close or equal to 90 degrees from horizontal (Plate 4). Furthermore, leaves folded at an angle equivalent to positions (B) and (C) in Plate 4, may intercept and retain significantly more spray

than leaves at position (D). Such a differential retention may result in variable performance of the same herbicide sprayed at different times. Such a variability in the performance of bentazon in control of Abutilon theophrasti (velvetleaf) was observed by Andersen and Koukkari (1978). They observed a low efficacy of bentazon in plants sprayed when leaves were held vertically compared to those sprayed when the leaves were in a near horizontal position. The authors concluded that the degree of control was directly related to the amount of spray retained and to the leaf angles of the plant. Judging from the retention results in the present study, it appears that spray reduction due to leaf folding could lead to low efficacy of some foliage applied herbicides in O. latifolia.

4.3 Control Studies

The growth stages of O. latifolia plants used in this study appeared to correspond to the second phase observed in the growth analysis studies previously reported. During this growth phase, the aerial parts (source) actively translocate photoassimilates to the most active sinks which are mainly bulbils and tubers (Chawdhry and Sagar, 1973).

Assessment after two weeks: All treatments stopped the production of new bulbils within this initial two week period. The control plants produced an average of ten new bulbils within the same interval. At the time of application of the treatments, the average fresh weight per plant was 10.6g (Section 3.3).

Fresh weight assessment (Table 8) showed that bulbils gained 2.61g, 3.95g and 4.18g per plant in defolition, paraquat and glyphosate treatments respectively within the two week period. Such gain in weight could

only have resulted from stored food reserves present in the contractile roots, since regrowth of the new vegetative shoot system was limited in the defoliation and paraquat treatments (1.61g and 0.11g respectively) and was completely absent in the case of glyphosate. Fryer and Makepeace (1977) state that destruction of the foliage results into transfer of food reserves from the contractile roots to the bulbils.

Bulbils excavated from the glyphosate treatment had a brown-black appearance but were not decayed. The tetrazolium test was carried out four weeks after excavating the bulbils and the results (negative for viability) indicated that the bulbils were dead.

In germination tests, only 5% or 6 out of 120 bulbils (from all six glyphosate pots or replicates) did not decay. These germination tests also revealed that mechanical (defoliation) and chemical (paraquat) defoliation have differing effects with respect to the dormancy of the bulbils. This difference can be explained from the considerations of moisture status of the entire plant. In defoliated plants, the water content of underground organs is not upset immediately since roots may continue to absorb water from the soil. By contrast, paraquat treated plants are rapidly dessicated due to the ability of this herbicide to translocate in the xylem (Slade and Bell, 1966). The resultant low moisture status in the underground structures caused the onset of dormancy in all bulbils from the paraquat treated plants but not in the defoliated plants in which 54% of the bulbils germinated. Further, some bulbils were severed from the parent bulb in paraquat but not in defoliated plants. The level of sprouting was similar in the defoliated and control plants. A higher water status also led to a faster regrowth

(15-fold increase in dry weight) in the defoliated plants compared with paraquat treated plants (Table 8).

Assessment after 4 weeks: All three treatments significantly reduced the number of bulbils per plant compared to the control. Within a 2 week period (between the first and second assessment), defoliated plants produced 16.5 more bulbils per plant than the paraquat treated plants which may primarily be attributed to the availability of more foliage regrowth in the defoliation plants. However, the fresh weights of these two treatments did not differ significantly (Table 8). The regular watering regime (2-3 days) appears to have resulted in a constant and high level of bulbils which did not enter into the state of dormancy in either defoliation or control plants shown in germination results (Section 3.3).

Viability and germination tests confirmed that glyphosate was translocated into the bulbils and the rest of the plant. Sprankle et al., (1975) demonstrated that glyphosate is translocated primarily in the phloem with the photoassimilates following the established source to sink relationship. Using yellow nutsedge (Cyperus esculentus) at 10-leaf stage as well as a young and an old plant of both quackgrass (Agropyron repens) and Canada thistle (Circium arvense), these authors found that the herbicide moved acropetally in the treated shoot and basipetally into the untreated shoots, developing tillers, and the growing rhizomes. They concluded that glyphosate moves to the areas of highest metabolic activity. A similar pattern in translocation appears to have occurred in O. latifolia plants in the present study. Glyphosate, which was applied at the second phase of growth, moved acropetally to kill the petioles and

leaves as well as basipetally into the tubers and developing bulbils. Chawdhry and Sagar (1973) suggested that ^{14}C-labelled assimilates found in the stolons were only in transit to the bulbils. This suggestion may be offered to explain the cause of stolon decay in the glyphosate treatment. The decay of stolons did not necessarily result from the toxic effects of glyphosate since 50% and 100% of the stolons in defoliation and paraquat treatments, respectively, also decayed.

5. GENERAL DISCUSSION AND CONCLUSION

The success of Oxalis latifolia as an aggressive perennial weed can mainly be attributed to the nonsynchronized emergence of plants due to differences in the level of dormancy of bulbs in the soil as well as rapid vegetative growth and production of these perennating structures. Presently, no soil-applied herbicide is capable of killing dormant bulbs and no selective foliage-applied herbicides have been found.

In growth analysis studies, young O. latifolia plants established vegetative growth very rapidly due to the presence of large food reserves stored in fleshy scales (Jackson, 1960). The scales support the initial development, particularly the growth of adventitious roots (Chawdhry and Sagar, 1973). This rapid establishment makes O. latifolia competitive with young crop seedlings during their early growth stages (Church and Henson, 1969). In the present study, stolons were produced within two weeks after planting. The main function of the stolons was noted to be the initiation, followed by the spread or dispersal of the bulbils. The primary stolons also had the ability to form secondary stolons, which terminated to secondary bulbils, thus increasing the reproductive capacity per plant. Both primary and secondary stolons thus aid in diageotropic dispersal of the bulbils though over short (less than 8.1 cm) distances. Fryer and Makepeace (1977) have noted that the main dispersal of O. latifolia bulbs is achieved through cultivation which detaches and relocates the bulbils away from the mother plant. Once detached, bulbils may be dispersed by machinery or birds but fresh

infestations in a new site normally result from bulbs carried over in plant roots, peat or manure.

In the present study, production of bulbils started 6 weeks after planting. The period of growth between planting and the start of formation of the bulbils, was classified as the first or vegetative phase. Any attempts made to control O. latifolia by cultural means (defoliation) or contact-type herbicides, should therefore be applied within the first phase of growth since the plants can be killed without leaving any viable new bulbils. Probably, it is more appropriate to apply such control measures towards the latter stages (3 or 4 weeks after emergence), to ensure that most of the plants have emerged. Achieving synchronization of emergence has been noted to be the main limitation where defoliation is used as the principal control method (Parker, 1966). The second limitation is that at least four consecutive defoliations are necessary before the regenerative ability of O. latifolia plants is exhausted (Chawdhry and Sagar, 1974).

The second or reproductive phase, (weeks 7 to 12) was characterized by the initiation and growth of the primary bulbils. A proportion of the bulbils produced shoot structures (leaves) of their own. The role of these leaves may be to increase the assimilatory or photosynthetic area of the mother plant since bulbils did not form any roots to enable them to grow and reproduce independent of the mother plant. Attempts to control O. latifolia plants at the second phase of growth may be unsuccessful unless a translocated herbicide such as glyphosate is applied. In these studies glyphosate (1.068 $kg.ha^{-1}$) was translocated through the stolons to accumulate in the bulbils, arresting their growth and inhibit-

ing their regenerative capacity.

Although prolific production of peduncles bearing inflorescence, occurred during the reproductive phase of the present study, no seed was set, probably due to the tristylic nature of flowers (Pierce, 1973). All flowers examined in the current study were of the short-stylar type. The peduncles bearing inflorescence were noted to account for up to 20% of the total plant biomass during the tenth week. It is difficult to appreciate the function or biological value associated with the production of these seedless reproductive structures. In O. latifolia, the competition of peduncles with the bulbils for photoassimilates may reduce the reproductive capacity per plant. Further, lack of sexual reproduction implies that all individual plants in any given population would lack genetic variability and thus the species should remain as a clone. Perpetuation of a clone can depend on the stability of the habitat. Changes in the habitat which are unfavourable to the clone such as viral infection, natural predators, drastic weather changes or a strong competitor may lead to extinction of the species in that particular habitat (Jaime and Koller, 1985). Such theoretical consideration should however be placed in a practical context. In the case of O. latifolia, existence as a clone does not appear to limit the spread, survival and the aggressiveness of the species as a weed.

Control of O. latifolia using foliage-applied herbicides at any stage of growth could be greatly affected by the wettability of the leaves. Results of the present study have shown that the leaf surface of this species has a thick epicuticular wax deposit (platelet type). Wettability of the leaves was enhanced by the inclusion of a non-ionic

surfactant, Tween-20. In the presence of a surfactant, the main factor governing wettability of the foliage was found to be the drooping or folding nature of leaves. Folding reduced the amount of spray retained by up to 74 per cent. This folding could thus reduce the total amount of herbicide absorbed and the potential for a herbicide to be translocated and subsequently accumulate at the ultimate site of action. The performance of both contact and translocated herbicides will be reduced where leaf folding has taken place at the time of herbicide application. From the literature previously cited, it is apparent that an inadequate degree of control has been reported for most herbicides including glyphosate (Cox, 1978; Sakira, 1981) applied under field conditions. Based on the problems in spray retention reported in the present study, it is probable that the poor performance of some foliage-applied herbicides may in part be attributed to low a degree of spray retention.

Spray retention is likely to be most important where a contact herbicide requiring good coverage of a weed is applied rather than a translocated herbicide.

Thus, it is evident from the studies reported herein that O. latifolia does possess several biological characteristics which makes it a difficult weed to control. These include,

(1) rapid establishment and prolonged food storage ability of the mother bulb,

(2) extensive reproduction and perennation by the underground bulbs, and

(3) nyctinastic movement may reduce potential herbicide retention on difficult to wet leaves.

Further research might usefully examine bulb dormancy, a long-term (from one growing season to the next) analysis of growth and the influence of crop competition on the growth and development of O. latifolia. Continued screening of herbicides, the search for long-term cultural and biological means of weed supression are also desirable means to achieve the control of O. latifolia in cropping systems.

BIBLIOGRAPHY

Andersen, R.N. and Koukkari, R.N. 1978. Response of Velvetleaf (Abutilon theophrasti) to bentazon as affected by leaf orientation. Weed Sci. 26: 393-395.

Baker, E.A. 1982. Chemistry and morphology of plant epicuticular waxes. In The Plant Cuticle. edited by D.F. Cutler, K.L. Alvin and C.E. Price. Academic Press, London, pp. 140-165.

Ball, N.G. 1969. Nastic Responses. In Physiology of Plant Growth and Development. Edited by M.B. Wilkins. McGraw Hill, London. pp. 277-300.

Chawdhry, M.A. 1974a. Growth study of Oxalis latifolia H.B.K. E. Afr. Agric. J. 39: 402-406.

Chawdhry, M.A. 1974b. Laboratory screening of herbicides for control of Oxalis latifolia H.B.K. E. Afr. Agric. J. 40: 65-71.

Chawdhry, M.A. and Sagar, G.R. 1973. An autoradiographic study of the distribution of ^{14}C labelled assimilates at different stages of development of Oxalis latifolia H.B.K. and O. pes-caprae L. Weed Res. 14: 430-437.

Chawdhry, M.A. and Sagar, G.R. 1974a. Dormancy and sprouting of bulbs of Oxalis latifolia H.B.K. and O. pes-caprae L. Weed Res. 14: 349-354.

Chawdhry, M.A. and Sagar, G.R. 1974b. Control of Oxalis latifolia H.B.K. and O. pes-caprae L. by defoliation. Weed Res. 14: 293-299.

Church, J.M.F. and Henson, H.M.G. 1969. Chemical control of Cyperus rotundus and Oxalis latifolia in Uganda. PANS 15: 578-583.

Cox, T.L. 1978. The effect of glyphosate on O. latifolia. In: Proc. 31st N.Z. Weed and Pest Control Conference. pp. 34-39.

Cox, T.I. and Kerr, R.M., 1981, Oxalis latifolia control programmes with oxadiazon and glyphosate. In: Proc. 8th Asian-Pacific Weed Sci. Soc. Conference. pp. 231-234.

Davies, P.J., Drennan, D.S.H., Fryer, J.D. and Holly, K. 1967. The basis of the differential phytotoxity of 4-hydroxy-3, 5-di-iodobenzonitrile. Weed Res. 7: 220-233.

Esler, A.E. 1962. Some aspects of autecology of Oxalis latifolia H.B.K. In: Proc. 15th N.Z. Weed Control Conference. pp. 87-90.

Estelita-Teixeira, M.E. 1982. Shoot anatomy of three bulbous species of Oxalis: Ann. Bot. 49: 805-813.

Fryer, J.D. and Makepeace, R.J. 1977. Weed Control Handbook. Vol. II British Crop Protection Council. Blackwell Scientific Publications, London, pp. 374-375.

Green, D.H., Stephen, D. and Hocombe, S.D. 1964. Experiments on the control of Oxalis species with herbicides in East Africa. In: Proc. 3rd E. Afr. Herbicide Conference. pp. 269-291.

Harper, R.D. and Appleby, A.P. 1984. Selectivity factors relative to asulam for Senecio jacobaea L. control in Medicago sativa L. Weed Res. 24: 85-92.

Hawton and Stobbe, 1971. Selectivity of nitrofen among rape, redroot pigweed, and green foxtail. Weed Sci. 19:42-44.

Hibbitt, C.J. 1969. Growth and spray retention of wild oat and flax in relation to herbicidal selectivity. Weed Res 9: 95-107.

Holloway, P.J. 1969a. The effects of superficial wax on leaf wettability. Ann. Appl. Biol. 63: 145-153.

Holloway, P.J. 1969b. Chemistry of leaf waxes in relation to wetting. J. Sci. Fd. Agric. 20: 124-128.

Holloway, P.J., 1970. Surface factors affecting the wettability of leaves. Pestic. Sci. 1: 156-163.

Hull, H.M. 1958. The effect of day and night temperature on growth, foliar wax content and cuticle development of velvet mesquite. Weeds 6: 133-142.

Hunt, R. 1978. Plant growth analysis. Edward Arnold Publishers Ltd. pp. 8-38.

Ivens, G.W. 1967. East Africa weeds and their control. Oxford University Press. East Africa. pp. 175-179.

Jackson, D.I. 1960. A growth study of Oxalis latifolia H.B.K. N.Z.J. Sci. 3: 600-609.

Jaime, K. and Koller, D. 1985. Asexual reproduction of weeds. In: Weed Physiology Vol. 1. Reproduction and Ecophysiology. CRC Press, Boca Raton, U.S.A. pp. 65-100.

Martin, J.T. 1969. Determination of the components of plant cuticles. J. Sci. Food Agric. 11: 635-640.

Michael, P.W. 1965. Studies on Oxalis pes-caprae L. in Australia I. Quantitative studies on oxalic acid during the life cycle of the pentaploid variety. Weed Res. 5: 123-132.

Parker, C. 1967. Pot experiments with herbicides on Oxalis latifolia Kunth. Proc. 8th Br. Weed Control Conference. pp. 126-134.

Parsons, W.T. 1973. Noxious weeds of Victoria. Inkata Press, Melbourne. Australia. pp. 216-220.

Pierce, J.R. 1973. Oxalis pes-caprae L. in Western Australia, its life history, distribution, morphological variation and weed potential. Dept. of Agric. Western Aust. Tech. Bull No. 20.

Price, C.E. 1982. A review of the factors influencing the penetration of pesticides through plant leaves. In: The Plant Cuticle. edited by D.F. Cutler, K.L. Alvin and C.E. Price. Academic Press, London. pp. 237-252.

Radford, P.J. (1967). Growth analysis formulae - their use and abuse. Crop. Sci. 7:171-175.

Ram, C.B. and Tuwari, 1979. Chemical control of Tipatia weed (Oxalis latifolia H.B.K.) in apple orchard. Indian J. Agric. Res. 13: 203-207.

Robb, S.M. 1962. Oxalis latifolia Kunth. New Phytol. 62: 75-79.

Sakira, W.A. 1981. Field evaluation of glyphosate on Oxalis latifolia Kunth in Robusta coffee. In: Proc. 8th E. Afric. Weed Control Conference. pp. 317-328.

Schafer, D.E. and Stobbe, E.H. 1973. Selectivity of benazolin in wild mustard and rape species. Weed Sci. 22: 45-47.

Slade, P. and Bell, E.G. 1966. The movement of paraquat in plants: Weed Res. 6: 267-274.

Sprankle, P., Meggitt, W.F. and Penner, D. 1975. Absorption, action and translocation of glyphosate. Weed Sci. 23: 235-240.

Stephens, D. 1961. Observational trials of the control of Oxalis semiloba Sond. with herbicides: PANS: 7: 77-80.

Touchstone, J.C. and Dobbins, M.F. 1983. Practice of thin layer chromatography. Second edition. A-Wiley-Interscience Publications. p. 405.

Versluys, W.S. 1977. New plant disease record in New Zealand Puccinia oxalidis on Oxalis. New Zealand J. Agric. Res. 20: 429-430.

Walker, J. 1976. Rusts on Oxalis and Mahonia in Australia. In: Australian Plant Pathology Society Newsletter 5: 60-62.

Young, D. P. 1958. Oxalis in the British Isles. Watsonia 4: 51-69.