

## BIOLOGY OF CUTLEAF EVENINGPRIMROSE

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### ABSTRACT

Experiments were conducted at the Upper Coastal Plain Research Station (Location 1) and the Fountain Research Farm (Location 2), Rocky Mount, NC. A completely randomized design was employed with fifty cutleaf eveningprimrose seedlings selected at the 4-leaf growth stage and monitored from October to early April prior to planting. Dependent variables included leaf number, whole-plant diameter, leaf area, and above-ground dry biomass per plant and were determined bi-monthly. Statistical analysis was performed on data collected from the four harvested plants (4 reps) at each timing. Data were subjected to ANOVA with sums of squares partitioned to evaluate linear and nonlinear effects of time. Location was considered random and time effects were tested by the appropriate interaction with the random variable. Regression analysis was used to describe the growth trends over time. Cutleaf eveningprimrose leaf number increased exponentially over time. Lack of location effect ( $P > 0.05$ ) indicates that cutleaf eveningprimrose leaf number is not environmentally dependent. Cutleaf eveningprimrose diameter increased exponentially over time, but variation existed between locations. Location 1 is adjacent to a swine farm and had a higher fertility rate. This location is sprayed by lagoon effluent and thus future work will investigate cutleaf eveningprimrose growth under different soil nitrogen fertility regimes. Cutleaf eveningprimrose leaf area increased exponentially over time. Although leaf number per plant was not environment dependent, the rate of leaf expansion was much greater in the location of higher fertility and may explain trends in whole-plant diameter. Cutleaf eveningprimrose above ground dry biomass also exhibited an exponential trend similar to leaf area. Trends indicate that most of the above-ground biomass can be attributed to leaf material. This is not uncommon for rosette-forming plants, like cutleaf eveningprimrose, in the vegetative stage. Cutleaf eveningprimrose growth exhibited an exponential trend from October to early April. The normal sigmoidal growth trend likely did not occur because field preparation halted growth during the linear phase and prevented an asymptotic response. The growth rate is slow between October and mid-February and the rapid linear phase of growth occur after this period. Thus reduced-tillage fields planted late are more likely to be problematic with large cutleaf eveningprimrose plants. Leaf area, whole-plant diameter, and aboveground dry biomass did exhibit environmental dependency, but leaf number was not affected by location.

### INTRODUCTION

Historically, cotton has been grown in a conventional-tillage environment using primary and secondary tillage. Prior to the registration of postemergence (POST) herbicides with over-the-top selectivity in cotton, producers were required to use intensive soil-applied herbicide treatments and high use rates of relatively non-selective herbicides and specialized equipment for postemergence-directed (PDS) applications (Buchanan, 1992; McWhorter and Bryson, 1992; Wilcut et al., 1995, 1997). These operations require considerable fuel, labor, and time. Increasing economic inputs, low commodity prices, and concerns for declining soil organic matter, subsoil compaction, and water stress damage have led to interest in alternative tillage options such as strip-tillage production systems (Troeh et al., 1991; Wauchope et al., 1985). Strip-tillage cotton acreage is increasing across

North Carolina and the Southeastern Coastal Plain. There are several advantages for utilizing strip-tillage production systems. These advantages include: (1) water conservation and reduction of sand blasting on sandy soils, (2) elimination of seedbed preparation reduces tillage operations and the number of trips made across the field, and (3) soil tilth and water-holding capacity are improved over time (Bradley, 1995). Strip-tillage production systems work well where soils are prone to develop a hardpan or plow layer that impedes root growth (Sholar et al., 1995). With this shift away from fall and winter tillage it has allowed the establishment of cool-season weeds, such as cutleaf eveningprimrose (*Oenothera laciniata* Hill). Successful elimination of vegetation prior to planting cotton in reduced-tillage production is critical for adequate stand establishments, eliminating early-season weed interference and maintaining yields. Poor weed control has been cited as the major limitation to adoption of cotton in conservation-tillage cotton production (McWhorter and Jordan, 1985). Weed management in cotton often requires both soil-applied and postemergence-applied herbicides for maximum effectiveness (Buchanan, 1992; Wilcut et al., 1995). Soil-applied herbicides do not provide season-long weed control in cotton, therefore proper selection of POST herbicides and other inputs are crucial for maximum weed control, cotton yield, and economic returns (Crowley et al., 1979; Culpepper and York, 1997; Wilcut et al., 1995, 1997). In the past 5 yrs, advances in biotechnology and new postemergence over-the-top (POT) technology have broadened cotton growers' options for weed management strategies (Culpepper and York, 1997, 1999; Wilcut et al., 1996). Bromoxynil, glyphosate, and pyriithiobac control a broad spectrum of weeds POST (Askew and Wilcut, 1999; Culpepper and York, 1997, 1998, 1999; Dotray et al., 1996; Jordan et al., 1993a; Scott et al., 2001). Bromoxynil and glyphosate can only be used in their respective transgenic herbicide-resistant cultivars (York and Culpepper, 2000). However, with this technology, farmers have become more reliant on fewer herbicide applications and with delaying burndown applications. This has led to the presence of cutleaf eveningprimrose at cotton planting. Traditional burndown chemistry is being replaced with glyphosate applications that are not effective on large cutleaf eveningprimrose plants.

Cutleaf eveningprimrose is an herbaceous winter annual or biennial native to Eastern North America (Uva et al., 1997). Cutleaf eveningprimrose can be found throughout the southeastern US. It is found in cultivated fields, sandy waste areas, and roadsides throughout the Southeast. Cutleaf eveningprimrose is a member of the Onagraceae family. It is a basally prostrate or weakly ascending plant with stems branching at the base. It has a fibrous tap root system. In its juvenile stages its stems are simple or many branched from the base up to 8 dm long and are hairy (Uva et al., 1997). The leaves are alternating oblong to lanceolate (3-8 cm long), coarsely toothed to irregularly lobed, dull green with short hairs present. The hypocotyl is short, smooth and not evident above the soil the second leaf develops. The cotyledons are kidney-shaped with flat petioles on the upper surface. Mature plants have flowers single in the leaf axils, lacking stalks, with large, yellow petals fused basally into a long narrow tube. The fruit is a four-lobed capsule (2-4 cm long) that is cylindrical and hairy becoming smooth with age (Uva et al., 1997). Cutleaf eveningprimrose seed are thick ellipsoid, sharply angular seed varying in shape (1.2-1.4 mm long and 0.8 mm broad). The seed are pale-brown and are strongly pitted (Uva et al., 1997).

Reduction in fall and winter tillage allows for the establishment of cool season species, including cutleaf eveningprimrose (Fairbanks et al., 1995). In March and April, the presence of very diverse cool season annuals makes it hard to identify some of these species. With the use of natural cover (i.e., winter weeds), the need for an early burndown treatment will depend upon the weed species present along with the size of the weeds (York et al., 1999). Early burndown is advantageous with ryegrass (*Lolium mutliflorum* Lam.), cutleaf eveningprimrose, and wild mustard (*Brassica kaber* (D. C.) L. C. Wheeler), wild radish (*Raphanus raphanistrum* L.), or curly dock (*Rumex crispus* L.) are

present (York et al., 1999). Legume cover crops that reseed contribute nitrogen, avoid the cost and problems of annual seeding and suppress the growth of troublesome winter weeds such as cutleaf eveningprimrose or horseweed (*Conyza canadensis* (L.) Cronq.) (Dabney et al., 1993). However, they will not produce adequate suppression of summer weeds as rye since they are maturing and dying back at the same time that the summer weeds begin to grow (Dabney et al., 1993). Since cotton is a poor early-season competitor, it is important that weeds be controlled during early growth (McClelland et al., 1993). Preplant burndown practices are also beneficial for conservation of moisture, nutrients and time in preparation of difficult-to-manage seedbeds (King, 1994).

If control efforts, such as 2, 4-D [2, 4-(dichlorophenoxy) acid] are delayed until April or May, then cutleaf eveningprimrose can be difficult to control (Reynolds et al., 2000). Cutleaf eveningprimrose is becoming a hard-to-kill winter annual and is one of the most prevalent spring weeds on the Coastal Plain of North Carolina. Of the commonly found broadleaf weeds listed, cutleaf eveningprimrose and horseweed are the most troublesome. This is because these weeds are usually spread across entire fields and can be difficult to control. Silt or sandy loam fields generally have more horseweed and, especially, more cutleaf eveningprimrose (Guy, Jr., 1995). The growth characteristics and development of winter weeds determine the impact they may have on cotton growth. Some winter annuals such as henbit (*Lamium amplexicaule* L.), common chickweed (*Stellaria media* (L.) Vill.) and annual bluegrass (*Poa annua* L.) do not persist throughout the entire cotton-growing season whereas weeds such as horseweed or cutleaf eveningprimrose will interfere with cotton the entire growing season (Guy, Jr., 1995). To date there is very limited research on this weed and no research data concerning cutleaf eveningprimrose growth, development, or the environmental affects that promote this species. Since cultural and chemical control practices targeted at weed management depend on knowledge of the basic growth characteristics and life cycle of weeds, we initiated this study. The purpose of this investigation is to study the growth and development of cutleaf eveningprimrose, a recent problem weed in early season strip-till cotton production in North Carolina. Based on these experiments, we hope to first learn of the optimal condition in which cutleaf eveningprimrose thrive in and draw a correlation to early-season problems reported by local farmers in controlling this species. Studies of germination and seedling establishment requirements yield basic ecological information for soil emergence (Bhowmik, 1997). Such information can be used to characterize the competitiveness and the potential infestation range of the weed as well as enhance management practices, allowing biological, chemical, or mechanical control options to be properly timed (Bhowmik, 1997; Dyer, 1995; Potter et al., 1984; Wilson, 1988). Therefore, research was initiated to gain an understanding of the germination requirements of this problematic early-season weed in cotton.

The objectives of this research will be evaluated in laboratory and field studies. For the laboratory work, we hope to determine the optimal germination factors for cutleaf eveningprimrose including temperature (constant and alternating regimes) and depth of emergence. The objectives of the field studies will be to determine the growth and development of cutleaf eveningprimrose throughout the winter and spring until cotton planting. Factors to be evaluated will be plant diameter, leaf area and number and plant above ground dry biomass.

## METHODS AND MATERIALS

Cutleaf eveningprimrose seed was harvested from fallow fields near Rocky Mount, NC in mid-April 1999 and 2000. The seed were stored in refrigerator. The seed were sieved to remove any extraneous plant or floral material. The sieved seed were divided in an air column separator<sup>1</sup> and separated into light and heavy fractions. The heavy fraction, the majority of which were fully developed seed, was used in germination and emergence experiments. Seed tested for viability using

1% tetrazoleum chloride solution prior to each trial (Peters, 2000). Cutleaf eveningprimrose seed tested % viable by tetrazoleum chloride tests before each study was conducted (data not shown).

A randomized complete block design was used for experiments in seed germination chambers. Experiments performed on the gradient table precluded randomization as the zones of temperature were fixed in position (Larson, 1971). There were six flasks per temperature zone on the gradient table and each flask represented one replication. Studies in seed germination chambers<sup>2</sup> had four replications of treatments, each of which was arranged on a different shelf within the respective seed germination chamber.

Preliminary experiments indicated cutleaf eveningprimrose germinated dependent of light in experiments in growth chambers. Therefore, light was provided for 8 h to coincide with the length of the high temperature component of the temperature regime for all studies conducted in growth chambers. Observations were made during the 8 h light period.

### **Growth and Development.**

Field studies will be conducted near Upper Coastal Plain Research Station (Location 1) and the Fountain Research Farm (Location 2), Rocky Mount, NC in the fall of 2002 and 2003. Approximately 50 germinating cutleaf eveningprimrose plants were flagged and monitor throughout the fall and up to planting. Plant size, growth in diameter and leaf number will be taken bi-monthly throughout the season. In addition to these measurements, four plants from each site will be harvested during each visit. The roots will be removed from these plants and fresh weight determined. The plant's leaf surface area will be measured using a leaf surface area meter and the plants will then be placed into a dryer for 3-5 d. After drying, the plants will be removed and dry weight measurements determined. This data will be replicated at two locations in Rocky Mount, NC over two seasons.

### **Effect of Temperature.**

The effect of constant temperature will be evaluated by evenly spacing 20 cutleaf eveningprimrose seed in 25 ml Erlenmeyer flasks containing three pieces of filter paper<sup>4</sup> and 8 ml of deionized water. Experiments performed on the gradient table precluded randomization as the zones of temperature are fixed in position (Larsen 1965). The flasks will be arranged on a thermogradient table (Larsen 1965) in six lanes corresponding to constant temperatures of 15, 20, 25, 30, 35, and 40 C, with six replicate flasks per temperature lane. Flasks will be sealed using Parafilm to retain moisture. Light will provided by fluorescent overhead bulbs set for an 8 h light 16 h dark regime with a light intensity of  $30.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Daily germination counts will be made for the first 7 d, and then every 3 d until no seed germination was observed for two observations. Each seedling will then be removed when a visible radicle could be discerned (Baskin and Baskin, 1998). The study will be conducted twice and the data combined for analysis.

A second study will be conducted in growth chambers to determine cutleaf eveningprimrose response to diurnal temperature. A randomized complete block design with four replications of treatments will be used and the study was conducted twice. Each replication will be arranged on a different shelf within the respective germination chamber. Blocks will be considered study replication over time. Twenty-five cutleaf eveningprimrose seed will be evenly spaced in 110 mm diameter by 20 mm Petri dishes containing 2 pieces of germination paper<sup>2</sup> and 10 ml of deionized water. Four temperature regimes will be selected to reflect typical seasonal variation in North Carolina. The regimes 10/25, 15/30, 20/30, and 20/35 C, correspond to mean daily low and high temperatures for the months of May, June, July, and August, respectively, in Rocky Mount, NC

(Owenby and Ezell, 1992). The high temperature component of the regime will be maintained for 8 h. Light will be provided by fluorescent overhead bulbs set for a 8 h light 16 h dark regime with a light intensity of  $34.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Daily germination counts will be made for 7 d, and then every 3 d until no seed germination is observed for 7 continuous days. Each seedling will then be removed upon germination as previously mentioned. The study will be conducted twice and the data combined for analysis.

### Depth of Emergence.

A depth of emergence study will be conducted to examine the effect of burial depth on cutleaf eveningprimrose seed emergence. The study design will be a randomized complete block with treatments replicated four times in a glasshouse at an average daily temperature of  $33 \pm 5 \text{ C}$  and a nightly temperature of  $23 \pm 5 \text{ C}$ . Natural light supplemented with fluorescent lamps at a light intensity of  $300 \pm 20 \mu\text{E m}^{-2} \text{s}^{-1}$  will be used to extend the day length to 14 h in the glasshouse study and to simulate field conditions in June. Containers will be filled to a depth of 10 cm with a Norfolk loamy sand soil (fine-loamy, siliceous, thermic, Typic Paleudults). Containers will be 15 cm in diameter by 18 cm tall. Twenty cutleaf eveningprimrose seed will then be placed on the soil surface or covered to depths of 0.5, 1, 2, 4, 6, or 10 cm with the same soil. Pots will be sub-irrigated prior to planting to field capacity, and then surface irrigated daily to field capacity. Emergence counts will be recorded daily for the first 7 d, and then every 3 d until no seed germination is observed for 7 continuous days. Plants will be considered emerged when a cotyledon can be visibly discerned. The study will be conducted three times and the data combined for analysis.

### Statistical Analysis.

Data variance was visually inspected by plotting residuals to confirm homogeneity of variance prior to statistical analysis. Both non-transformed and arcsine-transformed data were examined, and transformation did not improve homogeneity. Analysis of variance (ANOVA) was therefore performed on non-transformed percent germination. Trial repetition and linear, quadratic, and higher order polynomial effects of percent germination over time were tested by partitioning sums of squares (Draper and Smith, 1981). Regression analysis was performed when indicated by ANOVA. Nonlinear models were used if ANOVA indicated that higher order polynomial effects of percent germination were more significant than linear or quadratic estimates. Estimation used the Gauss-Newton algorithm, a nonlinear least squares technique<sup>6</sup>.

Germination resulting from constant temperature treatments was described by a parabolic model of the form:

$$y = \beta_0 + \beta_1 \text{temp} + \beta_2 \text{temp}^2 \quad [1]$$

where  $\beta_0$ ,  $\beta_1$ , and  $\beta_2$  are the intercept, first and second order regression coefficients, respectively, and  $y$  is the cumulative germination at temperature *temp*. A parabolic model was used to describe the germination of cutleaf eveningprimrose as the constant temperature used in the experiment allowed direct correlation of germination response.

ANOVA indicated higher order polynomial effects for germination resulting from alternating temperature treatments, solution pH treatments, and water potential treatments. Thus, the germination response for each treatment was modeled using the logistic function:

$$y = M [1 + \exp(-K(t - L))]^{-1} \quad [2]$$

where  $y$  is the cumulative percentage germination at time  $t$ ,  $M$  is the asymptote or theoretical maximum for  $y$ ,  $L$  is the time scale constant or lag to onset of germination, and  $K$  is the rate of increase (Roché et al., 1997). Estimation used the Gauss-Newton algorithm, a nonlinear least squares technique<sup>6</sup>. When a non-linear equation was fit to the data, an approximate  $R^2$  value was obtained by subtracting the ratio of the residual sum of squares to the corrected total sum of squares from one (Askew and Wilcut, 2001; Draper and Smith, 1981).

Depth of emergence data was subjected to an ANOVA using the general linear models procedure SAS<sup>6</sup>. No cutleaf eveningprimrose plants emerged from 10 cm, and consequently these data were not included in the analysis. Sums of squares were partitioned to evaluate planting depth and trial repetition. Both study replication and repetition were considered random variables and main effects and interactions were tested by the appropriate mean square associated with the random variable (McIntosh, 1983).

## RESULTS AND DISCUSSION

### Growth and Development.

All growth and development data were subjected to ANOVA with sums of squares partitioned to evaluate linear and nonlinear effects of time. Location was considered random and time effects were tested by the appropriate interaction with the random variable (McIntosh 1983). Regression analysis was used to describe the growth trends over time. Cutleaf eveningprimrose leaf number increased exponentially over time (Figure 6). Lack of location effect ( $P>0.05$ ) indicates that cutleaf eveningprimrose leaf number is not environmentally dependent. Cutleaf eveningprimrose diameter increased exponentially over time, but variation existed between locations (Figure 7). Location 1 is adjacent to a swine farm and had a higher fertility rate. This location is sprayed by lagoon effluent and thus future work will investigate growth under  $N_2$  fertility regimes. Cutleaf eveningprimrose leaf area increased exponentially over time (Figure 8). Although leaf number per plant was not environment dependent (Figure 6), the rate of leaf expansion was much greater in the location of higher fertility (Figure 8) and may explain trends in whole-plant diameter (Figure 7). Cutleaf eveningprimrose above ground dry biomass also exhibited an exponential trend similar to leaf area (Figure 9). Trends indicate that most of the above ground biomass can be attributed to leaf material. This is not uncommon for rosette-forming plants, like cutleaf eveningprimrose, in the vegetative stage. Cutleaf eveningprimrose growth exhibited an exponential trend from October to early April. The normal sigmoidal growth trend likely did not occur because field preparation halted growth during the linear phase and prevented an asymptotic response. The growth rate is slow between October and mid-February and the rapid linear phase of growth occurs after this period. Thus reduced tillage fields planted late are more likely to be more problematic with large cutleaf eveningprimrose plants. Leaf area, whole-plant diameter, and aboveground dry biomass did exhibit environmental dependency, but leaf number was not affected by location effects. Even though one location was a swine waste management area and had a higher fertility, trends in leaf number per plant were similar for both experiments.

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Figure 1. Influence of six constant temperature regimes on cutleaf eveningprimrose germination for 1999 seed lot.

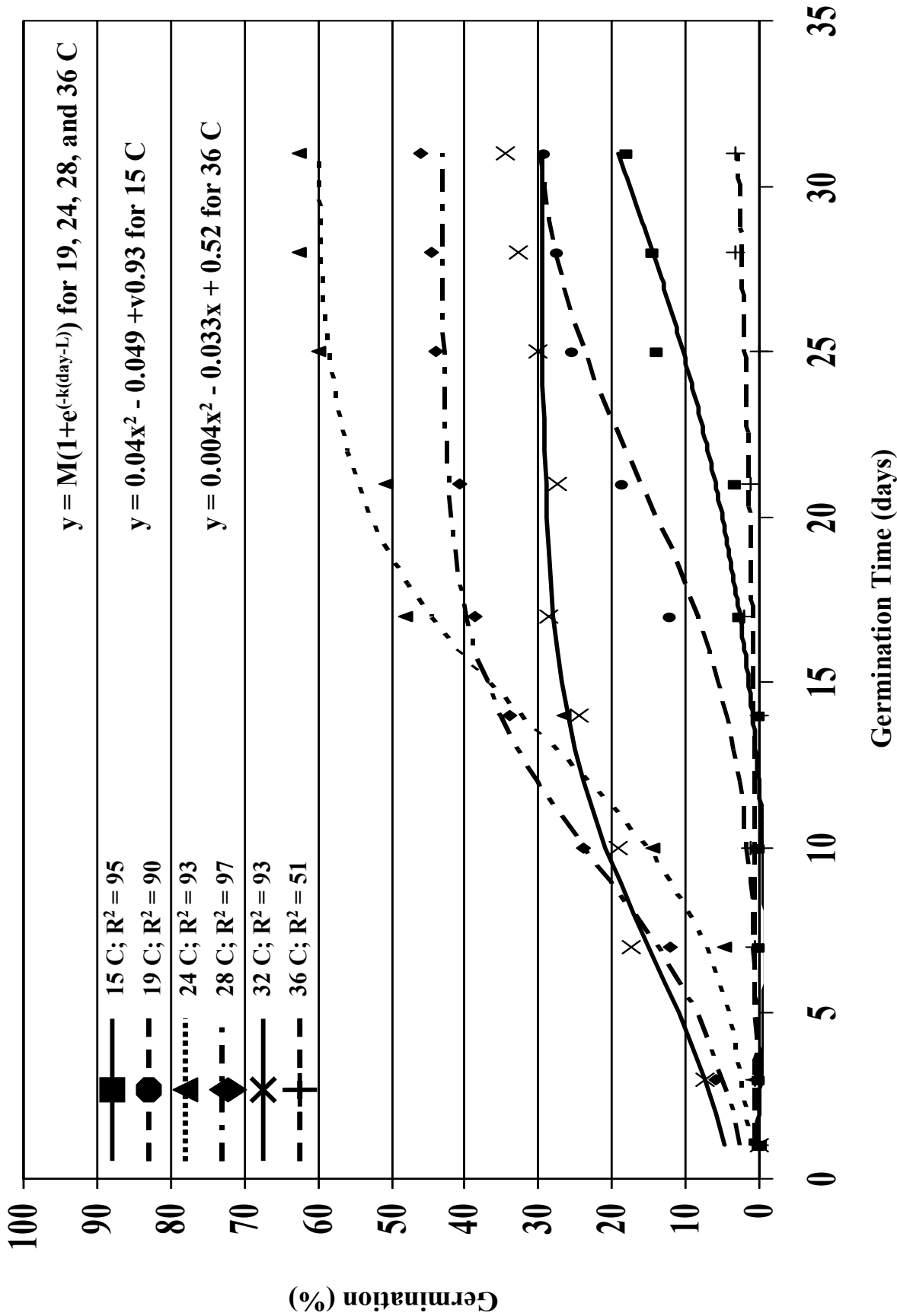


Figure 2. Influence of six constant temperature regimes on cutleaf eveningprimrose germination for 2000 seed lot.

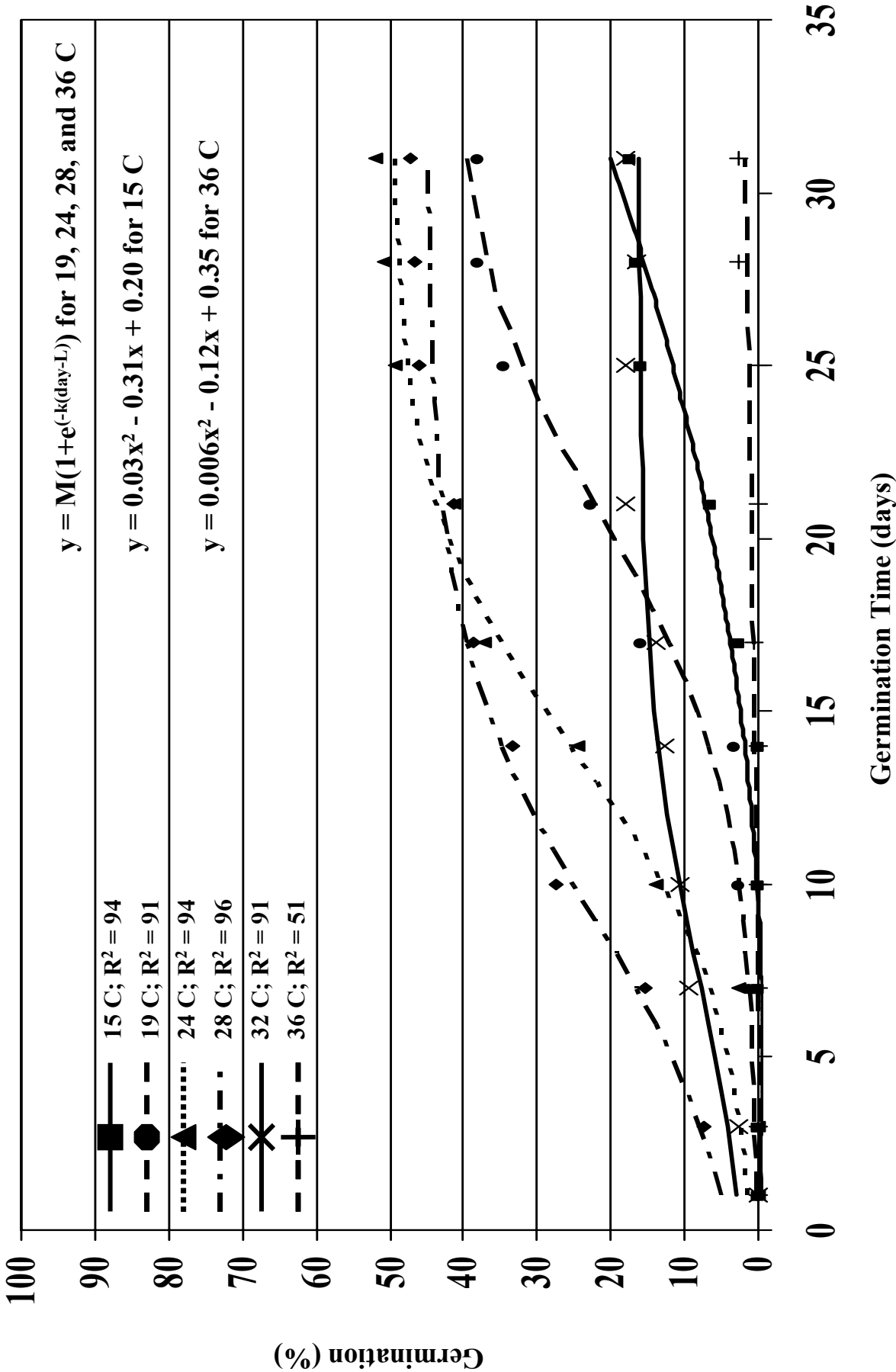


Figure 3. Influence of six temperature regimes on cutleaf eveningprimrose germination for 1999 seed lot.

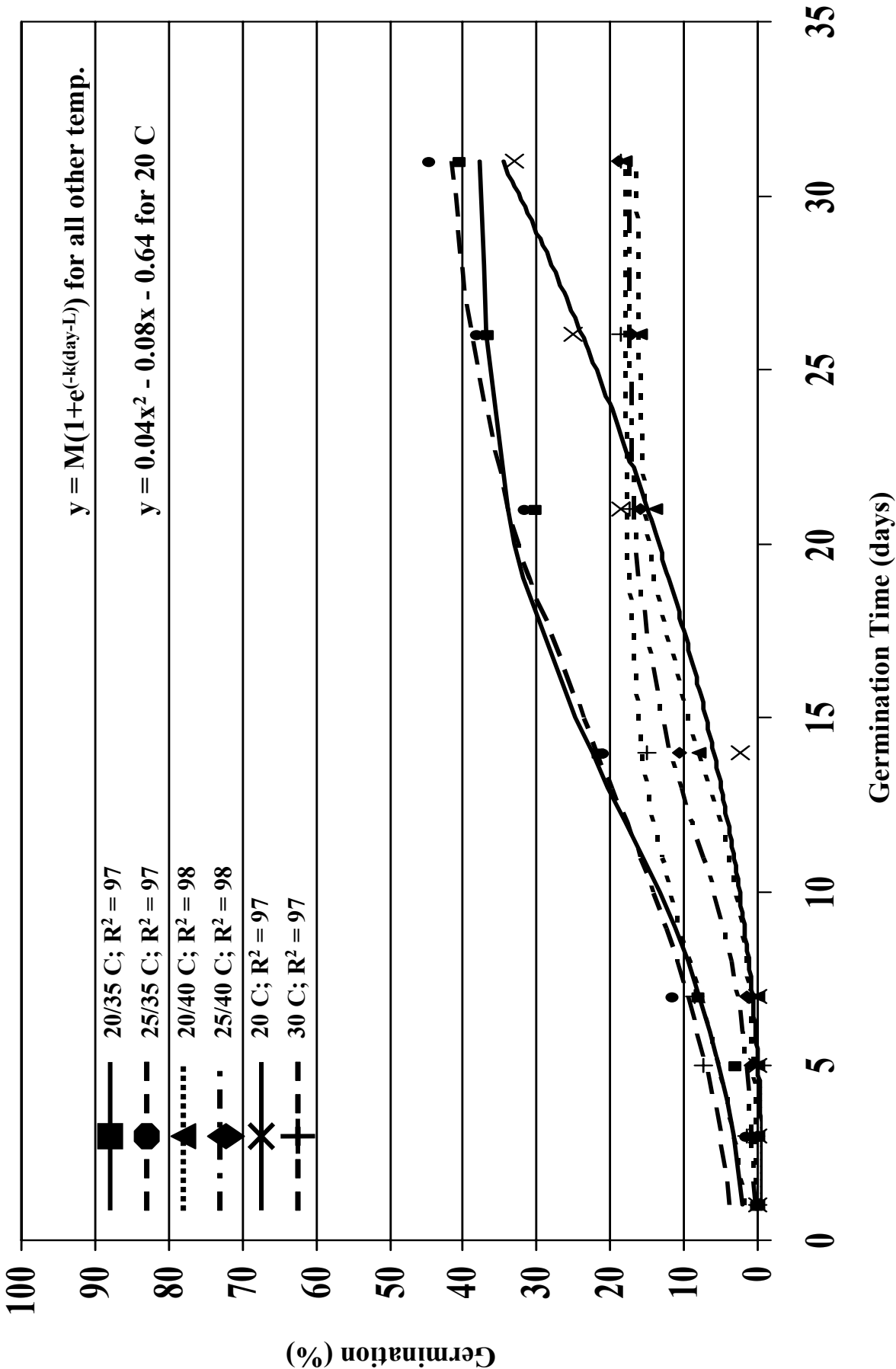


Figure 4. Influence of six temperature regimes on cutleaf eveningprimrose germination for 2000 seed lot.

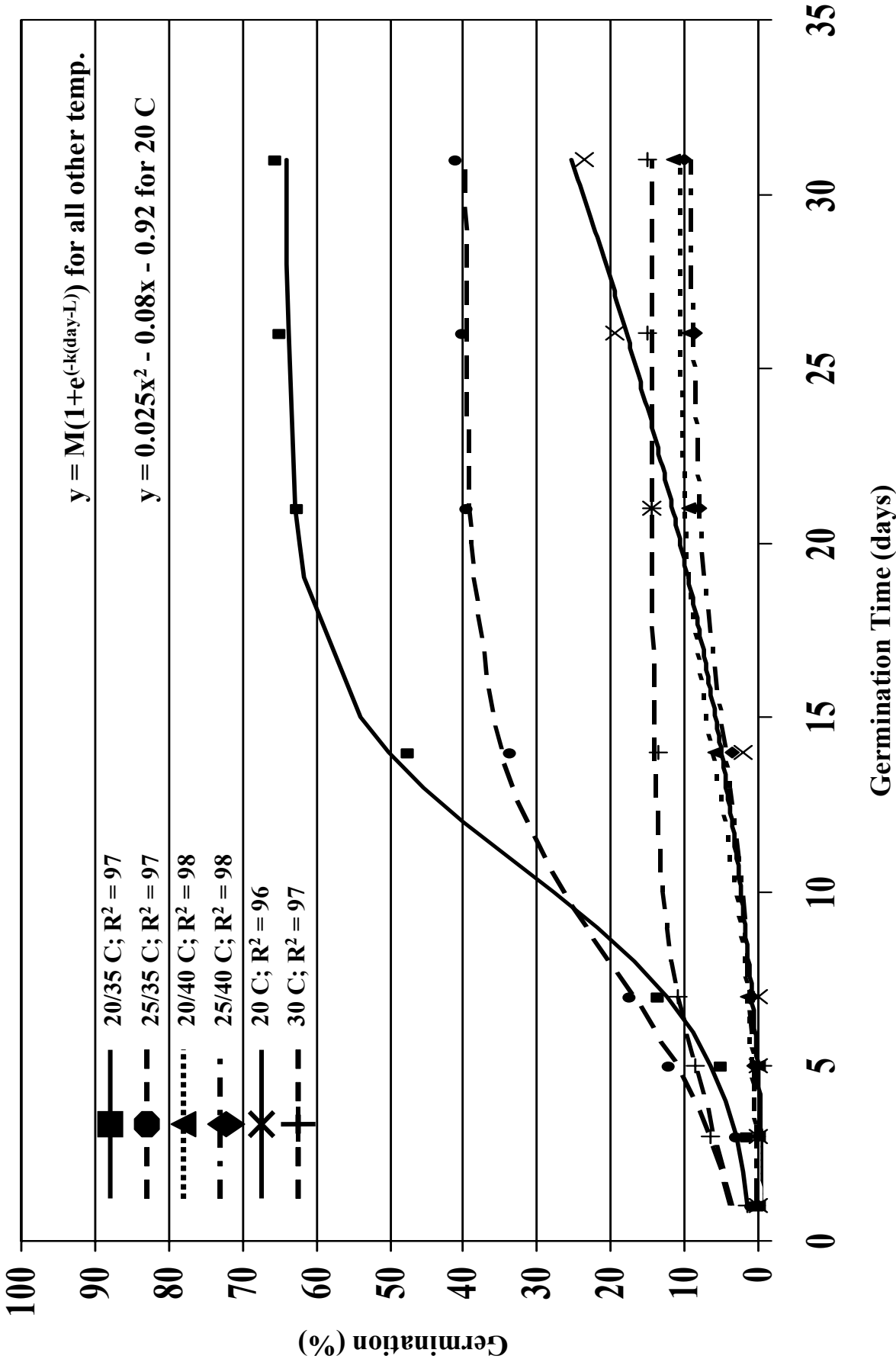


Figure 5. Four-Year Mean Maximum and Minimum Temperatures for North Carolina.

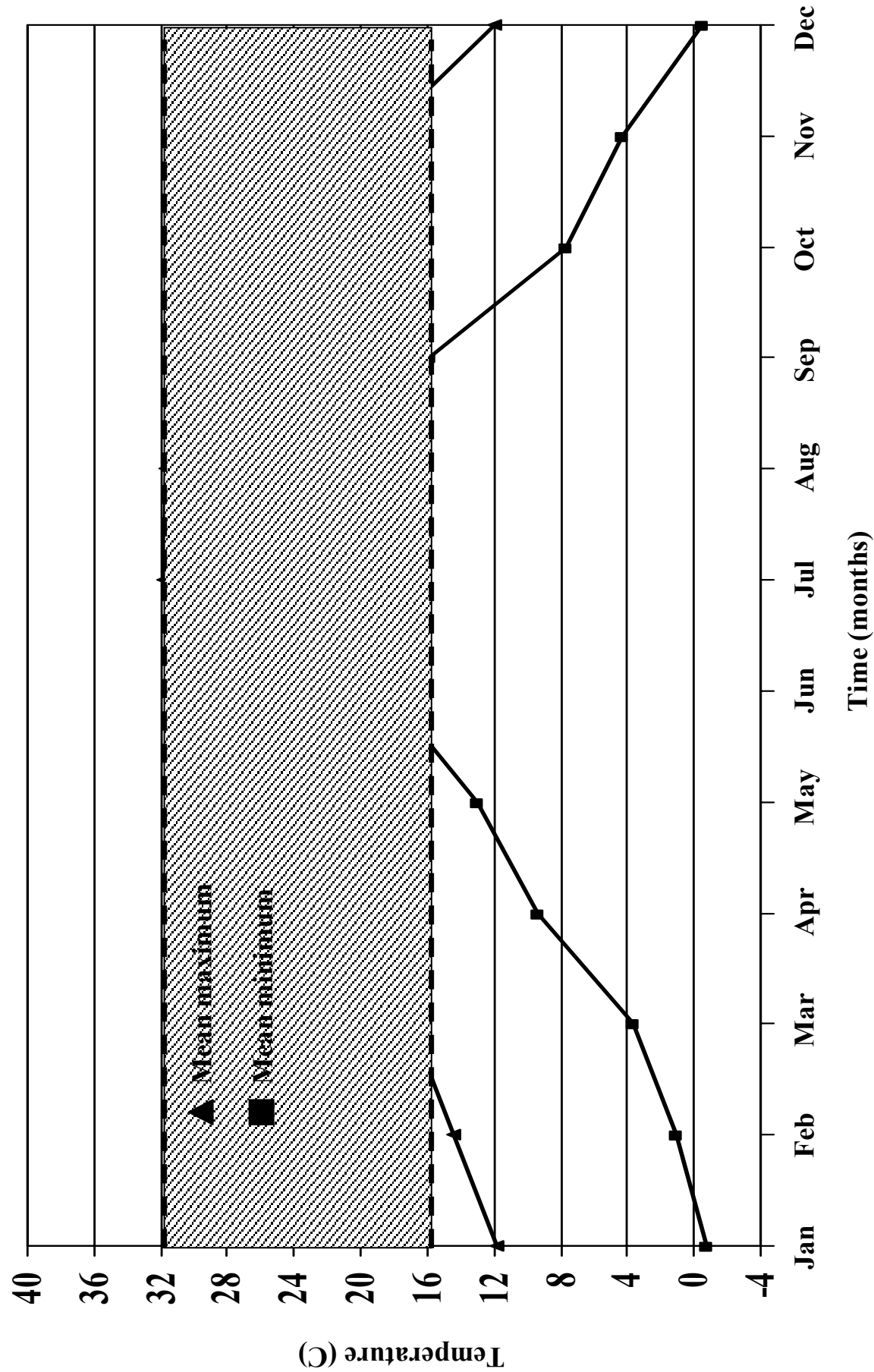


Figure 6. Average exponential increase in *O. laciniata* leaf number over time at two locations between October 2001 and April 2002.

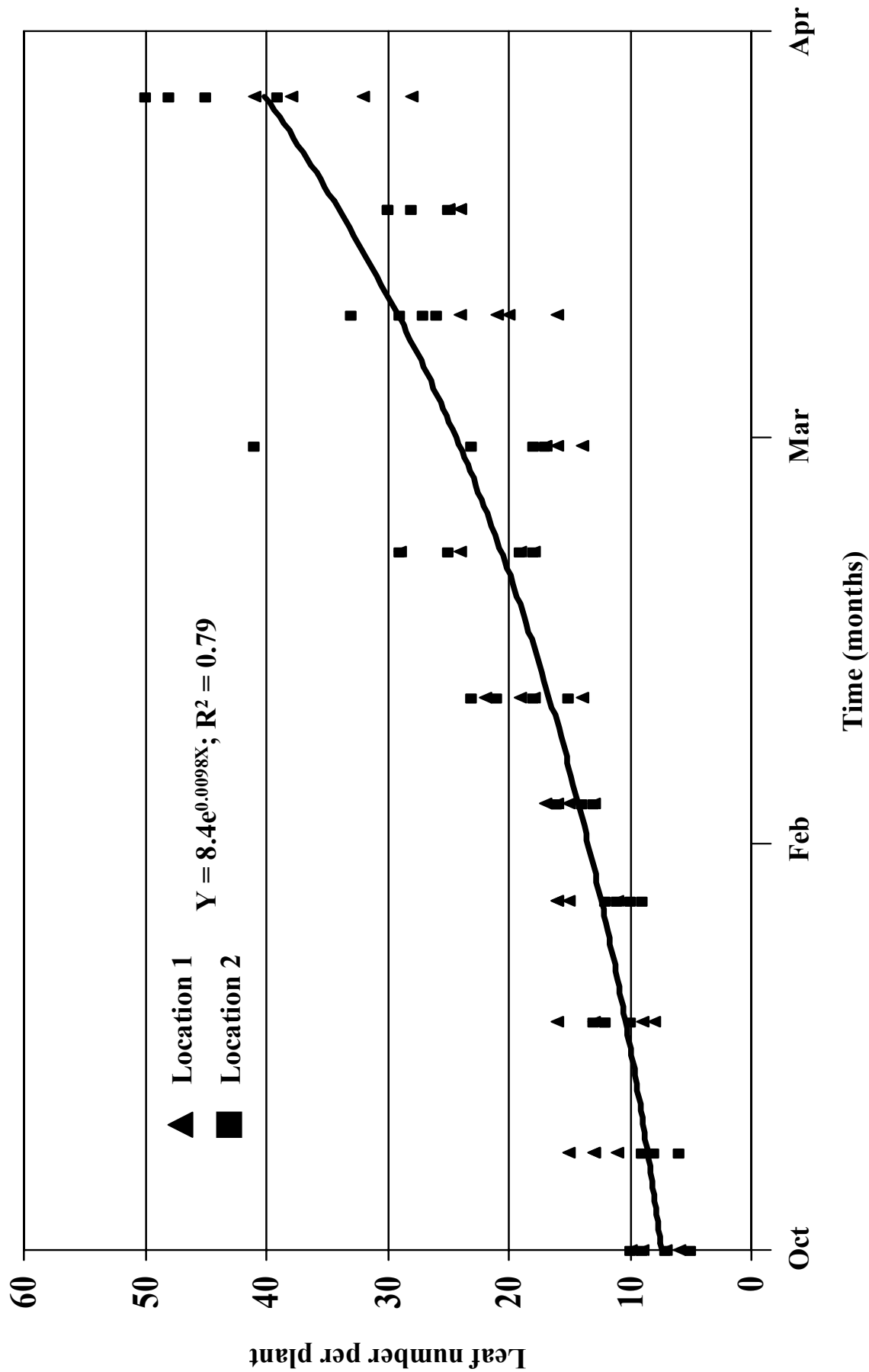


Figure 7. Exponential increase in *O. laciniata* diameter over time between October 2001 and April 2002.

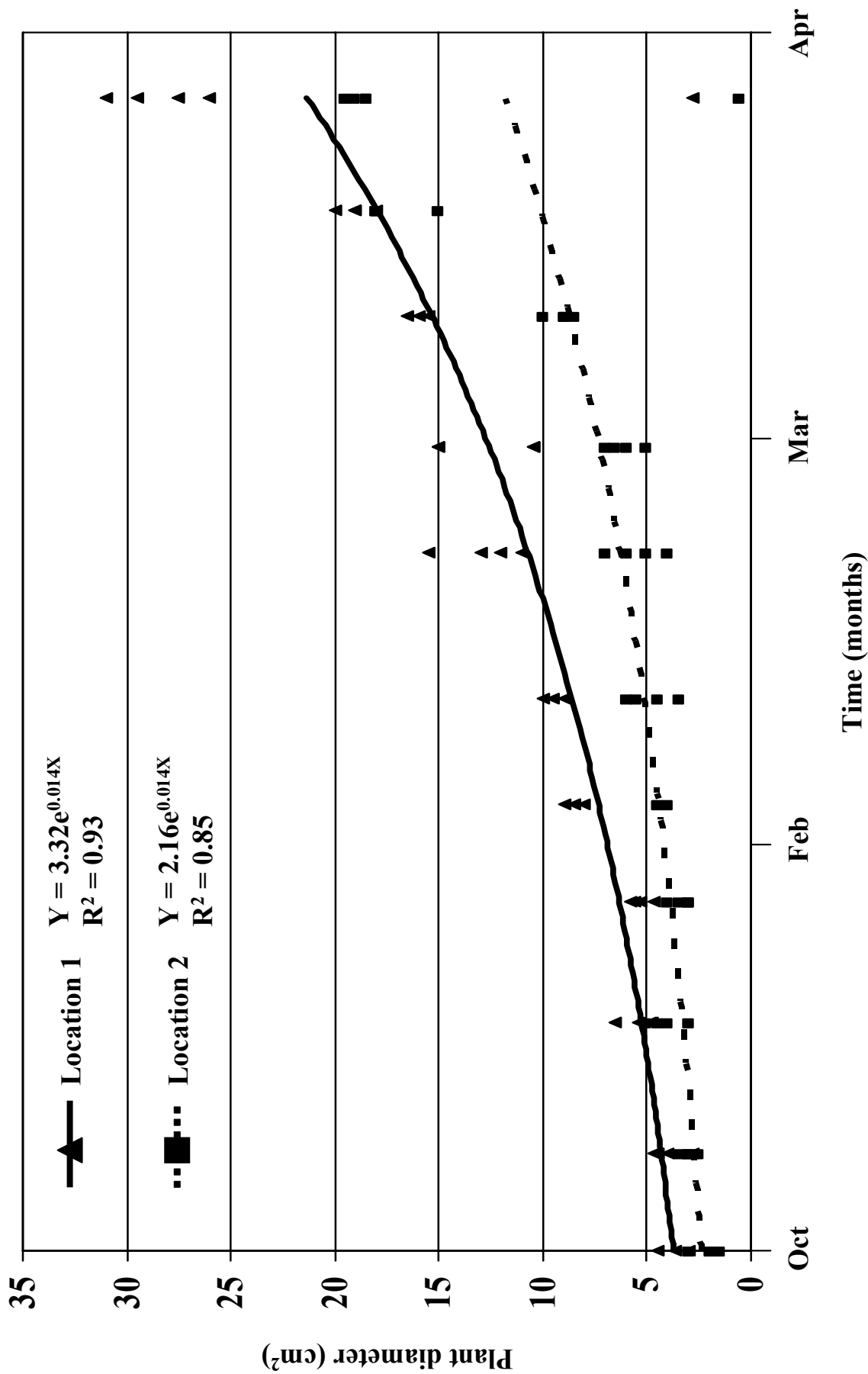




Figure 8. Exponential increase in *O. laciniata* leaf area over time between October 2001 and April 2002.

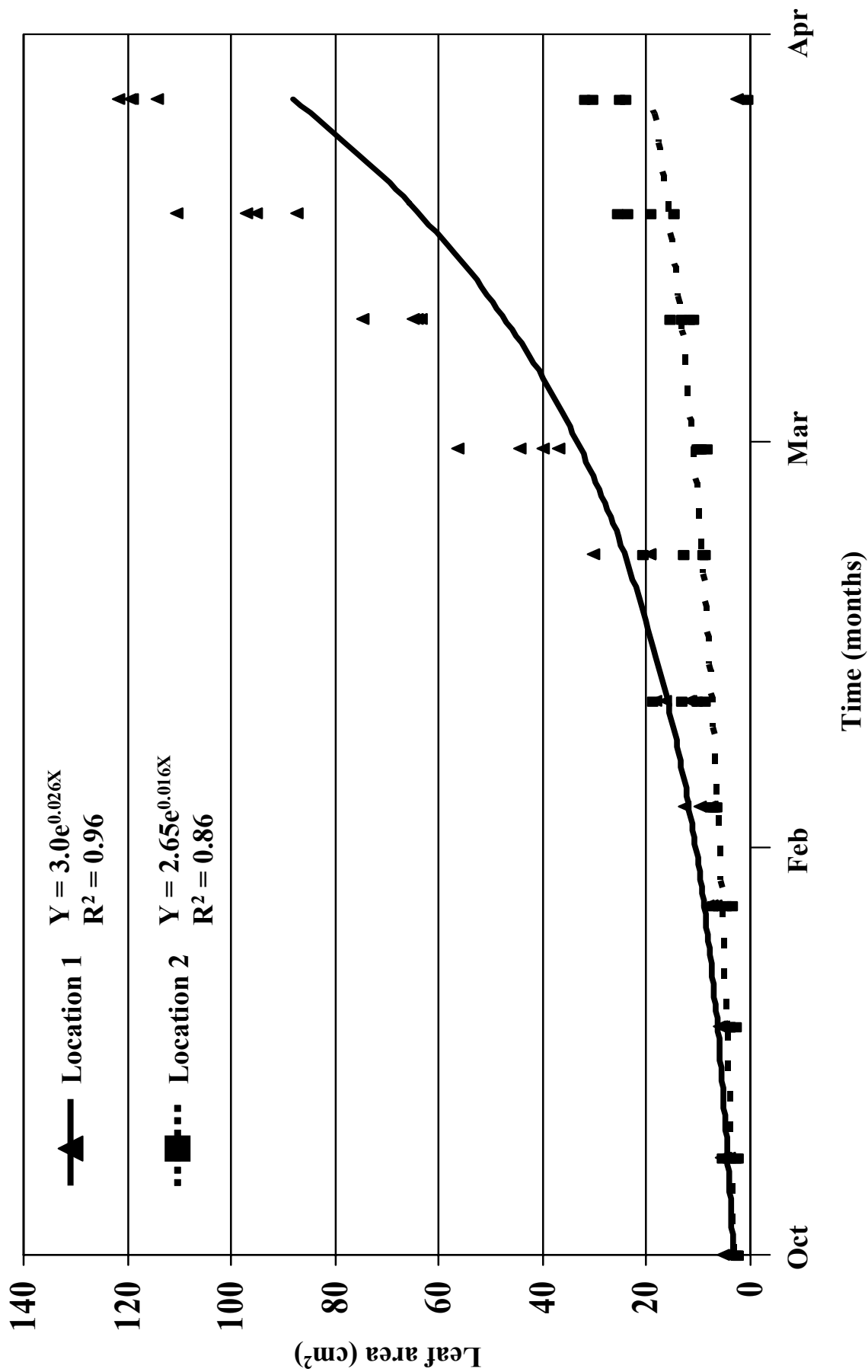


Figure 9. Exponential increase in *O. laciniata* dry biomass over time between October 2001 and April 2002.

