

IMPACT OF HEAT STRESS ON FIELD PENNYCRESS SEED YIELD AND POLLEN VIABILITY



WESTERN ILLINOIS UNIVERSITY

K. Parker and W.B. Phippen

School of Agriculture, Western Illinois University, Macomb, IL, USA

Western Illinois University

Alternative Crops Research

ABSTRACT

Field pennycress (*Thlaspi arvense* L.) is a new crop being investigated for its potential as a possible off season source for biodiesel. Related to the Brassica species of mustard and canola, winter annual field pennycress is also susceptible to heat stress during the reproductive stage of development. The objective of this study was to determine at what temperature seed development was inhibited in pennycress. The hypothesis of our experiment is that pollen viability becomes diminished as temperatures rise above 30°C as seen in the 2012 growing season.

A growth chamber experiment was conducted on spring pennycress 'Spring 32' over an 8 week period. Thirty plants in individual pots were grown to anthesis at 24°C day /18°C night. Five plants were transferred to 30, 32, 33, 34, and 35°C for 7 days and returned to normal conditions (24°C). Plant height, pod number, seed number, seed mass, dry biomass, seeds per pod, and harvest index were calculated for each plant. Pollen was collected from each plant after treatment, fixed in Carnoy's fixative, and examined for viability under a dissecting microscope. Pollen was also placed on growing media to evaluate percentage of pollen germination.

Plant height was not significantly impacted by increased temperatures when compared to plants which remained under 24°C conditions. However, temperatures above 30°C had a significant impact on pod and seed numbers. Pollen viability decreased by 84% at 30°C and 100% at 32°C. Pollen germination could not be determined due to the small size and poor visibility.

Pennycress's ability to handle heat stress will greatly impact which regions of the country are best suited for commercialization and aid breeders in evaluating lines for improved heat tolerance.

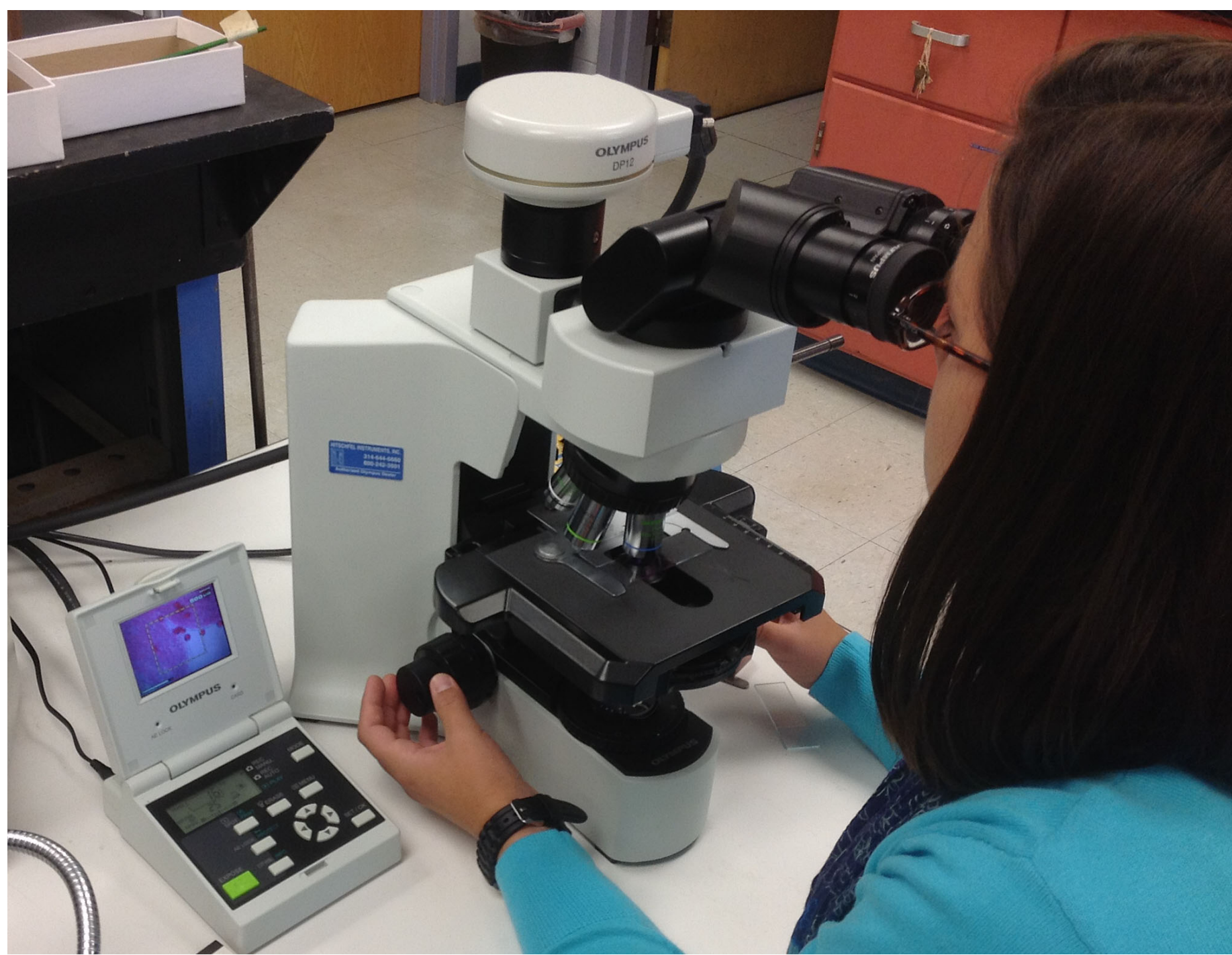


Figure 1. Determination of pollen viability by staining and observation under a phase-contrast microscope.

INTRODUCTION

Development of diverse and renewable feed stocks for the production of fuel has become a priority for the U.S. However, displacement of crops that produce the world's food from tillable land is a concern. One approach to simultaneously solve needs for food and fuel is to rotate both types of crops through the same land in one extended growing season. In recent years, it has been discovered that field pennycress (*Thlaspi arvense* L.) can be used as a domestic source of biodiesel fuel (Moser et al. 2009). Pennycress belongs to the Brassicaceae family and grows as a common weed throughout the temperate climate in North America. Pennycress is especially beneficial because it is a winter annual, grown in the off-season, and does not take land away from producing crops such as corn and soybean. Pennycress can be worked into a corn-soybean rotation, and it can also act as a cover crop.

Unfortunately, as with many winter annual crops, pennycress can be susceptible to heat stress in early spring. In studies done with Brassica species, it was found that at temperatures above 35/15°C day/night temperatures for 7 days during flowering caused yield reduction (Singh et al. 2008). Singh also reported that yield reduction is caused by pollen infertility which causes flower abortion. Heat stress during early flowering has a greater impact on yield than heat stress during pod development.

To improve the growth of pennycress as an agronomic crop, it is necessary to understand the response of pennycress to higher temperatures. In 2012, it was noticed that pennycress had decreased pod and seed set during the reproductive stage when temperatures rose in March and early April. The main objective of this study was to determine at what temperature pennycress plants display heat stress symptoms and what impact this would have on pollen viability.



Figure 2. Extraction of fatty methyl esters from pennycress seed.

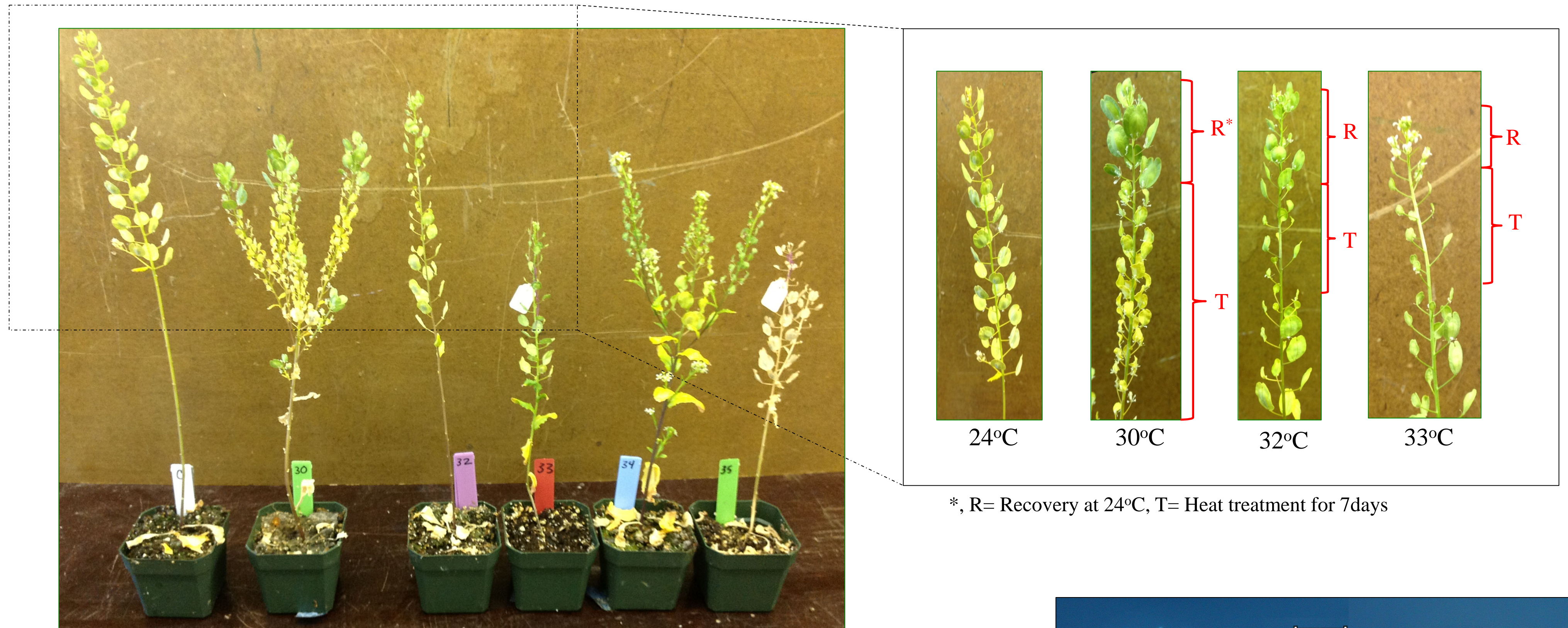


Figure 3. Pennycress plants grown under heat stress conditions for 7 days and returned to normal 24°C conditions.

Table 1. Mean spring pennycress response to heat stress during seed development.

Temp °C	Branch number	First branch height (cm)	First pod height (cm)	Final plant height (cm)	Pod number	Seed number	Number seeds per pod	Seed mass (g)	Dry Biomass (g)	1000 seed weight (g)	Pollen count
24	2.4ab*	2.95	14.5	28.6	68.2a	610.4a	9.2a	0.61a	0.77a	1.00a	662a
30	5.6b	6.5	16.2	31.6	70.8a	80.6b	1.3b	0.06b	0.92a	0.74b	522a
32	0.6ac	2	15.3	26.6	27b	0c	0c	0.3b	0	0	26b

* Within columns, means followed by the same letters are not significantly different at 0.05 probability level. Columns with no letters are not significant.

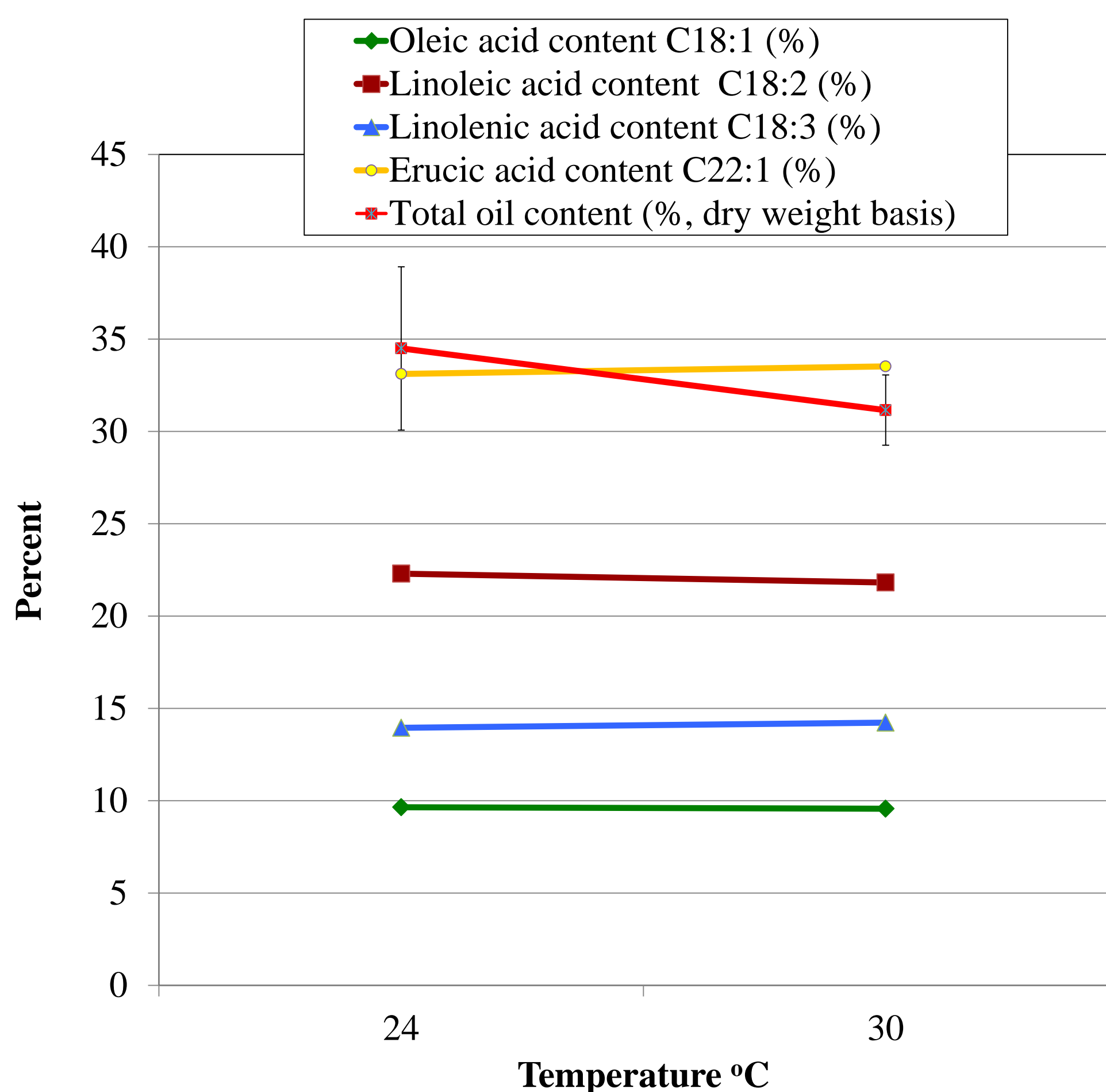


Figure 4. The effect of heat stress temperatures on fatty acid methyl esters and total oil content. Change in total oil content was not significant $p < 0.01$.

MATERIALS AND METHODS

Growth Chamber Experiment:

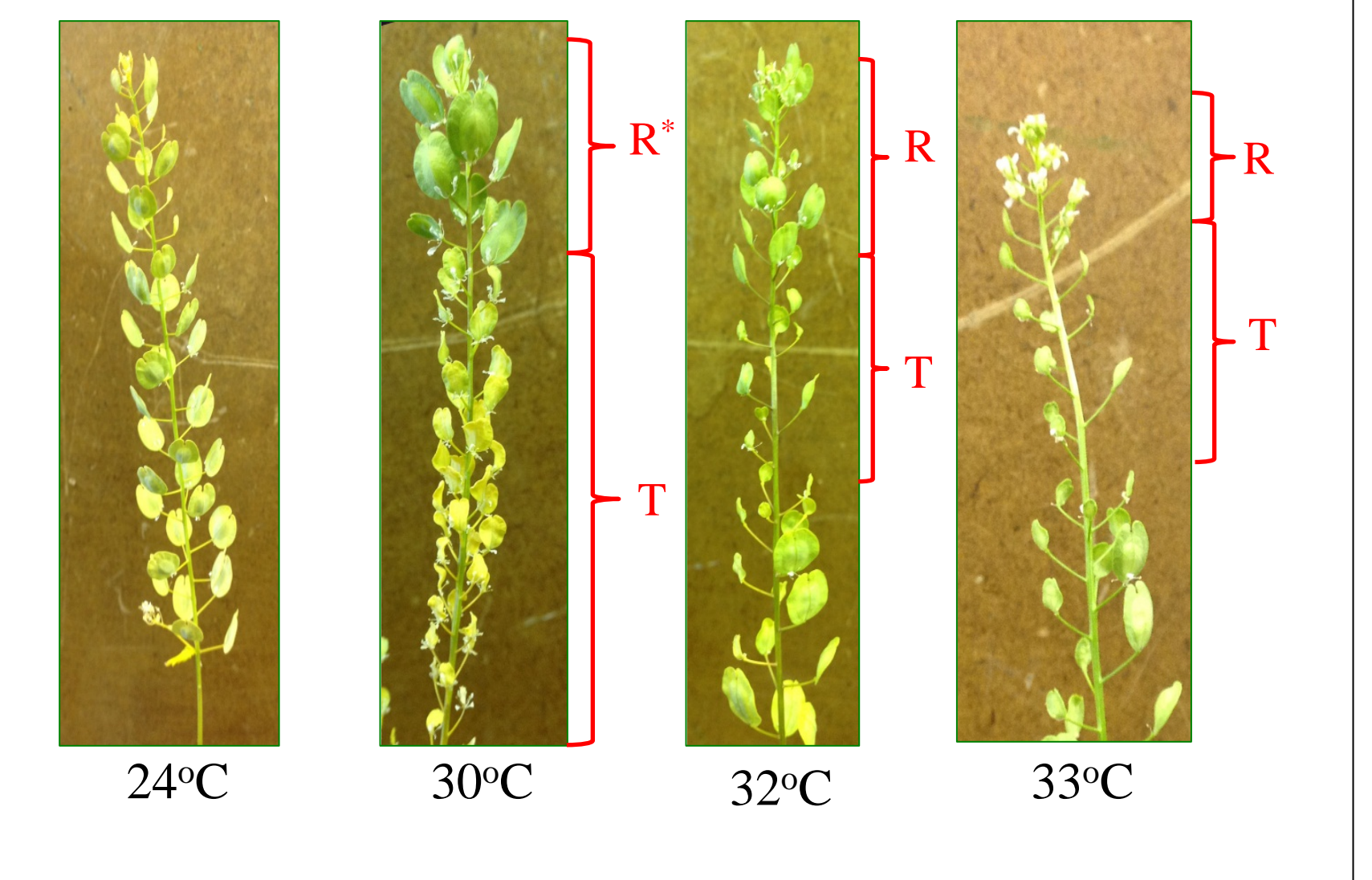
A growth chamber experiment was conducted on the non-dormant spring breeding line, 'Spring 32'. Seeds were planted in a germination soil mix and allowed to germinate under warm (24°C) and long day (18 hr) conditions. After emergence, a single seedling at the cotyledon stage was transplanted into an individual 7.5 cm pot and held at the same conditions for a period of three days to ensure successful transplanting. A total of 30 seedlings were transplanted. The seedlings were watered with a wicking system throughout the experiment. Once the plants began to flower, five plants were transferred to growth chambers at temperatures of 30, 32, 33, 34, and 35°C. After 7 days, plants were returned to control conditions at 24°C. After approximately 30 days, all plants were hand harvested at the time of full maturity.

Pollen Viability:

Pollen was collected on the last day of each heat treatment and placed on media as described by Fonseca and Westgate (2005). Pollen was allowed to germinate on the media for 3 hours at a temperature of 28°C. Pollen number and tube elongation was observed under an Olympus SZ61 dissecting microscope. Five open buds with mature pollen but non-dehiscent anthers from each plant were collected on the last day of heat treatment and placed in a centrifuge tube with Carnoy's fixative (6 alcohol: 3 chloroform: 1 acetic acid). The buds remained in the fixative for 2 hours to 12 months. Anthers were stained using a procedure described by Peterson et al. (2010) and observed under an Olympus BX41 phase-contrast microscope at 20x magnification (Figure 1).

Oil Analysis:

Total oil and fatty acid methyl esters content were determined utilizing an Agilent 6890 gas chromatograph with a flame ionization detector (Figure 2). Analysis of variance (ANOVA) was performed by Microsoft Excel software.



* R= Recovery at 24°C, T= Heat treatment for 7 days

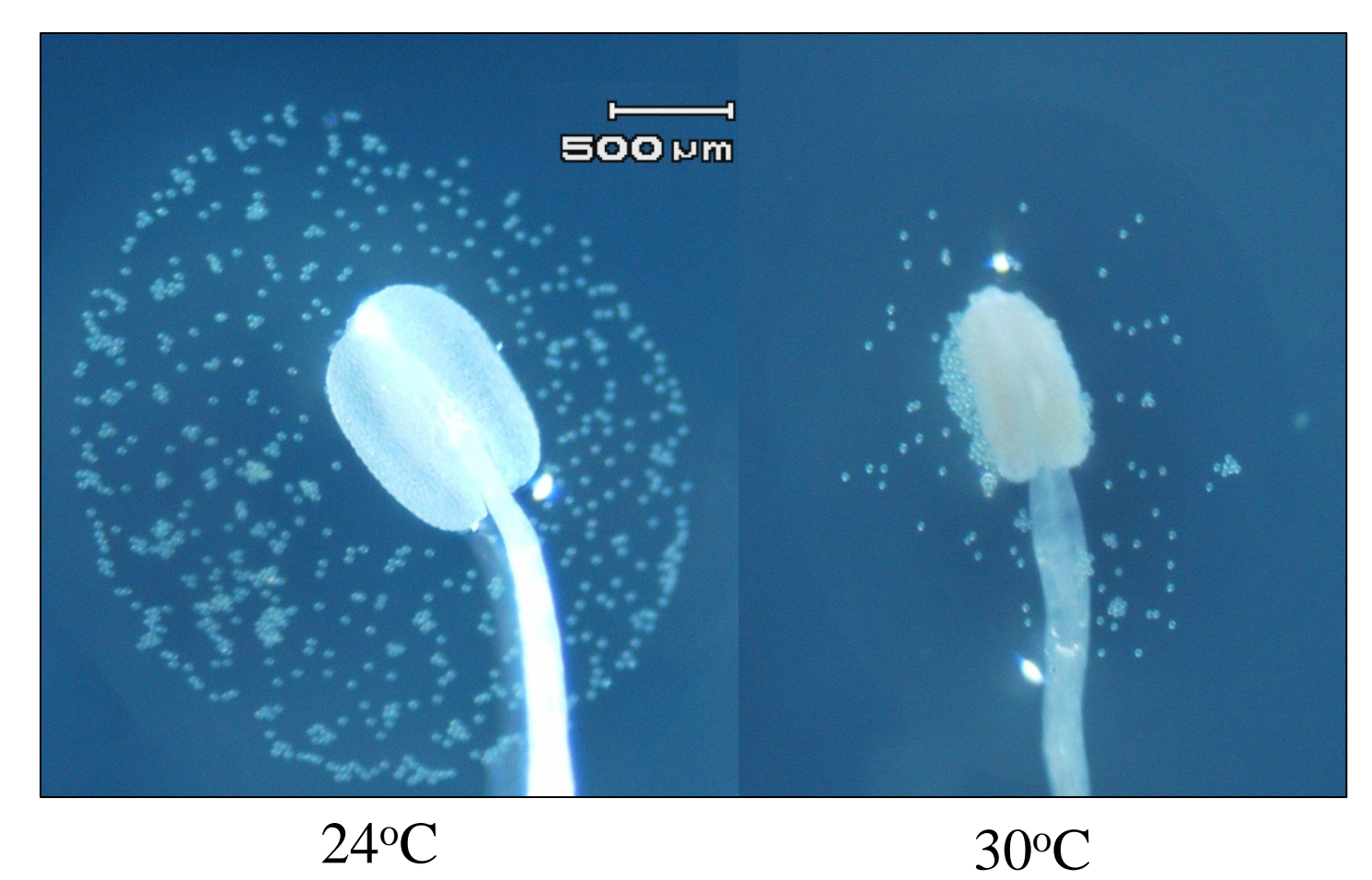


Figure 5. Pollen shed of pennycress anthers grown at 24°C and 30°C.

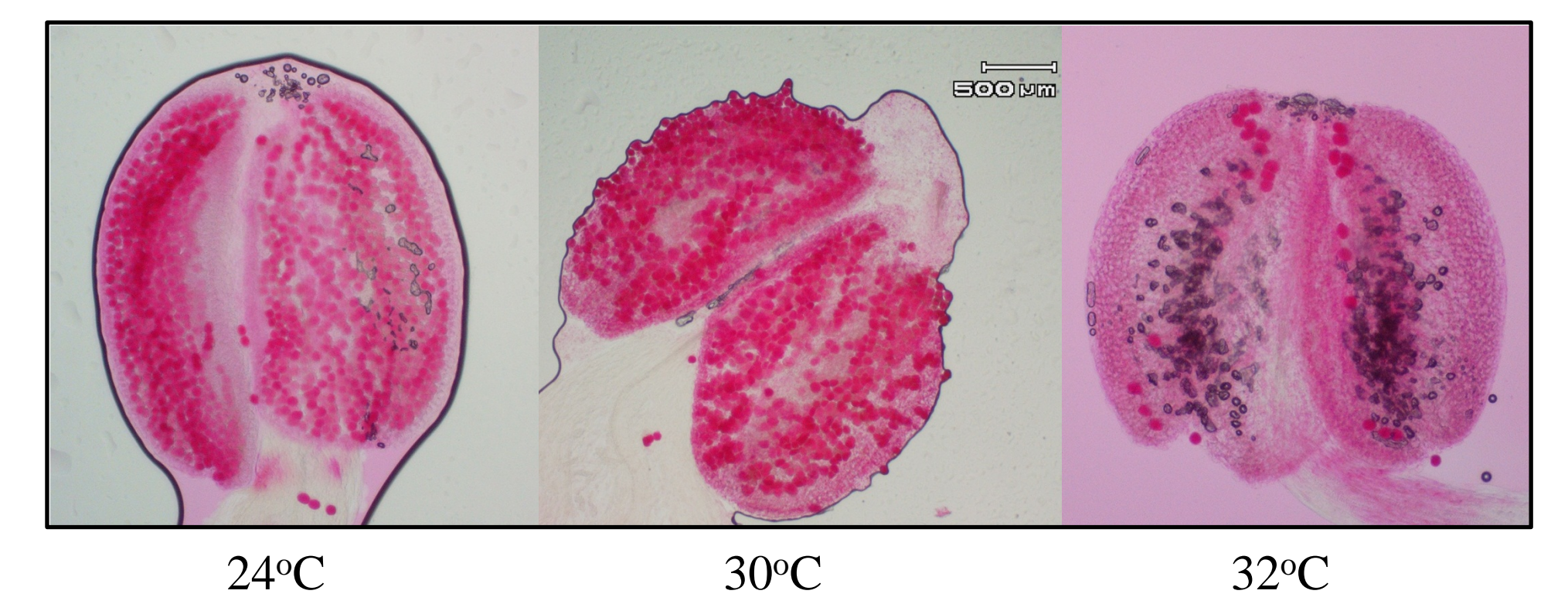


Figure 6. Staining of pollen grains in anthers subjected to increasing heat stress temperatures.

RESULTS

Overall, temperatures above 32°C had a significant negative impact on pennycress growth and development resulting in 0 seed production. Plants exposed to 30°C continued to grow but seed pods had an average of only 1 seed per pod (Figure 3). At 24°C, plants had an average of 68 pods and 610 seeds per plant (Table 1). Although total oil decreased slightly, change in total oil and oil constituents was not significant at temperatures of 24°C and 30°C (Figure 4). Unfortunately, pollen tube development was too small and too difficult to visualize pollen tube growth (Figure 5). Utilizing the pollen stain method, pollen count and viability at 30°C were not significantly different from the control (Figure 6).

CONCLUSIONS

Understanding heat stress symptoms that occur in pennycress (*Thlaspi arvense* L.) at temperatures above 30°C will enable producers to identify cooler regions for production and to modify their production practices to avoid high temperatures. In addition, plant breeders can use the above methodologies to screen the available collection of pennycress populations to identify potential new breeding lines for increasing heat tolerance or develop early flowering to avoid high temperatures. Improving heat tolerance in pennycress will greatly improve production of pennycress in variable environments and expand the current production areas.

LITERATURE CITED

- Fonseca, A.E., and M.E. Westgate. 2005. Relationship between desiccation and viability of maize pollen. *Field Crops Research* 94:114-125.
- Moser, B.R., G. Knothe, S. F. Vaughn, and T. A. Isbell. 2009. Production and evaluation of biodiesel from field pennycress (*Thlaspi arvense* L.) oil. *Energy Fuels* 23: 4149-4155.
- Peterson, R., J.P. Slovin, and C. Chen. 2010. A Simplified method for differential staining of aborted and non-aborted Pollen Grains. *Int. J. Plant Biology* 1:13-16.
- Singh, S.K., V.G. Kakani, D. Brand, B. Baldwin, and K.R Reddy. 2008. Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. *J. Agr. Crop Science* 194:225-236.

RESEARCH FUNDING

This project was supported by the Agriculture and Food Research Initiative competitive grant # (2010-85117-20535) of the USDA National Institute of Food and Agriculture.

