Long-term ecological research on chlorophyll cycling in the Yuanyang Lake Nature Preserve I. The grey prediction models on chlorophyll degradation of *Chamaecyparis* var. *formosana* leaf

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Abstract

We applied the grey system theory to evaluation of chlorophyll (Chl) degradation in *Chamaecyparis* Sieb. & Zucc. var. *formosana* (Hayata) Rehder needle-leaf in the Yuanyang Lake Nature Preserve of northern Taiwan. Pigment analysis was finished within 12 h after collecting the samples. Four grey prediction models for the degradation of Chl *a*, Chl *b*, and for the change of Chl *a/b* ratio and water content were established and compared with the results of linear and exponential regression analysis. The residual error and accuracy range show that the grey prediction process is much better than regression analysis. The degradation of Chl *a* and *b* contains two phases, one being fast and the other slow.

Additional key words: exponential regression; grey prediction; linear regression.

Introduction

Chlorophyll is the most abundant green pigment in nature. Its biosynthesis is a very complex process from its precursor glutamate to final product (Reinbothe and Reinbothe 1996). The Chls are degraded into small molecules, which are recycled in the nature after completing their mission (Matile *et al.* 1996). Since Chl molecules possess carbon and nitrogen atoms, the synthesis and breakdown of Chl must affect the carbon and nitrogen cycles in nature. Many environmental factors—such as irradiance, temperature, and humidity—may influence the rates of biosynthesis and degradation of Chl. Constructing a biosynthetic or degradative model of Chl under normal condition prior to studying the effects of environmental factors on the models

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is crucial. The process of Chl degradation can be considered as a system, but its information is uncertain and incomplete. We, therefore, applied the grey system theory to build a behaviour model of Chl degradation in the needles of *Chamaecyparis* var. *formosana* in the Yuanyang Lake Nature Preserve.

The grey system theory was first presented in China in 1982 and thereafter it has been increasingly and widely applied in many research fields to efficiently deal with uncertainty, multi-input, discrete values, and incompleteness of a system (Deng 1982, 1989). This theory can explain a system with uncertainty and incompleteness of information *via* grey methodologies, including grey generating, grey relational analysis, grey modelling, grey prediction, grey decision making, and grey control. One of the biggest advantages of grey system theory is that only four groups of values, with equal or nonequal intervals, are required to predict the dynamic behaviour of a whole system. Grey prediction model GM(1,1) is the main component of grey system theory and is used in most research (Deng 1989). Among the many applications published in the Journal of Grey System, approximately 8 % are related to topics in life sciences, such as grain yield (Luo and Zhang 1991), vegetable and fruit yields (Long *et al.* 1993, Ma *et al.* 1996), crop breeding (Zeng *et al.* 1993, Guo 1994), serum markers of liver fibrosis (Chen and Tan 1995), diseases (Wei and Xie 1994), population (Jin and Tang 1994), and ecologic environment (Che and He 1993).

In this paper, the authors construct four grey models to forecast the dynamic breakdown of Chl *a* and *b* and the alteration of water content and Chl *a/b* ratio in the needles of *Chamaecyparis* var. *formosana* collected from the Yuanyang Lake Nature Preserve. In every aspect, the grey system theory has a more precise forecasting ability than the regression analysis.

Materials and methods

Leaves: Fresh and fallen needles of *Chamaecyparis* Sieb. & Zucc. var. *formosana* (Hayata) Rehder were collected from the walkway surrounding Yuanyang Lake and immediately stored in the ice box. All of the following procedures were done in a 4 °C cold room. The leaves were divided into five groups according to their homology colour—*i.e.*, dark green, green, green-yellow, yellow-green, and yellow—and designated, respectively, stages 1, 2, 3, 4, and 5. Fresh mature leaf was treated as belonging to the first, dark green stage and was used as control.

Water content: Sample leaves were dried in an oven at 60 °C for four days. The leaves were weighed before drying and after it. Water content [%] = [(fresh mass − dry mass) (fresh mass)$^{-1}$]×100.

Chl determination: Following extraction of liquid nitrogen-frozen needles with 80 % acetone, the concentrations of Chl *a* and *b* and Chl *a/b* ratio were determined according to the spectrophotometric method of Porra *et al.* (1989). Absorbance was measured with a *Hitachi U-2000* UV-visible spectrophotometer.

Table 1. The original values of water content, chlorophyll (Chl) *a* and *b* contents [g kg^{-1}], and Chl *a/b* ratio at different degradation stages of *Chamaecyparis* var. *formosana* needle-leaves in the Yuanyang Lake Nature Preserve. The values are means of three determinations. DM = dry mass.

Degradation [d]	Water content [%]	Chl *a* [g kg^{-1}(DM)]	Chl *b* [g kg^{-1}(DM)]	Chl *a/b*
1	60.45±3.11	1.944±0.052	0.765±0.041	2.54±0.13
2	52.73±2.34	1.844±0.071	0.710±0.031	2.59±0.12
3	45.01±3.24	0.774±0.018	0.348±0.010	2.22±0.05
4	48.03±1.78	0.460±0.015	0.256±0.011	1.80±0.09
5	37.77±2.87	0.178±0.013	0.156±0.005	1.14±0.08

Grey prediction: The single series first-order linear dynamic model *GM(1,1)* of grey system theory (Deng 1989) was used to make grey prediction model of pigment degradation and changes in water content. A series of degradation time values of tested compounds designated as $X^{(0)}(i)$ was established to make the corresponding first-order accumulated generating operation (I - AGO). The AGO of grey generating is to accumulate new values by a series of original values. Only the mean of three determinations was adopted to do grey prediction analysis (Table 1). The last equation can be used to calculate the prediction value. Meanwhile, the accuracy and residual error were analyzed. The details of grey operation are presented in the Appendix.

Results and discussion

The Yuanyang Lake Nature Preserve is located in the northeastern mountains in Hsinchu County, Taiwan. The total area of this preserve is about 374 ha and the lake area is about 3.6 ha. The elevation of the lake is 1670 m. The marsh area encircling the lake is about 2.2 ha. The surrounding slopes are covered with a cypress forest. The climate is classified as temperate heavy moist: the average maximal and minimal temperatures are 15±5 and 0±5 °C, respectively, and there is almost 100 % relative humidity the whole year. Owing to the heavy moist climate and swampy lake topography, moisture is a limiting factor for the hydrophytic and hygrophytic plants in this nature preserve (Hwang and Fan 1996). At 20:40 h on October 15, 1998, a strong typhoon, Typhoon Babes, struck northern Taiwan and subsided at 03:05 h on October 17, causing heavy rain to pour into the Yuanyang Lake Nature Preserve, and fresh and mature leaves of many kinds of plants fell to the ground. At 12:00 h of October 21, we collected the needles of *Chamaecyparis* var. *formosana* from the ground.

Fig. 1 shows the temperature, relative humidity, and solar radiation of the Yuanyang Lake Nature Preserve between October 10 and 25, 1998, equivalent to solar days 283 to 298, respectively. During the period from the possible falling time and collection of needles, the air temperature fluctuated between 13 and 21 °C, the relative humidity between 92 and 96 %, and the solar radiation was less than 3.2 W m^{-2}, except during two very short days of October 17 and 18, when it reached 6 and 8 W m^{-2}, respectively. Since a grey prediction based on five points is more accurate than that based on four points, only the former type of model is shown in the figures to compare it with the regression analysis.

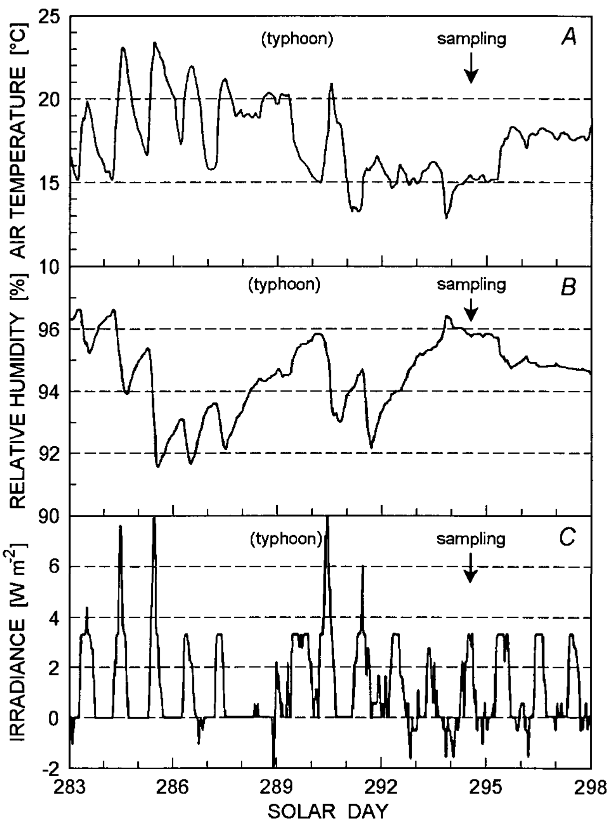

Fig. 1. Air temperature, relative humidity and solar radiation of the Yuanyang Lake Nature Preserve from October 10 to October 25 (solar days 283 to 298), 1998.

Dehydration grey model: The grey model of water content was predicted by four or five points, but only the five-point prediction model was shown as follows: y = 57.17 exp[-0.09 (t - 1)] (Fig. 2*A*). The water content of needle-leaves of *Chamaecyparis* var. *formosana* moderately decreased following the above five-point grey prediction equation. The average residual error of four- and five-point grey prediction and of exponential and linear regression analysis on water content was 7.79, 5.43, 4.74, and 4.94 %, respectively. Their maximal and minimal accuracies were between 96.79 and 83.77, 99.07 and 90.82, 98.62 and 90.50, and 97.96 and 91.18 %, respectively (Table 2). For water dehydration, both grey prediction models were very close to exponential and linear regression models, especially the five-point grey prediction model (Fig. 2*A*). Since the temperature was low, between 13 °C at night and 21 °C during the short daytime, and relative humidity was between 92 and 96 %, it is reasonable to assume that these needles dehydrated very slowly.

According to the five-point grey prediction model and exponential regression equation [y = 65.74 exp(-0.1 t)], water dehydration may take 123 and 111 d, respectively, to approach water content less than 0.1 %. However, it just takes 13 d according to the linear regression equation, y = 63.82 - 5.01 t, not shown in Fig. 1.

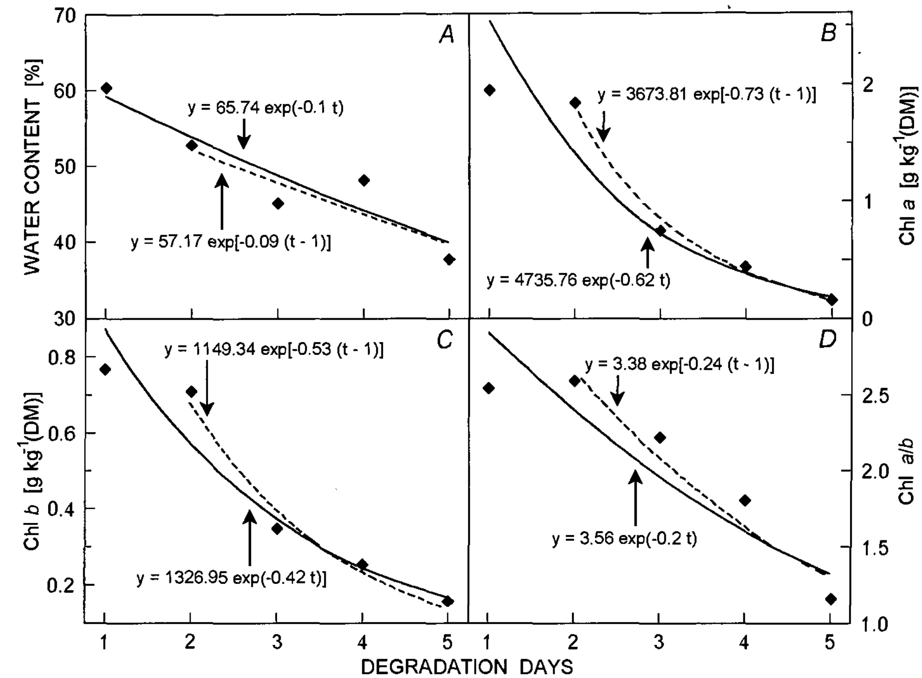

Fig. 2. The grey prediction and exponential regression analysis of the needle dehydration (*A*) and chlorophyll (Chl) *a* (*B*) and *b* (*C*) contents and their ratio in *Chamaecyparis* Sieb & Zucc. var. *formosana* (Hayata) in the Yuanyang Lake Nature Preserve. ♦ experimental values; - - - grey prediction; —— exponential prediction.

Chl *a* degradation grey model: The grey prediction model and exponential regression equation of Chl *a* degradation was as follows: y = 3673.81 exp[-0.73 (t - 1)] and y = 4735.76 exp(-0.62 t), respectively (Fig. 2*B*). The average residual error for four- and five-point grey predictions, exponential and linear regression, were 8.73, 8.89, 18.89, and 28.57 %, respectively. Their maximal and minimal accuracies were between 95.83 and 88.55, 95.80 and 88.96, 96.02 and 68.61, and 95.92 and 31.82 %, respectively (Table 2). No significant difference existed between four- and five-point grey predictions. However, the residual errors of both grey prediction equations were much smaller than for the two types of regression analysis, and the accuracy ranges of the former two were narrower than those of the latter two. According to the five-point grey prediction model, the Chl *a* in the fallen needles of *Chamaecyparis* var. *formosana* may disappear to the extent less than 0.1 % within approximately 22 d (Fig. 2*B*). However, the same process may take 25 d according to the exponential regression analysis. In spite of the grey prediction model or exponential regression analysis, the degradation process of Chl *a* can be divided into two phases, *i.e.*, it

Table 2. Comparison of residual error analysis of grey prediction, exponential and linear regression analysis of water, chlorophyll (Chl) *a* and *b* contents, and Chl *a*/*b* ratio of *Chamaecyparis* var. *formosana* needles in the Yuanyang Lake Nature Preserve.

	Degradation [d]	Grey prediction [%] four points	five points	Exponential regression [%]	Linear regression [%]
Water content	1	0	0	1.94	2.71
	2	3.21	0.93	1.38	2.04
	3	7.84	6.06	7.10	8.42
	4	3.89	9.18	9.50	8.82
	5	16.23	5.54	3.78	2.69
Max. accuracy		96.79	99.07	98.62	97.96
Min. accuracy		83.77	90.82	90.50	91.18
Average		7.79	5.43	4.74	4.94
Chl *a*	1	0	0	31.39	4.08
	2	4.17	4.20	25.28	16.95
	3	9.55	9.78	3.98	34.34
	4	11.45	11.04	12.74	19.31
	5	9.73	10.52	21.58	68.18
Max. accuracy		95.83	95.80	96.02	95.92
Min. accuracy		88.55	88.96	68.61	31.82
Average		8.73	8.89	18.89	28.57
Chl *b*	1	0	0	13.86	2.09
	2	3.85	4.60	19.30	13.45
	3	11.76	14.67	8.16	28.47
	4	13.29	8.00	3.23	9.47
	5	19.20	11.35	3.97	28.04
Max. accuracy		96.15	95.40	96.77	97.91
Min. accuracy		80.80	85.37	80.70	71.53
Average		12.02	9.65	9.70	16.30
Chl *a*/*b*	1	0	0	15.5	9.29
	2	0.40	2.96	7.23	6.68
	3	1.97	5.16	11.08	7.30
	4	1.17	7.65	9.91	5.61
	5	33.68	15.13	16.86	17.54
Max. accuracy		99.60	97.04	92.77	94.39
Min. accuracy		67.32	84.87	83.14	82.46
Average		9.31	7.72	12.05	9.28

degrades very fast for approximately 4 d, after which a very slow stage occurs for several more days. It is not the case that, as the linear regression equation ($y = 2515.76 - 491.82\ t$, not shown in Fig. 2*B*) suggests, that only 6 d are required to degrade Chl to the extent less than 0.1 % of initial content.

Chl *b* degradation grey model: The degradation behaviour of Chl *b* was very similar to that of Chl *a*, *i.e.*, $y = 1149 \exp[-0.53\ (t - 1)]$ and $y = 1326.95 \exp(-0.42\ t)$ for five-point grey prediction and exponential regression, respectively (Fig. 2*C*) The average residual errors of four- and five-point grey prediction models were 12.02 and 9.65 %, respectively, whereas those of exponential and linear analyses were 9.70 and 16.30 %, respectively. While the accuracy ranges of four- and five-point prediction models fluctuated between 96.15 and 80.80 %, and between 95.40 and 85.37 %, respectively, those of exponential and linear regression analyses were between 96.73 and 80.70 %, and between 97.91 and 71.53 %, respectively (Table 2). Even if the five-point grey model had a residual error similar to exponential regression, the former had a narrower accuracy than that of the latter. In the case of Chl *b* degradation, the grey prediction model still outperformed exponential regression analysis. According to the five-point prediction model, the Chl *b* in the fallen needles of *Chamaecyparis* var. *formosana* may totally disappear within approximately 28 d (Fig. 2*C*), taking longer than Chl *a*. Like Chl *a*, Chl *b* degrades very fast for the first 5 d and then enters a very slow stage, taking another 23 d to reach Chl *b* content of less than 0.1 %. If based on linear regression analysis, $y = 949.14 - 167.33\,t$ (not shown in Fig. 2*C*), it takes just 6 d to match the Chl *b* content.

Chl *a/b* ratio grey model: The change of Chl *a/b* ratio, an indicator of the relative accumulation of Chl *a* and *b*, may have resulted from the relative biosynthetic and degradative processes in this research. The grey prediction model for Chl *a/b* ratio alteration is $y = 3.38\exp[-0.24\,(t - 1)]$ and the exponential regression equation is $y = 3.56\exp(-0.2\,t)$ (Fig. 2*D*). While the average residual error of four- and five-point grey prediction equations was only 9.31 and 7.72 %, respectively, those for exponential and linear regressions were 12.05 and 9.28 %, respectively. Meanwhile, accuracy fluctuated between 99.60 and 66.32 %, and between 97.04 and 84.87 %, respectively, for four- and five-point grey predictions, whereas the fluctuation was between 92.77 and 83.14 %, and between 94.39 and 82.46 %, respectively, for exponential and linear regressions (Table 2). The combination of average residual error and accuracy range demonstrated that the grey prediction model is more precise than the regression analysis. The Chl *a*/*b* ratio may reach 0.1 % of initial value within approximately 35 d according to the five-point grey prediction equation, and may take 41 d according to the exponential regression (Fig. 2*D*). The linear regression analysis ($y = 3.14 - 0.36\,t$, not shown in Fig. 2*D*) shows that the Chl *a/b* ratio takes about 9 d to decline to less than 0.001. The values also indicated that during the first degradation stage, Chl *a* degraded much faster than Chl *b*, causing the Chl *a/b* ratio to quickly decline; and during the second stage, the degradation rate of both Chls converged, but that of Chl *a* was still slightly faster than Chl *b*, leading to the Chl *a/b* ratio decreasing slowly.

Conclusion: We applied the grey system theory to the Chl degradation behaviour of needles of *Chamaecyparis* var. *formosana* in the Yuanyang Lake Nature Preserve and compared the grey prediction models with the exponential and linear regression analyses. We showed that: (*1*) the grey system theory can be applied to the prediction of Chl degradation in the field; (*2*) this theory is more accurate than the linear and exponential regression analyses; (*3*) the degradation process of either Chl *a* or *b* can be divided into two stages, the first being fast and the second slow; (*4*) in the first stage, Chl *a* degrades more quickly than Chl *b* does, causing the Chl *a*/*b* ratio to decline very fast; (*5*) in the second stage, Chl *a* degrades slightly faster than Chl *b*, leading to a slow decrease in the Chl *a*/*b* ratio.

Both the external and internal factors influence Chl degradation patterns and rates. External factors such as low irradiance, short photoperiod, low temperature, and the extremely high relative humidity in the Yuanyang Lake Nature Preserve apparently retard the degradation rate. However, internal factors may also play an important role in the pattern and rate of Chl degradation. These include: (*1*) The location of Chl *a* and *b* in the thylakoid membrane, since most of Chl *a* is distributed in the margin of grana and in the stroma lamellar, where the Chl *a*/*b* ratio is high, and most of Chl *b* is located inside the grana, where the Chl *a*/*b* ratio is low. (*2*) Whether or not the Chl degradation enzymes including chlorophyllase and Mg-dechelatase are activated. Probably the unequal distribution of Chl *a* and *b* significantly affects their degradation rates. Chl *a* is generally more susceptible to degrading factors than Chl *b*, especially during the first stage; during the second stage the factors may attack the inside of grana and therefore more of Chl *b* is attacked. The attack difference may account for the two-stage decline in the Chl *a*/*b* ratio during the degradation process.

Since many factors affect the Chl degradation behaviour, it cannot be concluded at present which is the most important one. The grey relational analysis will be further applied to compare the possible factors.

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Appendix

The grey system theory was established by professor Deng Julong in 1982 and was originally based on fuzzy theory. The theory can deal with poor, incomplete, or uncertain problems of the systems. As far as the systems lacking information, such as structure message, operation mechanism and behaviour document, are referred to as grey systems. The "grey" means poor, incomplete, or uncertain information in a system. The goal of grey system and its applications is to bridge the gap existing between social science and natural science. So far, the grey system theory has many successful applications in areas such as agriculture, economy, medicine, geography, industry, military affairs, sports, management, material science, environmental protection, *etc.* (Deng 1989).

The aims of grey system theory are to provide theory, techniques, and ideas for analysis intricate systems, for example:
(*1*) To define and constitute grey process replacing the stochatic process and to find the read time techniques instead of statistical model to deal with the grey process.
(*2*) To turn the disorderly original data into a more regular series by grey generating techniques for the benefit of modelling instead of modelling with original values.
(*3*) To build a differential model—so called grey model (GM)—by using the least four values.
(*4*) To develop a novel family of grey forecasting methods instead of time series and regressive methods.

The grey operation used in this research is as follows:

(*1*) AGO (Accumulated Generating Operation)
Let $X^{(0)}$ be a nonnegative original data sequence,

$$X^{(0)}(k) = [X^{(0)}(1), X^{(0)}(2), \ldots\ldots\ldots\ldots X^{(0)}(n)]$$

$$X^{(1)}(k) = \text{AGO}\ X^{(0)}(k) = \sum_{i=1}^{k} X^{(0)}(i),\ k \in \{1,2,3 \ldots\ldots\ldots\ldots n\} \quad (1)$$

Taking AGO on $X^{(0)}$, we obtain a first order AGO sequence $X^{(0)}$.

(*2*) Mean generating operation, $Z^{(1)}(k)$

$$Z^{(1)}(k) = 1/2\{X^{(1)}(k) + X^{(1)}(k-1)\},\ k = \{2,3,\ldots\ldots n\} \quad (2)$$

(*3*) Grey differential equation of GM(1,1)

$$X^{(0)}(k) + aZ^{(1)}(k) = b,\ k = \{2,3,\ldots\ldots n\} \quad (3)$$

where a and b are called the developing coefficient and the grey input, respectively.

(*4*) Whitening equation of the grey differential equation is

$$\frac{dx^{(1)}}{dt} + ax^{(1)} = b \quad (4)$$

Define matrix $\hat{A}$, B, and Y_n as:

$$\hat{A} = \begin{bmatrix} a \\ b \end{bmatrix} = \left(B^T B\right)^{-1} B^T Y_N \qquad (5)$$

$$B = \begin{bmatrix} - & Z^{(1)} & (2) & 1 \\ - & Z^{(1)} & (3) & 1 \\ - & Z^{(1)} & (4) & 1 \\ & \vdots & & \vdots \\ - & Z^{(1)} & (n) & 1 \end{bmatrix} \qquad (6)$$

$$Y_n = [X^{(0)}(2), X^{(0)}(3), \ldots\ldots\ldots\ldots, X^{(0)}(n)]^T \qquad (7)$$

The solution of the whitening differential Eq. (4) is

$$X^{(1)}(n + p) = (X^{(0)}(1) - b/a)e^{-a(n+p-1)} + b/a \qquad (8)$$

where the parameter p is the prediction step size.

(5) Take I - AGO (Inverse Accumulated Generating Operation) on $X^{(1)}$.
The corresponding IAGO sequence $X^{(0)}$ is denoted as $X^{(0)}$ = I - AGO $X^{(1)}$

I - AGO: $\hat{X}^{(0)} = \hat{X}^{(1)}(k) - \hat{X}^{(1)}(k-1)$

$$\hat{X}^{(0)} = \hat{X}^{(1)}(n+p) - \hat{X}^{(1)}(n+p-1) = (X^{(0)} - \frac{b}{a})[e^{-a(n+p-1)}(1-e^{a})] \qquad (9)$$

where $\hat{X}^{(0)}(n+p)$ is the predicted value of $X^{(0)}(n+p)$.