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OPTIMUM TEMPERATURE FOR CITRUS SEED GERMINATION

R. E. ROUSE AND J. B. SHERROD
University of Florida, IFAS
Southwest Florida Research & Education Center
P.O. Box 5127
Immokalee, FL 34143-5002

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Abstract. **Seed of 17 citrus varieties that have been used commercially or tested as rootstocks were obtained from the Florida Department of Agriculture, Division of Plant Industry, Citrus Budwood Office at Winter Haven. Seeds were germinated on a temperature gradient table in sand media maintained at a temperature gradient from 60 to 100 degrees Fahrenheit (°F). Seed varieties were sour orange, Smooth Flat Seville, *Citrus obovoidea,* Gou Tou #1, Zhu Luan, Cleopatra mandarin, Sun Chu Sha, X-639, Ridge Pineapple, Carrizo citrange, Benton citrange, C-35 citrange, Swingle citrumelo, F80-18 citrumelo, Rangpur lime × Troyer citrange, rough lemon, and *Poncirus trifoliata*.**

Optimum temperatures for germination ranged from 77° for *P. trifoliata* to 90.5°F for Rangpur × Troyer. The mean of optimum germination temperatures for all varieties was 85.5°F. Mean germination time and percentage seed germinated were recorded and used in the formula to determine optimum temperature for germination.

Citrus rootstocks are propagated from seeds. Citrus seeds are easily obtainable, relatively inexpensive, plentiful, and grow true-to-type (Castle, 1981). However, seedling uniformity is not guaranteed and the difference between the time for germination of the first and last seed varies (Mobayen, 1980). These problems with seedling growth have led to studies on the factors that affect seedling germination and growth. Factors include the effects of cold and dry storage, seed size, maturity and size of the fruit from which the seeds are extracted, seed coat removal prior to storage, and temperature. Of all of these factors, the least is known about the effects of soil temperature on germinating seeds.

The few experiments that have studied the effects of soil temperature on seed germination have been limited in number to the more traditional varieties. The varieties studied have included trifoliate orange (*Poncirus trifoliata* L. Raf.) (Mobayen, 1980), Cleopatra mandarin (*Citrus reticulata* Blanco) (Mobayen, 1980), sour orange (*C. aurantium* L.) (Camp et al., 1933; Fawcett, 1929), rough lemon (*C. jambhiri* Lush) (Camp et al., 1933), and several varieties of grapefruit (*C. paradisi*) (Camp et al., 1933). The optimum temperatures for these varieties range from 78.8°F (26°C) to 86.0°F (30°C) (Mobayen, 1980; Camp et al., 1933). The minimum and maximum temperatures ranged from 42.8°F (6°C) to 59.0°F (15°C) and 102.2°F (39°C), respectively (Mobayen, 1980). Information is needed on hybrid varieties of seed currently used commercially and on seed varieties that show promise in current trials for use in the future.

The objective of this study was to determine the optimum temperature for germination of 17 citrus varieties used as rootstocks including standard, previously researched varieties and newer unresearched varieties. The optimum temperature is defined as the maximum number of seeds germinated in the minimum time.

Materials and Methods

Materials and equipment used in this experiment include: Sand Sifter (18 mesh), Sakrete all-purpose sand, aluminum trays, clear packaging tape (4 inch wide), phosphoric acid 85%, 0.5% sodium hypochlorite solution, Seed Processing Thermal Gradient Table (Enkhuizen, Holland), Fisher Scientific Isotemp Constant Temperature Circulator Model 801, Fisher Scientific Isotemp Refrigerated Circulator Model 901, Sierra Antifreeze, Campbell Scientific 21X Datalogger, two Campbell Scientific AM416 Multiplexers, thermocouple wire (type T), and an IBM-compatible personal computer. Plant materials included seed of 17 citrus varieties used as rootstocks. Varieties were: Carrizo citrange (*C. sinensis* L. Osbeck × *P. trifoliata*), Benton citrange, C-35 citrange, Swingle citrumelo (*C. paradisi* × *P. trifoliata* L. Raf.), F-80-18 (*C. paradisi* × *P. trifoliata* L. Raf.), Cleopatra mandarin, Sun Chu Sha (*C. reticulata* Blanco), sour orange, Zhu Luan, Gou Tou #1 [both putuative hybrids most likely involving pummelo (*C. grandis* L. Osbeck), mandarin, and sour orange (Barnett and Rhodes, 1976; Hodgson, 1967)] (Castle et. al., 1992), Smooth Flat Seville (also known as Australian sour orange), *Citrus obovoidea* (common name, Kinkoji), trifoliate orange, rough lemon, 'Pineapple' sweet orange (*C. sinensis* L. Osbeck), Rangpur lime (*C. limomia* Osbeck) × Troyer citrange, and X-639 (Cleopatra mandarin × trifoliate orange).

Sand was sifted and then washed four times with a phosphoric acid solution followed by a deionized water rinse after each acid wash. The sand was then rinsed two additional times with deionized water.

Aluminum trays were made from u-channel that were cut to 39 inch lengths. The sides were cut with a hacksaw to the depth of the base, 1.5 inches from each end, bent, pop-riveted, and sealed with silicone to form closed-end trays with dimensions of 36 × 2 × 1.5 inches.

Seeds were obtained from the Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Bureau of Citrus Budwood Registration in Winter Haven, Florida. Seed were stored in refrigeration until used. Seeds from seven varieties ('Pineapple' sweet orange, Sun Chu Sha, *C. obovoidea*, Swingle citrumelo, Benton citrange, X-639, Rangpur × Troyer, and C-35) were washed with a 0.5% sodium hypochlorite solution for five minutes and then rinsed with deionized water to remove mold.

The thermal gradient table that was used was monitored for two weeks prior to beginning the experiment to establish a constant temperature range of 64.4 to 107.6°F (18 to 42°C). A data logger with multiple thermocouples attached was set up to monitor the temperature at numerous points on the table every minute.

Seeds were placed along the length of the aluminum trays in single rows using tweezers (two rows per tray, each row a different variety). Four seeds were placed per 3 inches giving a total of 48 seeds of one variety per tray except for Rangpur × Troyer and *C. obovoidea* (only 24 seeds per variety per tray due to limited seed availability). A total of 33 trays were used providing for 3 replications of Rangpur × Troyer and *C. obovoidea* and 4 replication of the other 15 varieties. The seeds were then covered with sand and watered enough to wet the sand, but not enough to have standing water in the bottom of the trays. The trays were covered with 4" wide clear packaging tape and sealed along the edges to prevent water loss and maintain an atmosphere of 100% humidity. All of the trays were then placed on the thermal gradient table in a randomized-complete-block arrangement.

During the experiment, 13 thermocouples were placed in the sand at 3 inch intervals in each of four trays in four areas of the table to record the temperature of the seeds at one minute intervals throughout the entire trial. Seeds were grouped by 3" intervals and identified as a temperature location, giving a total of 12 temperature locations per tray. This provided for a total of 16 seeds at each of the temperature locations. Temperature data was averaged for each hour and stored on a personal computer.

Seeds were checked daily for germination. When germination occurred, the number of the day since planting was recorded for that particular seed. Seeds were removed from the trays twice during the experiment to prevent crowding and allow for better viewing of germinating seeds. The experiment was terminated after 28 days.

$$
\begin{aligned}
y^{Germ(\%)} &= ax^2 + bx + c \\
-\ y^{MDG} &= ax^2 + bx + c \\
\hline
y^{Diff} &= ax^2 + bx + c
\end{aligned}
$$

$$
\begin{aligned}
\frac{dy^{Diff}}{dx} &= 2ax + b \\
0 &= 2ax + b \\
x &= \text{Max / Min or} \\
&\quad \text{Optimum Temp}
\end{aligned}
$$

Figure 1. Derivation of optimum temperature.

Results and Discussion

The temperature at specific locations across the gradient of the thermal gradient table remained constant throughout the trial. Minimal temperature deviation from the mean occurred when the table was opened daily for data recording. Temperature variance for all locations was less than ±0.9°F (±0.5°C). All data recorded show that the thermal gradient table is uniform and reliable.

Correlation analysis had shown that the best regression equation for describing the effect of temperature on time for citrus seed germination is a second degree polynomial (parabolic curve) (Wiltbank et. al., 1995). In addition to using the time to germination response to evaluate optimum germination temperature, this study also considered percentage germination. Using the second degree polynomial relationship, equations of each variety were generated describing the effect of temperature on time (mean days) of germination ($y^{MDG} = ax^2 + bx + c$) and the effect of temperature on percentage germination ($y^{Germ(\%)} = ax^2 + bx + c$). By subtracting the mean days to germination equation from the percentage germination equation for each variety, the resulting equation describes the difference between the two equations. The apex of this equation ($y^{Diff} = ax^2 + bx + c$) describes the point at which the difference between the mean days to germination equation and percentage germination is maximized. The apex (maximum or minimum point on the parabolic line) is determined by setting the first derivative of y^{Diff} equal to zero and solving for x. The x value is the optimum germination temperature based on time to germination and percentage germination factors (Fig. 1).

The calculated optimum germination temperatures for each variety are shown in Table 1, along with the range of temperatures in which seeds germinated and the range of days to germination. The individual equation graphs of mean days to germination and percentage germination by temperature are shown for each variety in Fig. 2. With few exceptions, the optimum temperature for germination was near 30°C (86°F). This point of optimum germination, defined as the temperature at which the greatest number of seeds germinated in the least number of days, was the temperature within the overlap of the two curves at which the curves are the furthest distance from each other.

Table 1. Optimum Temperature for Germination, Germination Temperature Range, and Days to Germination Range of 17 citrus varieties.

Variety	Optimum Temperature (°F) for Germination	Germination Temperature (°F) Range	Days to Germination Range
Rangpur × Troyer	90.6a[z]	72.7-105.6	5-28
Citrus obovoidea	88.0ab	76.1-105.6	10-28
Carrizo citrange	87.6ab	66.0-105.6	5-28
Benton citrange	86.5ab	69.3-105.6	5-28
Rough lemon	86.1ab	68.5-104.0	9-28
Sour orange	86.0ab	69.3-105.6	7-28
Swingle citrumelo	86.0ab	66.0-105.6	5-27
Gou Tou #1	85.8ab	66.7-104.0	5-28
Smooth Flat Seville	85.7b	71.8-105.6	7-28
Cleopatra mandarin	85.5b	70.2-104.0	11-28
F80-18 citrumelo	85.3b	69.3-105.6	5-28
Zhu Luan	85.0b	66.0-105.6	8-28
'Pineapple' sweet orange	84.9b	67.6-105.6	8-28
C-35 citrange	84.5b	66.0-104.7	7-28
Sun Chu Sha	84.0b	71.8-105.6	9-28
X-639	84.0b	71.1-105.6	5-28
Poncirus trifoliata	77.4c	68.5-105.6	5-27

[z]Means separation by Duncan's Multiple Range Test, 5% level.

Percentage germination increased as temperature increased up to the optimum temperature and decreased as temperature increased above the optimum temperature (Fig. 2). Temperatures above optimum were detrimental to seed germination, and contin-

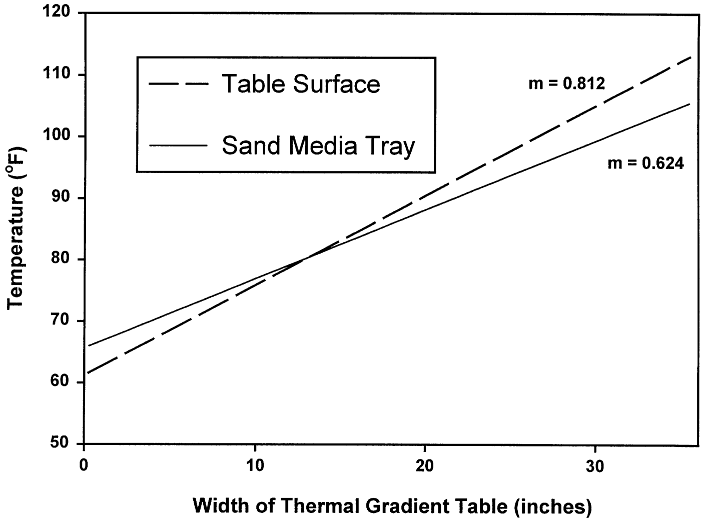

Figure 3. Temperatures measured on the thermal gradient table surface and in the sand media tray.

ued increase of temperature above optimum resulted in increased reduction in germination.

Mean days to germination decreased as the temperature increased up to the optimum temperature. Continued increase of temperature above the optimum temperature resulted in increased mean days to germination. This shows that temperatures above optimum were detrimental to germination of citrus seed.

All data recorded supports the conclusion that the temperature gradient table was uniform and reliable. There was very high correlation between temperature on the table surface and in the sand where the seeds germinated (Fig. 3).

These results are consistent with previous published (Wiltbank, et. al., 1995) and current unpublished data. Research in this area is continuing to support these results. Further studies are evaluating seed treatments, pre-germination conditioning, seed size, and growth regulator effects on seed germination.

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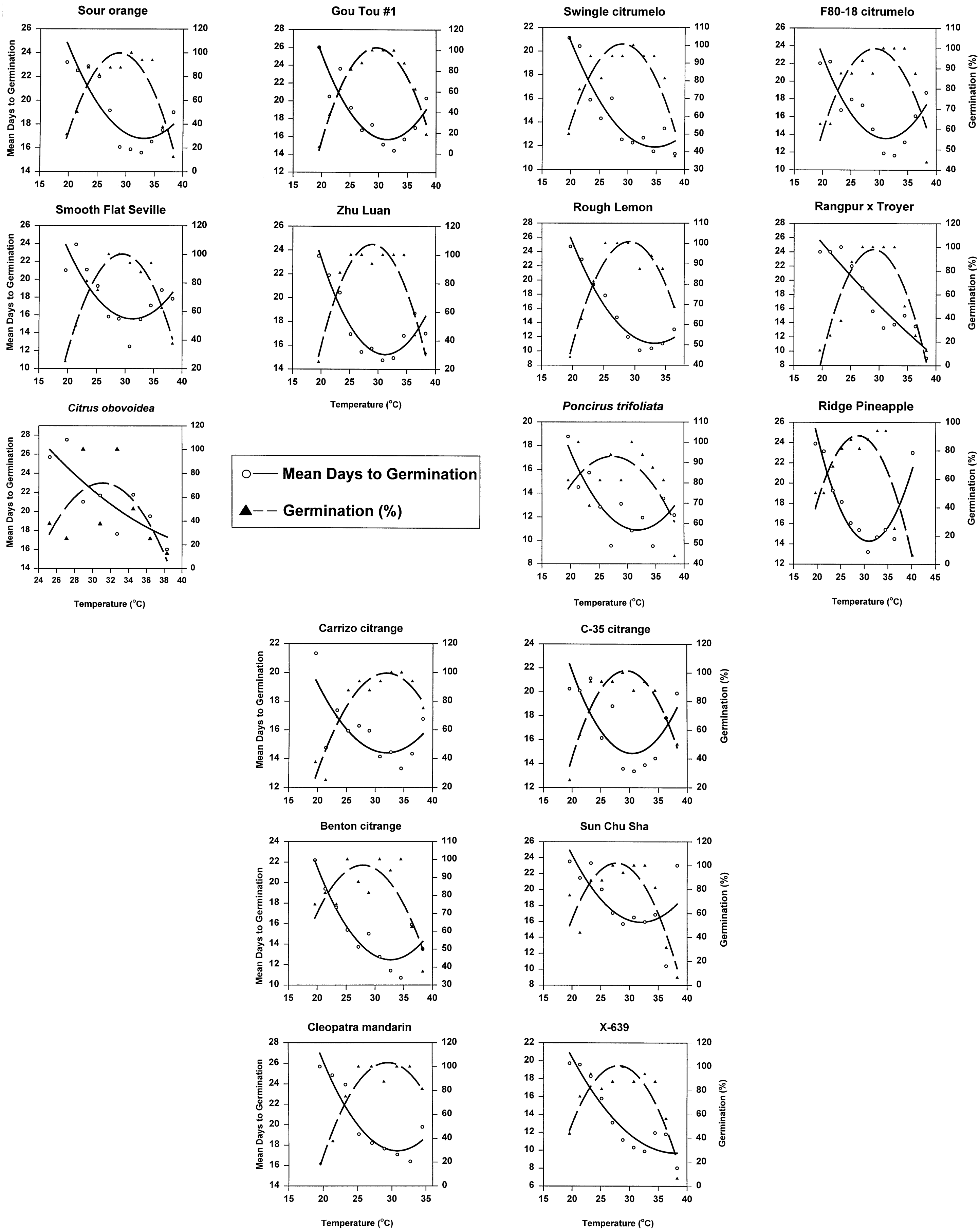

Figure 2. Graphs of mean days to germination and percentage germination vs. temperature (°C) for each citrus cultivar.