Modelling leaf development in *Oxalis latifolia*

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Abstract

This study was carried to develop functions that could explain the growth of *Oxalis latifolia*, in both early stages and throughout the season, contributing to the improvement of its cultural control. Bulbs of the Cornwall form of *O. latifolia* were buried at 1 and 8 cm in March 1999 and 2000. Samples were destructive at fixed times, and at each time the corresponding BBCH scale codes as well as the absolute number of growing and adult leaves were noted. Using the absolute number of adult leaves (transformed to percentages), a Gaussian curve of three parameters that explains the growth during the season ($R^2 = 0.9355$) was developed. The BBCH scale permitted the fit of two regression lines that were accurately adjusted for each burial depth ($R^2 = 0.9969$ and $R^2 = 0.9930$ respectively for 1 and 8 cm). The best moment for an early defoliation in Northern Spain could be calculated with these regression lines, and was found to be the second week of May. In addition, it was observed that a burial depth of 8 cm does not affect the growing pattern of the weed, but it affects the number of leaves they produce, which decreases to less than a half of those produced at 1 cm.

Additional key words: BBCH scale, control by defoliation, Gaussian curve, growth, linear regression.

Resumen

Modelización del desarrollo foliar de *Oxalis latifolia*

El presente trabajo se llevó a cabo con objeto de desarrollar funciones que expliquen el crecimiento de la forma de Cornwall de *Oxalis latifolia*, tanto en las etapas iniciales como a lo largo de toda la temporada de crecimiento, y que contribuyan a su control. Se plantaron bulbos de *O. latifolia* a 1 y a 8 cm en marzo de 1999 y 2000. Los muestreos fueron destructivos y se anotaron tanto el código de la escala BBCH como el número absoluto de hojas en crecimiento y de hojas adultas. Con el número absoluto de hojas adultas, transformado en porcentaje, se desarrolló una curva de Gauss de tres parámetros ($R^2 = 0,9355$) que explica el desarrollo de esta mala hierba a lo largo del año. La escala BBCH permitió desarrollar dos regresiones lineares bien ajustadas para cada profundidad ($R^2 = 0,9969$ y $R^2 = 0,9930$ respectivamente para 1 y 8 cm). Mediante dichas regresiones lineares se pudo calcular que el momento adecuado para realizar una primera defoliación de la mala hierba en el norte de España es la segunda semana de mayo. Por otro lado, se pudo observar que una profundidad de 8 cm no afecta al ritmo de crecimiento de los individuos, pero sí al número de hojas que desarrollan, disminuyéndolo a menos de la mitad que a 1 cm.

Palabras clave adicionales: control por defoliación, crecimiento, curva de Gauss, escala BBCH, regresión linear.

Introduction

Studies directed to predict emergence based on modelling using growing degree days (GDD) or hydro-thermal time (HTT) functions, are valuable tools in order to control weeds (Forcella *et al.*, 2000). Weed emergence is considered a key process as it determines the amount of weeds and the timing of their appearance in the field (Gardarin *et al.*, 2009). These functions are used mostly to predict emergence of annual weeds, but the presence of underground stems (bulbs or rhizomes) in perennial weeds needs particular attention as these

Abbreviations used: BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry), DAP (days after planting), GDD (growing degree days), HTT (hydrothermal time).

weeds can regrowth when losing leaves. In the particular case of *Oxalis latifolia*, which can easily emerge one leaf and get recovered (Royo-Esnal and López, 2008c), constant defoliation for several weeks is needed to achieve an optimal control of it (Esler, 1962; Chawdhry and Sagar, 1974).

O. latifolia is a common weed that invades maize fields, orchards and gardens in temperate and irrigated Mediterranean Spain (Villarías, 2000; Valenciano *et al.,* 2005; Royo and López, 2008c). Eradication of the weed in a field is almost impossible (Royo-Esnal and López, 2008b) because starvation of the bulb is needed in order to kill it (Valenciano *et al*., 2005). Thus, the combination of different methods is proposed for the control of *O. latifolia* (Royo-Esnal and López, 2008b). An early defoliation, either mechanical or chemical, has been considered from long time ago as an initial point in order to control *O. latifolia* in crop fields (Parker, 1966; Chawdhry and Sagar, 1974). This early defoliation will not kill all the bulbs, but it will retard the formation of new vegetative growth (Chawdhry and Sagar, 1974) and, therefore, its competitive capacity against the crop (Royo-Esnal and López, 2008b). Defoliation should be done after bulbs have developed up to 4 or 5 leaves, but before bulbs have recovered the spent storage or without leaving any viable bulbils (Chawdhry and Sagar, 1974; Marshall and Gitari, 1988; Royo-Esnal and López, 2008b). This weakest moment of the weed seems to be 42 to 56 days after planting (DAP) (Prathibha *et al.*, 1995). But spaced emergence, that may last for some weeks (Parker, 1966), and the inevitable climatic condition variations from year to year (Ghersa, 2000) make even more difficult the decision of the best moment for the early defoliation in the field.

For these reasons, a function predicting the moment when *O. latifolia* bulbs are weaker and could be better controlled seems to be extremely practical. The aim of this work is to develop a useful and simple function that could predict this weed's phenological weakest stage for a first defoliation that would contribute, together with other methods, to a better control of the weed.

Material and methods

Oxalis latifolia Cornwall bulbs were collected from a maize field near Hernani (Northern Spain, temperate climate; 43°19'17"N-1°51'54"W) in February during 1999 and 2000. Bulbs were washed immediately and left to dry on blotting paper at laboratory conditions

for three days; after this time, bulbs were weighted and 690 of them in 1999 and 630 in 2000, between 0.500 and 1.200 g —Kruskal-Wallis test non significant (Field, 2000)— were selected to be planted in a 24 m long $\times$ 1.5 m wide $\times$ 0.8 m deep cement vat provided with sandy clay loam soil, 2.15% organic matter, pH 7.84 and 0% carbonates. The vat had been used as a plant nursery for several years. Until the experiment was laid out, each spring the soil of the vat had been turned over and homogenized.

Plantation took place on 7th March 1999 and 15th March 2000, in the open air without any protection. Bulbs were planted in a grid manner, so as to keep each one 15 cm away from each other. 115 bulbs/plot and 105 bulbs/plot were planted at 1 and 8 cm depth in 1999 and in 2000 respectively, with three replicates disposed randomly in the vat.

Bulbs were sampled weekly until middle June and fortnightly after this date (with some exceptions). For each sample, five places where a bulb was planted were excavated. Samples were taken into the laboratory, washed with tap water on a 1.7 mm sieve and dried on blotting paper. This allowed the calculation of the extended BBCH scale stages in which *O. latifolia* bulbs were at each moment, from sprouting to adult leaf stages and, thus, a better comprehension of the early growth of the weed. The extended BBCH scale is a system for a uniform coding of phenologically similar growth stages where, for weeds growing from underground organs, the following code is followed: 07, bud breaking; 08, shoot growing towards soil surface; 09, emergence or leaf breaking through soil surface; 10, first true (adult) leaf emerged; 11, first true leaf unfolded; 12, two true leaves unfolded; 13, three true leaves unfolded...; 19, nine or more true leaves unfolded (Hess *et al.*, 1997). As the BBCH scale only considers nine true leaves and *O. latifolia* usually develops more of them, the total number of leaves was also noted to analyze the evolution throughout the season.

Statistical analysis

Data for maximum number of leaves produced were submitted to a t-student test after being square root transformed.

Daily rainfall and maximum and minimum air temperatures were obtained from a meteorology station located approximately at 3 km from the experimental field. Growing degree days (GDD) were calculated as

the accumulation of mean of the daily minimum and maximum air temperatures (Gupta, 1985; Benvenuti and Macchia, 1993), thus considering 0°C as base temperature for growth.

Results of the total number of adult leaves throughout the season were presented against GDD and different formulae and polynomials of different degrees were tried to fit the evolution of the weed development. Among these formulae Gaussian curve with three parameters [Eq. 1] was found to be the simplest function with high accuracy:

$$ae^{-0.5(x-x_0)/2c^2} \qquad [1]$$

where a is the maximum leaf development, x_0 is the amount of GDD needed for maximum leaf development, and c^2 is the variance. The mean BBCH stages from sprouting to middle June were also analyzed against GDD and different formulae were applied to see the early growth pattern of *O. latifolia*. Middle June was considered as the latest moment for soil disturbance in order to weaken the bulbs with a first defoliation.

Results

Seasonal leaf development

In 1999, bulbs started to sprout on the 18th of April at both 1 and 8 cm and in one week time these leaves appeared on the surface. After a month, on the 24th of May, bulbs buried at 1 cm developed twice as much leaves as the 8 cm buried ones (1.4 and 0.7 respectively) and at the time bulbs presented the maximum number of leaves (29th August), 1 cm buried bulbs showed 3.7 times more leaves than the 8 cm ones (12.1 and 3.3 respectively, $p < 0.01$). After this maximum bulbs continued developing leaves, but those senescent and dead ones were more abundant, thus the number of leaves per bulb decreased constantly until the bulbs lost all of them and no more leaves were developed (Fig.1, left).

In 2000, bulbs buried at 1 cm developed leaves one week earlier than the 8 cm ones (23rd April and 1st May respectively). This year the relation between leaves produced by 1 cm and 8 cm buried bulbs was maintained constant between 1.6 and 2.0, even when the maximum number of leaves were developed on the 8th of August (10.3 and 5.7 leaves respectively; relation 1.8, $p < 0.01$). After this date, the same tendency of developing and losing leaves was observed until middle November, when all of them disappeared (Fig. 1, right).

Figure 1 shows that the tendency in number of leaves is maintained independent of the burial depth. Despite this, number of leaves differed significantly between 1 and 8 cm. Bulbs buried at 1 cm always showed higher number of leaves independently of the time of the year, and at their maximum, they developed an average between the two years of 11.2 leaves while 8 cm buried bulbs showed an average of 4.5.

Transformation of the number of leaves to percentage and the analysis against GDD permitted an equalization of the values between years and burial depths (Fig. 2). A Gaussian curve of three parameters applied
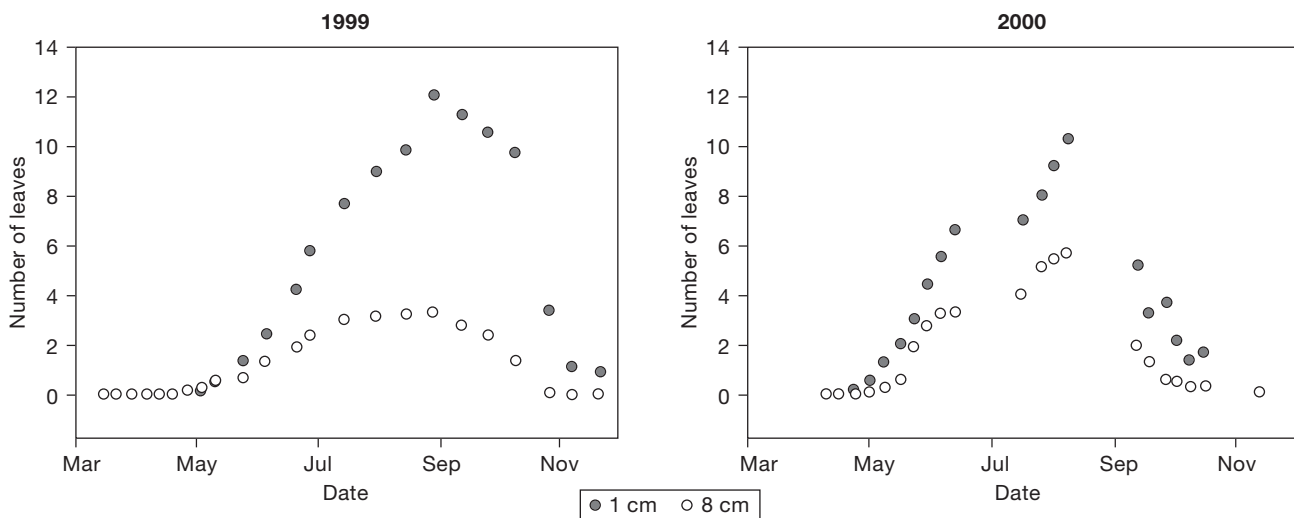
Figure 1. Total number of adult leaves developed by *O. latifolia* bulbs buried at 1 cm and at 8 cm.
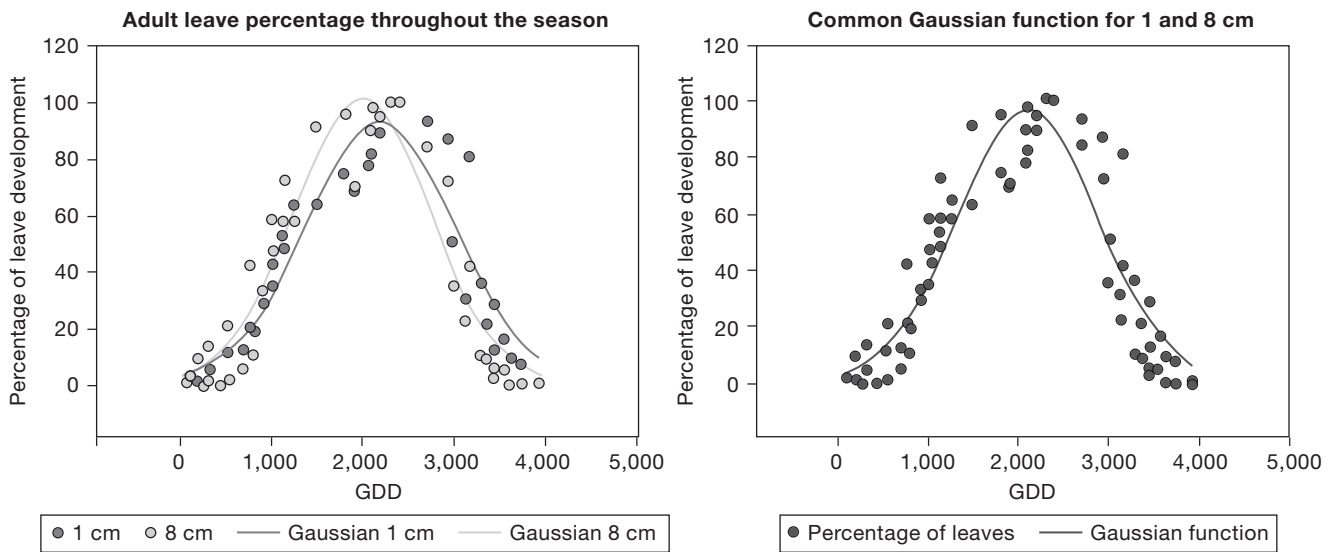
Figure 2. Leaf appearance (expressed as percent of maximum number of leaves) as a function of GDD. Fits are Gaussian functions. Left, discriminated by depth; right, not discriminated by depth.

to each burial depth proved the similar tendencies with high accuracy ($R^2 = 0.9491$ and $R^2 = 0.9486$ respectively for 1 and 8 cm, Table 1). This allowed the joint data analysis of both burial depths in order to calculate a common function with minor decrease of accuracy ($R^2 = 0.9380$). According to this function, *O. latifolia* bulbs would develop half of their leaves at about 1,000 GDD and would reach the higher leaf percentage between 2,000 and 2,100 GDD.

Modelling early leaf development with a BBCH scale

Figure 3 shows the results of the transformation of data into BBCH scale presented against GDD. Linear regressions resulted to be the best and most simple functions describing the leaf development in early stages. Accuracy of the functions was higher than in the previous section (R^2 of 0.9969 and 0.9930 respec-

tively for 1 and 8 cm, Table 2). According to these functions, bulbs buried at 1 cm develop the first leaf at 495 GDD and 8 cm buried ones at 729 GDD, while the second leaf would be developed at 620 GDD and 914 GDD respectively.

Discussion

Loss of dormancy in *O. latifolia* Cornwall form bulbs follow an exponential pattern (Royo-Esnal and
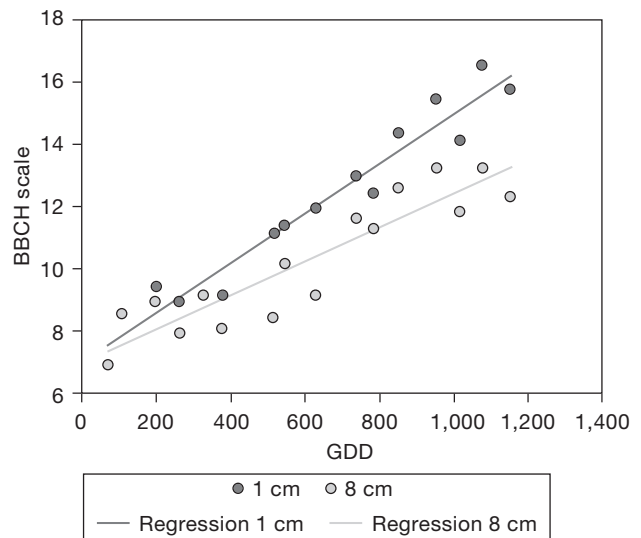
Table 1. Gaussian curves with three parameters for the percentage of leaves developed throughout the growing season by 1 cm and 8 cm buried bulbs, as well as for both burial depths considered together, and their respective R^2

Burial depth	*a*	*b*	x_0	R^2
1 cm	93.1243	812.1140	2204.6398	0.9448
8 cm	101.2952	744.2353	2004.1601	0.9442
Combined	96.3498	782.3914	2098.3842	0.9355

Figure 3. Regression lines for leaf appearance (BBCH scale), for bulbs buried at 1 and 8 cm.

Table 2. Regression formulae for early leaf development at BBCH scale for 1 and 8 cm buried bulbs; their respective R^2 and GDD necessary to reach 1 and 2 leaves stages (11 and 12 BBCH scale). Regression lines are represented in Figure 3

Burial depth	Regression line	R^2	GDD (stage 11)	GDD (stage 12)
1 cm	$y = 0.008x + 7.0390$	0.9969	495.1	620.1
8 cm	$y = 0.0054x + 7.0624$	0.9930	729.2	914.4

López-Fernández, 2008a). So, it is assumed that most individuals belonging to a particular depth will arrive to different phenological stages almost simultaneously or with little variation. But, even when Royo-Esnal and López (2007) confirm the delay in leaves emergence in those 12 to 32 cm buried bulbs, 8 cm seems not to be deep enough to see clearly this tendency and transformation of adult leaves data into percentages showed that the growing pattern during the season is similar at both 1 and 8 cm depths. Despite this, burial depth has a significant effect on the number of adult leaves developed throughout the growing season. Shallower buried bulbs produce more than twice as much leaves as 8 cm buried bulbs, confirming that the main difference between 1 and 8 cm buried bulbs is the number of leaves they produce.

The general Gaussian curve developed for the seasonal evolution of *O. latifolia* describes quite well the growth of the weed in its early stages. But the regression lines obtained using the BBCH scale are much more accurate and the inclusion of data belonging to sprouting and under-surface growing leaves strengthen the method. Moreover, the values used for the regression lines belonged only to the early growing period and these additional data also collaborated to a more accurate formula. Storkey and Cussans (2000) also found a high linear relation between early growth (relative growth rate in their case) and GDD, but this relation was maintained until 550-880 GDD, depending on the species. In the case of *O. latifolia* this linear relation could be increased at least until 1,200 GDD.

When considering the two regression lines, the most sensitive stages of *O. latifolia* to chemical control described by Prathibha *et al.* (1995) would occur at about 500 GDD (stage 11 at 1 cm, but stage 9.7 at 8 cm) and 900 GDD (stage 12 at 8 cm, but stage 14.2 at 1 cm), that is, from 18th April to 14th May in 1999 and from 26th of April to 22nd of May in 2000, which is almost a one month time. If the control is done too early deep bulbs would not have reached surface and they could recover easier, while if it is done too late many bulbs

that emerged leaves earlier could have recovered from the energy lost in the leaf emergence. So, the prediction for an effective defoliation should be more accurate. The fact that deep buried bulbs produce fewer leaves can be the result of more difficulties in order to emerge (Royo-Esnal and López, 2007), thus more energy is needed for leaf development. If this reasoning is correct, at the time 8 cm individuals are able to emerge their first leaf, their bulbs would be at the weakest moment because energy has been spent but not recovered. When 8 cm bulbs have developed the first leaf (730 GDD), 1 cm bulbs would have almost three adult leaves (stage 12.9). Chawdhry and Sagar (1973) demonstrated by an autoradiographic study that the first photosynthesized assimilates are destined to new leaf production, that means that the bulb does not store any or, at the most, very few of these. So, 1 cm bulbs would be as weak as 8 cm buried bulbs. Thus, stage 11 of 8 cm buried bulbs could be a good moment for this first defoliation. The 730 GDD mark was reached on 5th May in 1999 and on 11th May in 2000, one week difference between both years. This would mean that the second week of May could be the best moment for *O. latifolia* control in areas of similar climatic conditions to the Basque Coast.

Acknowledgments

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