Effect of burial on productivity and extinction of *Oxalis latifolia* Kunth

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The effect of burial on productivity and extinction of *Oxalis latifolia* bulbs has been studied. Linear regression formula showed that complete extinction of bulbs would be possible only at a burial depth of 32 cm. *O. latifolia* also sacrificed a number of its descendents in order to maintain its mean weight at increasing depth, mainly at depths greater than 28 cm, where a change in productivity distribution was observed. A comparison of these results with field population weights showed that a combination of agro-ecosystem conditions and deep burial would improve control of the weed.

Keywords: Bulb, depth, *Oxalis latifolia*, survival.

OXALIS *latifolia* is a South American bulbous plant[1] that grows in disturbed soils on mountain tops and quarries[2]. It has spread throughout the tropical, Mediterranean and temperate climates and is considered as a weed in 37 countries, where it affects 30 different crops[3]. Yield and economic loss caused by *O. latifolia* have been reported in cotton[4], potato[5], soybean[6], apple[7] and maize[8]. In Spain it is an undesired weed, specially in orchards[9] and maize fields[10,11], but it also appears in gardens and plant nurseries (authors' pers. obs.).

Many strategies have been adopted to control this weed. Most of them have been herbicide treatments, among which glyphosate[12–14], oxadiazon[4,12,15,16], oxifluorfen[15–18] and trifluralin[19,20], have given the best results.

Although most research has been directed to chemical control of the weed, depletion of the bulbs has been reported as the best way to fight against *O. latifolia*[9,21]; continuous leaf production without any accompanying photosynthesis and re-storage of the bulb (defoliation). Defoliation may be achieved directly – mechanically[22] or chemically[23,24] – or indirectly, with a deep burial that delays or prevents the emergence of leaves[25–27]. Despite a weak petiole[28], leaves can emerge from a 30 cm burial[29], even if most bulbs would die at shallower depths (only 20% of the bulbs survived when buried at 25 cm)[25]. Burial depth also improves chemical control of the weed; bulbs buried at 8 cm depth are more sensitive to pre-emergent herbicides such as oxadiazon and aclonifen than shallow ones[30]. Bulbs buried deeper must penetrate a thicker soil layer, which would improve contact of the herbicide with the growing foliar meristem. The developing leaves would be killed near the surface; the bulb is weakened because it does not recover the energy used for production of leaves. Moreover, bulbs buried deeper are exposed more to leaching herbicides.

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The effect of burial on productivity of the bulbs is quantified until depletion. Now, productivity of *O. latifolia*, which in Europe does not produce fruits or seeds, is only measurable by the increase/decrease in weight of the apical and lateral bulbs with respect to the parental bulb[27,31,32].

It is reasonable to think that big and heavy bulbs will be more difficult to control. The percentage of big bulbs – heavier than 0.700 g – in natural field conditions, i.e. in competition with crops and other weeds, varies from 2 to 5 in a population[33]. The effect of depth of burial of big bulbs on depletion down to death was studied. If weakening or consumption – extinction – could be achieved, the effectiveness of the treatment would be proven, that is, the death of the whole population. The relation between burial depth and productivity – until extinction depth – on a large field population of Cornwall form bulbs (heavier than 0.700 g) was studied.

O. latifolia Cornwall form bulbs were collected from a maize field near Hernani (northern Spain, temperate climate) in February 2001. In early April, 30 activated bulbs, with an initial weight (IW) of between 0.820 and 1.520 g, and Kruskal–Wallis (one-way ANOVA) found to be non-significant[34], were selected and planted on 14 April in a 24 m × 1.5 m cement vat provided with sandy clay loam soil, 2.15% organic matter, pH 7.84 and 0% carbonates. Since the vat had been used as a plant nursery for several years and also for other experiments in the previous two growing seasons, the soil had been turned over and had been homogenized. Soil homogeneity and the small size of the plot (Figure 1) made planting easier because randomiza-

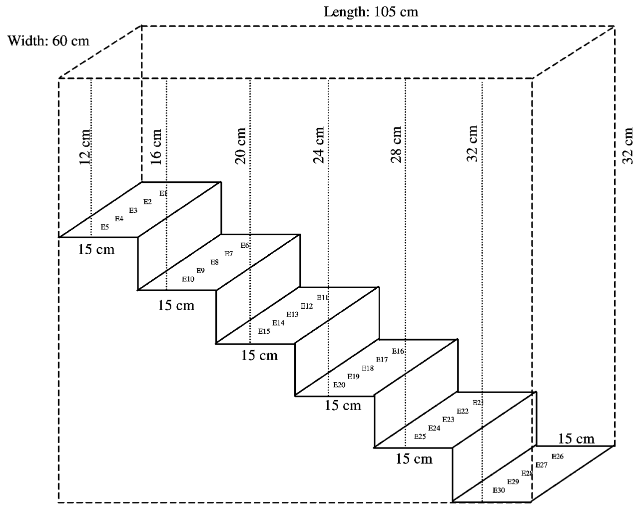

Figure 1. Planting method. E1–E30 represent each planted bulb. Bulbs planted at shallow depths are represented on the left side, while the deeper ones are on the right side.

Table 1. Mean value of different variables; and statistical results and extinction depth (depth at which bulbs would die according to the regression formula)

Bulb	Depth (cm)	IW	AW	LW	LN	LMW	PRO
E1–E5	12	1.109	7.73	23.24	67.4	0.391	2691
E6–E10	16	1.224	7.075	23.41	54.8	0.467	2391
E11–E15	20	1.087	5.299	16.02	38.4	0.413	1861
E16–E20	24	0.963	3.905	8.763	17.4	0.357	1216
E21–E25	28	1.137	3.411	4.008	10.5	0.319	552.4
E26–E30	32	0.996	0.533	0.119	0.8	0.074	–40.8
F-test (regression)			**	**	**	n.s.	**
R^2			0.419	0.401	0.399	0.181	0.449
Formula, *Y* =			12.26–0.346*X*	41.05–1.293*X*	108.06–3.478*X*	–	4559.6–141.57*X*
Friedman†			**	**	**	†	*
Extinction depth (cm)			35.4	31.7	31.1		32.9

n.s., Not significant; *Significant at $P < 0.05$; **Significant at $P < 0.01$; †Friedman was used for all variables[34].

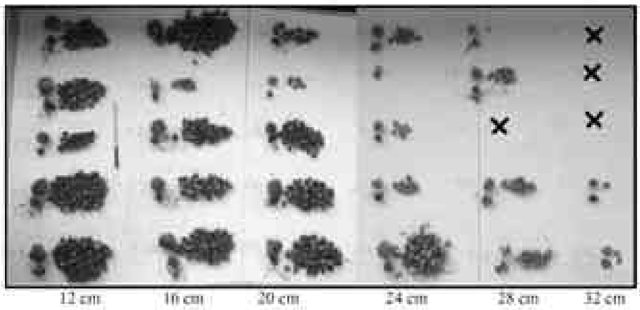

Figure 2. This follows the Figure 1 frame; bulbs planted at shallow depths are on the left, while the deeper ones are on the right. '×' represents dead bulbs.

tion had not been necessary. Rows of five bulbs were planted at 4 cm increasing depths, from 12 to 32 cm. Intra-row and inter-row planting followed as 10 and 15 cm respectively (Figure 1). Such distances had proved effective to avoid intra-specific competition[9]. Results were tested for the presence/absence of competition against productivity, due to the possible border effect in the sketch. The experiment was repeated twice.

The descendents of each parent bulb were carefully harvested on 14 October 2001, washed with tap water and allowed to dry for 3 days on a blotting paper. Then the new apical bulb was weighed (AW), as well as all the lateral ones (LW), whose number was also counted (LN). Mean weight of lateral bulbs (LMW) was calculated for each planted bulb (LW/LN). Productivity (PRO) was calculated as follows:

$$\text{PRO} = (\text{AW} + \text{LW} - \text{IW}) \times 100/\text{IW}.$$

Results were statistically analysed with linear regression among depths because each pack of bulbs grew under similar conditions[34]. Due to lack of normality of the samples and heterogeneity of variances, transformation of data to $\ln(x + 1)$ was required. As lowest productivity value was –100%, transformation was done as $\ln(x + 101)$. If significant regressions were found, differences between depths would be analysed as follows: first seeking any intra-specific competition, taking into account the productivity results of the outer rows and inner rows beside them; and accordingly, applying the convenient Friedman or Kruskal–Wallis test[34]. If necessary, Wilcoxon or Mann–Whitney *U* test[34] could be applied to find the significance between depths for each analysed variable.

Figure 2 shows bulbs obtained from the first replication after harvest with the same method of planting. The crossed mark (×) indicates that the bulb planted in that position had died without leaving any descendant.

Productivity of bulbs decreases as depth of planting increases; in fact, some bulbs died at 28 and 32 cm depth.

Linear regression of all variables against depth showed significant differences ($P < 0.01$) in all cases except for LMW. We have also found that differences among burials were highly significant or significant, and mortality increased with depth (Table 1).

Figure 3 represents the mean and standard deviation values of Table 1 for PRO, as well as the regression line and its formula.

The negative regression formulae of four variables allow us to calculate the depth at which their values would be 0 (–100% in the case of PRO), i.e. the depth at which all bulbs would die. In our field conditions, heavy bulbs (1 g mean weight) would die at approximately 33 cm depth (mean value of the four extinction depth data from Table 1). In order to simplify the analyses and as AW, LW and LN are part of the productivity formula, only PRO was taken into account for comparison between depths.

When analysing the intra-specific competition for PRO, lack of normality in one of the samples (inner rows) led us to a non-parametric related samples test, the Wilcoxon test[34], with the result of a slight significance ($P = 0.041$). Hence Friedman test is needed for depth comparisons. These comparisons show significant differences among samples at 32 cm depth and those buried down to 24 cm. Also there were significant differences in bulbs between

Table 2. Variation of productivity distribution with increasing depth. Numbers in parenthesis are the transformed values

Depth	AW	LW	AW/LW	LN	LMW
12	7.73	23.24	0.3	67.4 (1.00)	0.391 (0.84)
16	7.075	23.41	0.3	54.8 (0.81)	0.467 (1.00)
20	5.299	16.02	0.3	38.4 (0.57)	0.413 (0.88)
24	3.905	8.763	0.4	17.4 (0.26)	0.357 (0.76)
28	3.411	4.008	0.9	10.5 (0.16)	0.319 (0.68)
32	0.533	0.119	4.5	0.80 (0.01)	0.074 (0.16)

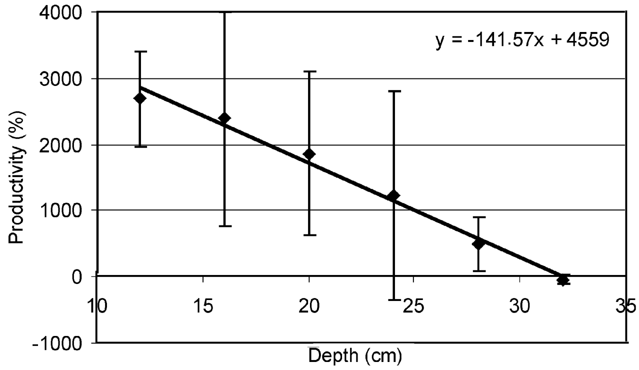

Figure 3. Decrease in productivity with depth.

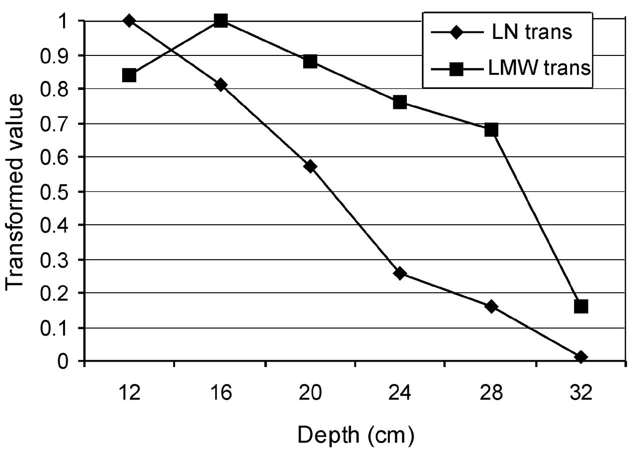

Figure 4. Graphical representation of LN and LMW transformed values vs depth.

12 and 28 cm depth. These data confirm the large difference among bulbs at 32 and 24 cm depths, while 28 cm depth could be considered as transition burial. These results, together with regression lines, indicate that the effect of depth is progressive but not significant for burial differences of 4 cm, under our experimental conditions. Though studies have shown that bulbs can be produced down to 30 cm depth[29], it should be pointed out that such bulbs would be weakened and would hardly survive a second growing season. In some cases complete control of the weed had been obtained after burying the bulbs at about 40 cm depth in the soil[25] and after a five-year combination of foliar herbicide application and 25 cm cultivation[12]. These burial depths would also improve the control of other weed species[35]. The 30 cm burial depth would certainly provide good control of the weed.

Summarizing, the results show that there is a close relation between decrease in productivity and increase in burial depth. Bulbs die depleted at nearly 33 cm depth: the killing effect of this factor is illustrated. Nevertheless, 32 cm depth is not enough for complete extinction of bulbs.

In Figure 3, the constant of the regression formula shows that each gram of a 0 cm buried parental bulb would produce as much as 45.59 g in the descendants. The coefficient shows that this productivity would decrease by 1.42 g for each burial centimetre. Thus each burial centimetre causes a 1.42 g loss in the biomass of the descendants in this experiment. From Table 1 it can be observed that extinction (–100%) is reached at 32.9 cm.

Table 2 shows variations of productivity distribution with increasing burial depth. Down to 20 cm depth, the apical bulb receives one-third of the produced biomass. Meanwhile, in deeper burials the proportion is exponentially increased, and the apical bulb can receive 4.5 times more biomass than the lateral ones. That is, when depth increases, productivity decreases, and the apical bulb becomes dominant. This influences multiplication as lateral bulbs disappear at 31.7 cm depth, while apical bulbs may live, according to the regression formula, up to a 35 cm depth (Table 1). Thus, when the extinction burial is almost achieved, scarce productivity is concentrated in the main bud (apical bulb).

With the results, the biology of *O. latifolia* can be further studied. In order to compare lateral productivity and burial, the results of LN and LMW were transformed by dividing each by its own highest value. Values in parenthesis are the transformed ones (Table 2); Figure 4 is their graphical representation.

It can also be observed that the decrease in LN was sharp (Figure 4), while LMW was constant down to a toleration depth (28 cm), after which a strong fall occurs. It can be concluded that the weed tries to maintain the weight of its descendants by sacrificing their number. Decrease in LMW indicates the approaching to the extinction depth of the weed. Decrease in LN and an increase in their size from 1 to 12 cm depth have been observed in previous studies[26]. This would mean that *O. latifolia* always sacrifices lateral bulbs with increasing depth, but the following also occur: increase in their size (weight) from 1 to 12 cm, maintenance of weight from 12 to 28 cm and sharp decrease from 28 to 32 cm.

Summarizing, the decrease in productivity due to depth has been 1.42 g/cm under our experimental conditions. It is distributed as follows: 25% of the biomass produced goes to the apical bulb and 75% to the lateral ones; weight of apical bulbs decreases by 0.350 g for each burial centimetre (Table 1), and LN decreases by 3.5 bulbs every burial centimetre (1.070 g/cm, Table 1). On reaching the extinction depth, this regularity is broken and the weed stores productivity in the main bud. This behaviour tries to ensure the difficult survival of the individual.

Table 3 shows AW, LMW and LN values at 12, 28 and 32 cm depth. These results are in conformity with earlier studies[24,25,33].

Potential development of the weed with slight intraspecific competition is shown in Table 3 (12 and 28 cm rows), which contrasts with those that grew near extinction depth (32 cm). In such circumstances, decrease in AW, LMW and LN values was observed.

The experiments have been carried out with the heaviest bulbs of the infesting population[33], which represent only 2–5%. Now, if it is assumed that the remaining population (95–98%) has smaller bulbs, a 32 cm burial depth would kill all of them. Burial to a certain shallower depth, if it does not kill the entire population during the first growing season, would certainly weaken it. This control would be improved with cover mulches[22,36] and/or pre-emergence herbicides like oxadiazon, whose effectiveness has been largely proven[12,13,30].

The weakening effect of depth depends on the size of the bulbs as well as the resistance of the soil against emergence of the petiole and limb. This effect is different from the one achieved in the agro-ecosystem due to competition of other weeds and of the crop and its management. A combination of both factors – depth and agro-ecosystem – would also improve in the control *O. latifolia*, which could reach extinction at shallower burials. This would ensure the control of bulbs distributed in the profile of the soil.

Summarizing, the weakening effect of deep burial should be considered for the control of *O. latifolia*. A combined action of pre-emergence herbicides, agro-ecosystem and burial would be a more effective control than burial alone.

Table 3. AW, LWM and LN values at different depths

Depth (cm)	AW (g)	LMW (g)	LN
12	7.730	0.391	67.4
28*	2.728	0.255	8.4
32*	0.533	0.059	0.8

*Means with dead values.

From 45.59 g of productivity for each initial weight when growing on the surface, *O. latifolia* produces 1.42 g less biomass for every centimetre it is buried in the soil. The apical bulb loses 0.350 g/cm and number of lateral bulbs decreases at the rate of 3.5 bulbs/cm.

Biomass distribution down to 20 cm depth is 25% for apical bulbs and 75% for lateral bulbs. This changes from a depth of 24 cm, when the apical bulb receives higher proportion of biomass. Near the extinction depth, scarce productivity is concentrated only in the main bud.

Lateral bulbs became extinct at a depth of 31.7 cm, while extinction of apical bulbs would occur at 35.4 cm depth. LMW remains constant down to 28 cm depth: *O. latifolia* sacrifices the number of lateral bulbs in favour of their weight. A 35 cm burial depth would kill 100% of an infesting population.

Thus a combination of pre-emergence herbicides, agro-ecosystem and burial, would provide effective control of *O. latifolia*.

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ACKNOWLEDGEMENT. We thank Sue Pexton for English revision of the paper.

Received 2 January 2006; revised accepted 16 November 2006

Isolation and identification of five alcohol-defying *Bacillus* spp. covertly associated with *in vitro* culture of seedless watermelon

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Five distinct bacterial clones (3 × WMARB-1 to 5) were isolated from the spent alcohol used for tool-disinfection during the subculturing of apparently clean, long-term micropropagated triploid watermelon (*Citrullus lanatus* L.) cultures that harboured bacteria in covert form. The isolates belonged to aerobic, Gram-positive, endospore-forming bacilli. Four of these were identified as *Bacillus fusiformis* (3 × WMARB-2), *B. pumilus* (3 × WMARB-3), *B. subtilis* (3 × WMARB-4) and *B. flexus* (3 × WMARB-5) through 16S rDNA sequence analysis (approx. 1450 bp), while isolate 3 × WMARB-1 was identified through fatty acid profiling as *B. megaterium*. These as well as other spore-forming organisms that were employed as control (*B. thuringiensis* and *Brevibacillus* sp.) showed survival in 70% or absolute alcohol from overnight to several days, while non-spore forming checks, including Gram-negative *Escherichia coli*, *Pantoea*, *Sphingomonas*, *Agrobacterium* spp. and Gram-positive *Microbacterium* sp. were killed within a few minutes. The alcohol tolerance property of *Bacillus* spores proved to be a threat to plant tissue cultures owing to the likelihood of unsuspected lateral spread of contamination through inadequately flamed tools when alcohol is used as a sterilant, compounded by their covert survival in tissue-culture medium, and in general microbiology, wherever alcohol is used as a surface disinfectant.

Keywords: *Citrullus lanantus*, endospore resistance, fatty acid profiling, microbial contamination, plant tissue culture.

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