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ORIGINAL PAPER

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On the use of non-linear regression with the logistic equation for changes with time of percentage root length colonized by arbuscular mycorrhizal fungi

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Abstract For the regression of sigmoid-shaped responses with time t of colonization C of roots by arbuscular mycorrhizal fungi, $C=C_p/1+[e^{-k(t-t_i)}]$ is the most useful form of the logistic equation. At the time of inflection t_i the slope is maximal and directly proportional to the product of the colonization plateau C_p and the abruptness k of the curve. Coefficient k has a high value when the curve rises abruptly following and preceding long shallow phases. The logistic equation has a curve that is symmetrical about t_i such that $C=C_p/2$ at inflection. Although the logistic equation can generate a good fit to many data sets for changes in colonization with time, there are cases that are not sigmoid and the logistic equation does not apply. For sigmoid curves, the lag in the development of colonization is directly related to both t_i and k but not to the plateau and not to the value of the maximum slope. Higher values of k or t_i reflect longer lag. When considered alone, t_i and k do not fully summarize the lag in colonization, and so a numerical method to combine them is presented here which allows lag to be compared between curves. In this method, the lag is evaluated by calculating the time during early colonization when the slope equals half of the value of the maximum slope. In summary, use of the logistic equation for regression of sigmoid curves of colonization with time allows numerical comparison between curves of the lag, the period of steep ascent, and the plateau. The logistic equation does not model directly the fundamental processes at work in the development of the mycorrhizae. Instead, it can be used as described here to gain insight into the colonization process by comparing the dynamics of that colonization for different species under various conditions.

Keywords Lag · Inflection · Plateau · Sigmoid · Abruptness

Introduction

Temporal changes in the percentage root length colonized by arbuscular mycorrhizal (AM) fungi have been reported in various types of studies. Examples include effects of AM fungi on plant growth and metabolism (Fredeen and Terry 1988; Schubert et al. 1992; Scharff et al. 1997), competitive interactions between AM fungi (Wilson and Trinick 1983; Pearson et al. 1993), patterns of colonization by different isolates under similar conditions (Wilson 1984), fungus propagation methods (Hung and Sylvia 1988; Hung et al. 1991), and effects of environmental conditions and soil management on mycorrhizae (Abbott and Robson 1982; McGonigle and Miller 1993; Bendavid-Val et al. 1997; Nehl et al. 1998).

The increase in the percentage root length colonized by AM fungi in a developing root system was summarized by Mosse et al. (1981) as being a sigmoid curve composed of three phases. These phases were described as a lag phase, a phase of rapidly increasing colonization, and a plateau phase resting typically at less than 100%. Wilson and Tommerup (1992) discussed expected effects of inoculum density and placement on changes with time in colonization of roots by AM fungi.

Models involving root length have been applied to data for colonization of roots by AM fungi (Buwalda et al. 1982a, b; Walker and Smith 1984; Smith and Walker 1985; Sanders 1986). Changes with time in percentage root length colonized by AM fungi result from the combined effects of changes in total root length and colonized root length. In turn, changes in total root length reflect root growth and the loss of root with time, and changes in colonized root length reflect the growth and the loss with time of fungus structures within the roots. Modeling of the fundamental processes at work in the development of mycorrhizae, therefore, requires separate but related models of the growth of roots and of the

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growth of the fungi within them. However, a model for AM fungus growth in roots based on properties that can be routinely measured is not currently available. The experimental approach frequently used is estimation of percentage root length colonized by inspection of root-line intersections (Giovannetti and Mosse 1980; McGonigle et al. 1990).

Another approach is to use non-linear regression to analyze percentage colonization with time. The purpose of such non-linear regression is not to model directly the fundamental processes at work in the development of mycorrhizae. Instead, this numerical method is used to describe and summarize the measured changes in percentage root length colonized. It is hoped that this process of summary and description will facilitate better understanding of the dynamics of the colonization process and, thereby, provide clues for further research. The logistic equation has been used for non-linear regression of sigmoid-shaped responses of colonization of roots by plant pathogens (Gilligan 1990) and by AM fungi (Brandon et al. 1997; Pattinson and McGee 1997). However, failure to get a fit to the data using the logistic equation can occur when the data do not correspond sufficiently well to a sigmoid shape. The logistic curve is symmetrical, and this can lead to poor fit for some data. When the logistic curve is not applicable, a linear model might be a good alternative. However, such analyses must be tailored to each case. A sigmoid-shaped response was not found in some studies in established ecosystems (Oliveira et al. 1997), because it only applies to developing root systems (Mosse et al. 1981).

The aim here is to summarize briefly the use of the logistic equation with sigmoid-shaped responses in the colonization of roots by AM fungi, and to develop calculations based on the logistic equation to assess the lag during the early period of colonization. These calculations are applied to a case study that had obvious variation in the lag phase and to data with contrasting features that were selected from the literature.

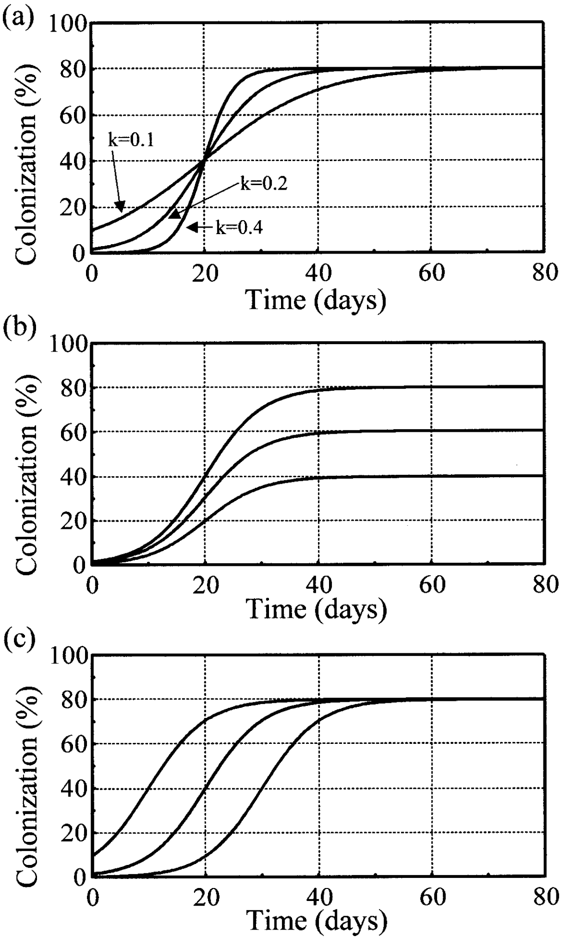

Fig. 1 Illustration of the effects of (**a**) k (the abruptness), (**b**) C_p (the plateau), and (**c**) t_i (the time of inflection) on the logistic curve $C=C_p/1+[e^{-k(t-t_i)}]$ (see Appendix for details). Values are: (a) C_p=80% and t_i=20 days, with values for k (day^{-1}) as shown; (b) k=0.2 day^{-1} and t_i=20 days, with values from top to bottom for C_p of 80%, 60%, and 40%; and (c) C_p=80% and k=0.2 day^{-1}, with values from left to right for t_i of 10, 20, and 30 days

Materials and methods

After summarizing the use of the logistic equation for non-linear regression of changes with time in colonization of roots by AM fungi (Eqs. 1–8 in Appendix), the effects on lag of coefficients k and t_i and of the plateau were investigated by generating hypothetical curves (Fig. 1). These curves were generated using the 2D-graphs custom-function option of Statistica 5.0 for Windows (Statsoft, Tulsa, Okla.). As an example, the custom function entered was y=80/<1+{exp[–0.2*(x–20)]}> to generate the central line of Fig. 1a.

A method was then developed (Eqs. 9–16 in Appendix) to determine lag in terms of the effects of coefficients k and t_i combined, because the hypothetical curves indicated (see below) that the lag in colonization is related to them both. t_i and k were inserted into Eq. 16 with w=5.828 to calculate the time during early colonization when the slope was equal to half of the value of the maximum slope. This method was applied to a case study with data modified after McGonigle et al. (1999) for colonization of American ginseng (*Panax quinquefolius* L.). These data were selected because a large difference in the lag phase was evident between plants. American ginseng has *Paris*-type (Smith and Smith 1997) colonization, and the values previously presented (McGonigle et al. 1999) were separate percentages of root length colonized by arbuscular coils and by non-arbuscular coils. At each point of inspection under the microscope, co-occurring arbuscular coils and non-arbuscular coils were recorded as arbuscular coils only (McGonigle et al. 1999), so that these data are additive, and they were combined here to give the percentage root length colonized (%RLC). All curve fitting used non-linear estimation within the statistics software. Using codes a (C_p), b (k), and c (t_i), a typical computer entry was *v2*=*a*/<1+{exp[–*b**(*v1*–c)]}> under the user-specified regression option with the variables arranged as *v2* for colonization and *v1* for time.

The logistic equation and the calculations based on it as presented here were applied to studies selected from the literature (Sanders et al. 1977; Jakobsen 1987; Rosewarne et al. 1997; Smith and Smith 1981 as presented in Smith and Read 1997). The data from these studies were for different combinations of soil, plant, and environment, and they serve to illustrate the variability between studies that occurs for the phases lag, steep ascent, and plateau. Data were taken from the following graphs: the lower-left part of Fig. 5 in Sanders et al. (1977); the line for non-treated and non-inoculated soil in Fig. 1 in Jakobsen (1987); the upper line in Fig. 3 of Rosewarne et al. (1997); and the data of Smith and Smith (1981) as presented in Fig. 2.12c in Smith and Read (1997). Sanders et al. (1977) inoculated onion *(Allium cepa* L.) with *Scutellospora calospora* (Nicol. and Gerd.) Walker and Sanders isolate BR in a low-P soil in pots. Jakobsen (1987) reported colonization for field-grown pea (*Pisum sativum* L.) colonized by indigenous fungi in non-fumigated soil. Fertilization raised the soil-P level, but colonization was similar in fertilized and non-fertilized pots, and so the pooled data were given (Jakobsen 1987). Rosewarne et al. (1997) grew recently germinated tomato (*Lycopersicon esculentum* L.) seedlings for 14 days in a sterile low-P soil-sand mix. These seedlings were then transplanted into similar pots with established leek (*Allium porrum* L.) nurse plants that had originally been inoculated with an isolate of *Glomus intraradices* Schenck and Smith. The data of Smith and Smith (1981) are for *Trifolium subterraneum* L. raised in pots with an experimentally manipulated propagule density in the soil.

Results and discussion

The form of the logistic equation in Eq. 1 can be changed by multiplying the term to which the base of natural logarithms is raised. However, multiplying this term prevents t_i from being read directly from the equation because it becomes embedded in the product of k and t_i that forms a different coefficient. Multiplying also makes the equation unnecessarily complicated by having k repeated.

The effects of the plateau and of coefficients k and t_i on lag

Coefficient k has units of the reciprocal of time and can be said to be the abruptness of the curve. When k is high the ascent is abrupt and the lag in the curve is long (Fig. 1a). When k is constant, C_p is related directly to the slope at inflection (Fig. 1b) as seen in Eq. 8. High values of t_i strongly increase the lag in the curve (Fig. 1c). The degree of lag in a curve, in so far as it relates to the progress with time of the attainment of the maximum slope, thus depends on t_i and k but not on the height of the plateau and not on the value of the slope at inflection. In this sense, the three curves in Fig. 1b all have the same degree of lag.

Application of the calculations to a case study

Regressions of the data for colonization of American ginseng (Fig. 2) using Eq. 1 and related calculations are summarized in Table 1. The plateau was relatively low for the plants in 1995, but it was higher for the first-year plants in 1996 because the slope at inflection was greater than in 1995 (Table 1). Although the t_i values differed by only 20 days between the first- and second-year plants, the time when the slope was half of the value of the maximum slope indicated that the lag in colonization lasted 25 days longer for first-year plants than for second-year plants growing alongside them in 1996 (Table 1).

Application of the calculations to data selected from the literature

The regressions using Equation 1 and related calculations for the data selected from the literature (Fig. 3) are summarized in Table 2. The time taken to reach 90% of the plateau was calculated for these data using Eq. 17, 18, and 19 in the Appendix. The rapid development of colonization for the tomato plants associated with leek nurse-plants (Rosewarne et al. 1997) was reflected in only 8.4

Table 1 Regression coefficients for the logistic equation $C=C_p/1+[e^{-k(t-t_i)}]$ (see Appendix for details) for colonization of American ginseng (*Panax quinquefolius* L.) in field plots (modified after McGonigle et al. 1999) as shown in Fig. 2. Calculated values are given for the maximum slope (at t_i) and the time during early colonization when the slope equals half the value of the maximum slope (f=0.50)

Year	Plant growth year	C_p (%)	k (day^{-1})	t_i (day)	Slope at t_i (% day^{-1})	Time when f=0.50 (day)
1995	1st. year	40.2	0.092	63.2	0.92	44.0
1996	1st. year	71.4	0.085	58.9	1.51	38.2
1996	2nd. year	65.5	0.067	39.4	1.09	13.1

Table 2 Regression coefficients for the logistic equation $C=C_p/1+[e^{-k(t-t_i)}]$ (see Appendix for details) for colonization data selected from the literature as shown in Fig. 3. Calculated values are given for the maximum slope (at t_i), the time during early colonization when the slope equals half the value of the maximum slope (f=0.50), and the time when 90% (g=0.90) of the plateau has been reached

Source	C_p (%)	k (day^{-1})	t_i (day)	Slope at t_i (% day^{-1})	Time when f=0.50 (day)	Time when g=0.90 (day)
Sanders et al. (1977)	61.1	0.298	35.6	4.6	29.7	43.0
Jakobsen (1987)	85.4	0.110	29.7	2.4	13.7	49.7
Rosewarne et al. (1997)	92.4	0.765	5.5	17.7	3.2	8.4
[a] Smith and Smith (1981)	74.3	0.269	13.4	5.0	6.8	21.6
[b] Smith and Smith (1981)	33.0	0.323	18.0	2.7	12.5	24.8

[a] 4.0 propagules g^{-1} dry soil as taken from Smith and Read (1997)
[b] 0.4 propagules g^{-1} dry soil as taken from Smith and Read (1997)

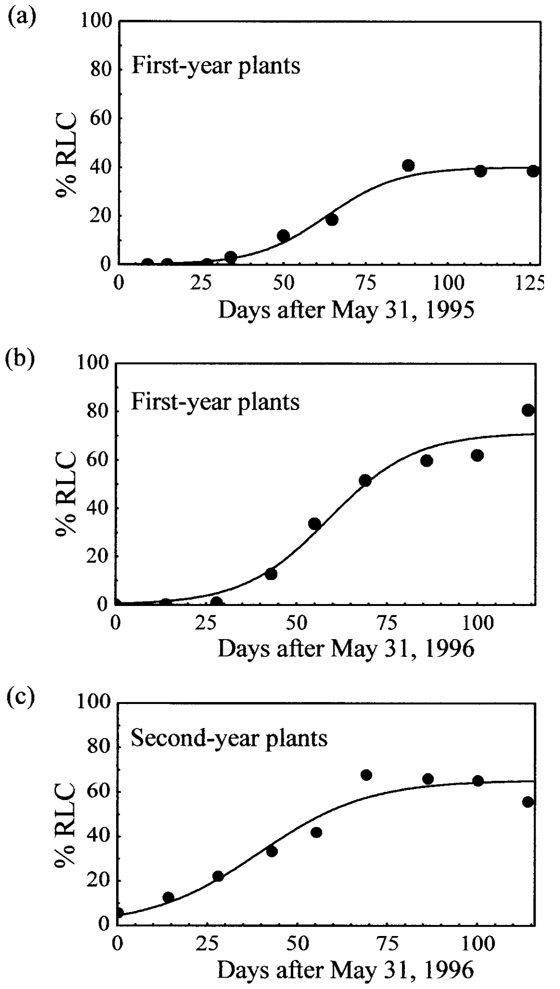

Fig. 2a–c Regressions using $C=C_p/1+[e^{-k(t-t_i)}]$ (see Appendix for details) for percentage root length colonized (*%RLC*) against days after 31 May in each year for American ginseng (*Panax quinquefolius* L.) in the field (modified after McGonigle et al. 1999). **a** First-year plants in 1995, **b** first-year plants in 1996, **c** second-year plants in 1996. All plants grew in the same experimental plots. The occurrence of the first-year plants in 1996 was caused by delayed germination of some seeds randomly across the site, which was in turn caused by varying post-harvest times needed for maturation between seeds

days being needed to reach 90% of the plateau. This rapid development of colonization was associated with a steep slope at inflection that in turn was related to a high value for the abruptness k (Table 2). Comparing the data of Sanders et al. (1977) and Jakobsen (1987), a difference in t_i of 6 days was associated with a difference in lag of 16 days because of different values for the abruptness (Table 2). The slope at t_i was 4.6% day^{-1} for the data of Sanders et al. (1977) compared with 2.4% day^{-1} for the data of Jakobsen (1987), and 90% of the plateau was reached only about 1 week later in the former than in the latter (Table 2). The data of Smith and Smith (1981) indicate that high propagule density can produce a considerably higher plateau than low propagule density over a similar time period (Table 2). The action of the high propagule density in increasing C_p was through an increase in the slope at inflection (Table 2). The reduced lag found with high relative to low inoculum density was associated with earlier inflection together with less abruptness for high versus low propagule density (Table 2).

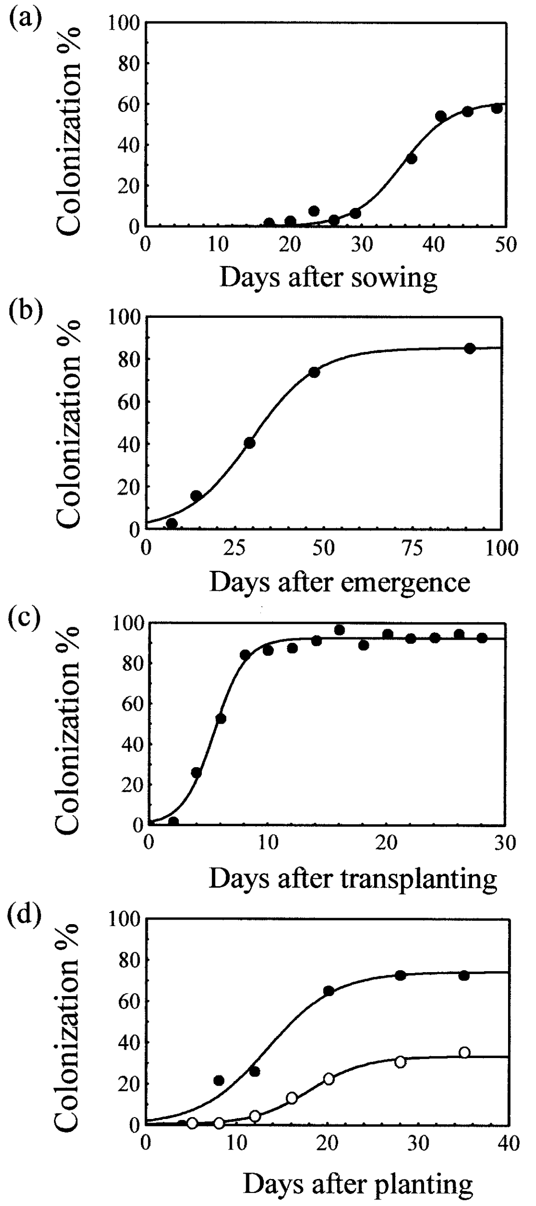

Fig. 3a–d Regressions using $C=C_p/1+[e^{-k(t-t_i)}]$ (see Appendix for details) for percentage colonization data selected from the literature for various soil-plant systems. **a** Sanders et al. (1977) for *Allium cepa* in inoculated pots, **b** Jakobsen (1987) for *Pisum sativum* in the field, **c** Rosewarne et al. (1997), for *Lycopersicon esculentum* in the presence of colonized *Allium porrum* nurse plants in pots, and **d** Smith and Smith (1981) as presented by Smith and Read (1997) for *Trifolium subterraneum* in pots with soils of high (*closed circles*) and low (*open circles*) inoculum density

Relationships between the logistic equation and the biology of colonization

The values of the slope at inflection and of k and t_i reflect the dynamics of the developing mycorrhiza. The height of the plateau relates to k and the value of the slope at inflection, whereas k and t_i reflect the lag in colonization. The value of the maximum slope and values of coefficients k and t_i thus summarize the interplay between various features of the process of colonization. One important feature is the extent to which colonization occurs from primary inoculum sources versus the extent to which it is secondary and caused by hyphae running along root surfaces and from root to root within a root system (Friese and Allen 1991). The influence of established hyphal systems as an inoculum source on the colonization process versus spores and other types of inoculum also can be expected to be strong (Jasper et al. 1989; Evans and Miller 1990). Other factors affecting the colonization process include inoculum density (Wilson and Tommerup 1992), frequencies of entry points (Fitter 1991), the patterns in space and time of the growth of hyphae around roots (Friese and Allen 1991), and growth rates of roots and the rate of spread of hyphae within them (Brundrett et al. 1985). Experiments are now needed to elucidate how these factors relate to the values of the maximum slope, k, and t_i in the logistic curve.

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Appendix

The logistic equation

$$C = \frac{C_p}{1+e^{-k(t-t_i)}} \tag{1}$$

The logistic equation written as Equation 1 has colonization C as a percentage that increases with time t to a plateau C_p with k as a coefficient describing the abruptness of the curve and with t_i as the point of inflection.

Calculation of the slope at inflection

The first- and second-order derivatives of Eq. 1 are given in Eqs. 2 and 3.

$$\frac{dC}{dt} = \frac{C_p\, k\, e^{-k(t-t_i)}}{\left[1+e^{-k(t-t_i)}\right]^2} \tag{2}$$

$$\frac{d^2C}{dt^2} = \frac{2C_p\, k^2\left[e^{-k(t-t_i)}\right]^2}{\left[1+e^{-k(t-t_i)}\right]^3} - \frac{C_p\, k^2\, e^{-k(t-t_i)}}{\left[1+e^{-k(t-t_i)}\right]^2} \tag{3}$$

A simplified notation is used here where w is substituted as shown in Eq. 4, so that the function and its first- and second-order derivatives can be rewritten as in Eqs. 5, 6, and 7.

$$w=e^{-k(t-t_i)} \tag{4}$$

$$C = \frac{C_p}{1+w} \tag{5}$$

$$\frac{dC}{dt} = \frac{C_p\, k\, w}{(1+w)^2} \tag{6}$$

$$\frac{d^2C}{dt^2} = \frac{2C_p\, k^2\, w^2}{(1+w)^3} - \frac{C_p\, k^2\, w}{(1+w)^2} \tag{7}$$

At inflection, dC/dt in Eq. 6 is a maximum and dC^2/dt^2 in Eq. 7 is zero. When $t=t_i$ then $w=1$ by Eq. 4 and $C=C_p/2$ by Eq. 5. The slope at inflection can be found by replacing w in Eq. 6 with unity to give Eq. 8.

$$\frac{dC}{dt} = \frac{C_p\, k}{4} \tag{8}$$

A method to evaluate lag

The method considers the combined effects of k and t_i on the lag by calculating the time when a chosen proportion (f) of the slope at inflection has been reached, as shown by changing Eq. 8 to Eq. 9. Combining Eqs. 6 and 9 to give Eq. 10, it is then possible to proceed to Eq. 14, which has the quadratic solution shown in Eq. 15.

$$\frac{dC}{dt} = \frac{C_p\, k\, f}{4} \tag{9}$$

$$\frac{C_p\, k\, w}{(1+w)^2} = \frac{C_p\, k\, f}{4} \tag{10}$$

$$4w=(1+w)^2 f \tag{11}$$

$$[4w/f]=1+2w+w^2 \tag{12}$$

$$0=1+2w-[4w/f]+w^2 \tag{13}$$

$$0=1+\{[2(1-[2/f])]w\}+w^2 \tag{14}$$

$$w = \left\{-[2(1-[2/f])] \pm \sqrt{\left\{\left[(2(1-[2/f]))^2\right]-4\right\}}\right\}/2 \tag{15}$$

For simplicity, a value of $f=0.50$ was chosen here to compare curves. That is to say, the degree of lag in a curve was summarized by calculating the time when the slope was equal to half of the value of the maximum slope. Values of $w=5.828$ or $w=0.1716$ are calculated using $f=0.50$ in Eq. 15. The value is $w=5.828$ for the left-hand side of the curve. Using the value of w corresponding to the chosen f value, the value of t can be calculated by rearranging Eq. 4 to give Eq. 16.

$$t=[(\ln w)/(-k)]+t_i. \tag{16}$$

Calculation of the time when a given proportion of the plateau has been reached

Consider a proportion (g) of the plateau as in Eq. 17.

$$C=gC_{\mathrm{p}} \tag{17}$$

Calculation of the time at which g has been reached proceeds by combining Eq. 5 and Eq. 17 to give Eq. 18, which becomes Eq. 19.

$$\frac{C_{\mathrm{p}}}{1+w}=gC_{\mathrm{p}} \tag{18}$$

$$w=(1/g)-1 \tag{19}$$

The time when a given proportion of the plateau has been reached is calculated by inserting the value of w from Eq. 19 into Eq. 16.

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