

EFFECT OF HEAT TREATMENT ON THE RESPIRATION RATES OF STIGMAS OF SELF-INCOMPATIBLE AND SELF-COMPATIBLE *OENOTHERAS*

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SUMMARY

Measurements were made of the rates of respiration of self-incompatible stigmas of *Oenothera* which had been heat treated for 5 minutes at 45°C and 50°C, and of self-compatible stigmas at 50°C. Fifty-degree treatment, which causes partial inactivation of self-incompatibility, lowered the respiration rate to about one-half that of the untreated controls. Forty-five-degree treatment, which does not affect self-incompatibility, had little effect on the respiration rate. Heat treatment had less effect on the respiration rates of self-compatible *Oenotheras*. The correlation between the effect of heat treatment of lowering respiration rate and overcoming self-incompatibility indicates that normal metabolic level may be necessary for self-incompatibility.

1. INTRODUCTION

It has been shown that heat treatment causes partial inactivation of incompatibility in the stigmas and styles of certain species of *Oenothera* (HECHT 1961, 1964; BALI 1963; BILLS 1968). In order to further our understanding of the physiological effect of heat treatment, respiration rates were determined for the heat-treated and untreated excised stigmas of several self-compatible and self-incompatible races of *Oenothera* species.

2. MATERIALS AND METHODS

Mature buds which shed pollen when opened were harvested and cut a few mm above the ovary with a razor blade. The bud was then opened and the stigma and its attached style was removed from the floral tube. Initially these were placed on damp filter paper in petri plates. Some of them were then heat treated by immersion in glass-distilled water in 600-ml beakers kept at a constant temperature in a water bath. Treatment was for 5 minutes at 50°C and 45°C; controls were allowed to remain in the petri plates at room temperature. Some of the stigma-styles were treated within a few minutes after their removal from the buds; others were incubated in the petri-plate moist chambers for 16 hours at 27°C prior to being treated.

Respiration rates were determined at both 0 and 24 hours after the heat treatments and at these same times for control lots, using a Gilson Differential

Respirometer. Six manometer flasks were utilized in each experiment: three contained heat-treated stigmas and three unheated stigmas. Each flask contained an equal number of stigmas, usually eight. However, this number had to be doubled to obtain essentially comparable respiration rates in the case of *Oe. rhombipetala* which has small stigmas. The stigmas were cut from the styles and floated on 2 ml of water in each flask. One side arm contained 10% KOH as a CO₂ trap; the temperature in the respirometer was maintained at 26°C for all runs. The manometers were read every 5 minutes, and the rates recorded were the changes in the manometer readings over 40-minute intervals. The respiratory rate recorded for each treatment was the average of the rates of the three flasks. Many of the experiments were repeated a number of times as noted in the tables, thereby greatly reducing the chance that a leaky manometer would affect the results.

Three clones of *Oe. organensis* are designated as S₃S₄, S₃S_{3,5}, and S₂₇S₂₈, being derivatives of plants studied many years ago by Dr. STERLING EMERSON (1940). Those designated as 146-9, 146-11, and 148-1 were obtained from seeds collected by Drs. Emerson and Robert Bandurski in 1958 from locations in the Organ Mountains of New Mexico, different from the sites where Emerson's earlier collections had been made. The seeds of *Oe. rhombipetala* came from Purcell, Oklahoma, *Oe. affinis* from Santa Fe, Argentina, *Oe. lamarckiana* from Pullman, Washington, whereas the *Oe. hookeri* was many generations removed by repeated selfing of a race of this species which was originally collected by H. de Vries in Berkeley, California.

3. RESULTS

Respiratory rates in terms of µl of O₂ evolved during 40-minute intervals for stigmas of three incompatible clones of *Oenothera organensis* are presented in table 1. These clones, designated as S₃S₄, S₃S_{3,5}, and S₂₇S₂₈, exhibited comparable rates of respiration in both the treated and the control lots.

Rates of respiration were reduced by about one-half by the 50°C treatment, whether measured immediately or after a delay of 24 hours. Treatment at

Table 1. Respiratory rates (µl/40 min) of self-incompatible *Oenothera organensis* before and after treatments in warm water for 5 minutes. (Figures in parentheses indicate number of experiments, each involving c. 48 stigmas in six respirometer flasks.)

| | 50°C treatment | | 45°C treatment | |
|-------------------|----------------------|-------------------|----------------------|-------------------|
| | Prior incubation (4) | No incubation (3) | Prior incubation (5) | No incubation (1) |
| Untreated | 74.6 | 65.0 | 57.0 | 66.3 |
| Treated | 37.2 | 40.4 | 49.5 | 60.9 |
| Untreated/treated | 0.50 | 0.62 | 0.87 | 0.92 |

45°C for 5 minutes does not inactivate the incompatibility substance and proved to have much less effect on the respiration rate of the treated stigmas. Three other clones of *Oe. organensis* were tested for effect of heat treatment on respiration rate. These three clones were not completely self-incompatible but differed in the degree of self-incompatibility from each other. Clone 146-9 was almost self-incompatible, 146-11 was more self-compatible than 146-9, while 148-1 showed a high degree of self-compatibility. Despite their differences in self-incompatibility the three clones had very similar responses to heat treatment with respect to respiration rates. With 50°C heating the incubated stigmas gave heated/nonheated ratios averaging 0.45, while those of the nonincubated averaged 0.53.

Another incompatible species, *Oe. rhombipetala*, gave a ratio of 0.43 with 5 minutes' heat treatment, whereas a 2.5-minute treatment resulted in a ratio of 0.63. This species has very small stigmas so that 2.5 minutes is sufficient for overcoming incompatibility (BALI 1963).

Table 2 shows the ratios of respiration rates for three species of self-compatible *Oenotheras*.

The self-compatible species as a group showed less response to heat treatment than did *Oe. organensis*. Heat-treated *Oe. hookeri* and *Oe. lamarckiana* showed a considerably lower respiration rate 24 hours after treatment. Perhaps the small stigmas of *Oe. lamarckiana* sustained more heat injury. However, it should be noted that *Oe. affinis* which has the largest stigmas of the three was affected the most in measurements made immediately after the heat treatments.

Table 2. Ratios of respiration rates ($\mu\text{l}/40$ min) of heat-treated (50°C) to not heat-treated stigmas of self-compatible *Oenothera* species. (Figures in parentheses indicate number of experiments.)

| | Incubated | | Not incubated |
|------------------------|--------------------------|---------------------------|--------------------------|
| | 0 hours after heating | 24 hours after heating | 0 hours after heating |
| <i>Oe. hookeri</i> | 0.89 (2) | 0.63 (2) | 0.87 (2) |
| <i>Oe. lamarckiana</i> | 0.82 (1) | 0.47 (1) | 0.76 (2) |
| <i>Oe. affinis</i> | 0.72 (2) | 0.75 (2) | 0.55 (2) |

4. DISCUSSION

The effect of heat treatment on the self-incompatibility mechanism of *Oenothera organensis* was found to be correlated with its effect on the respiration rate. All of the plants of *Oe. organensis* which were first collected and studied were found to be self-incompatible. Since the partially self-compatible clones were found some 20 years later, it is suggested that they may have been derived from incompatible clones. The fact that these partially self-compatible clones showed

basically the same type of response to heat treatment as the original self-incompatible clones suggests that the general character of the response of respiration to heat was retained when partial self-compatibility evolved. This respiratory correlation suggests that incompatibility rather than compatibility is the active state in this species, and that the incompatibility mechanism is lost when the metabolism is disturbed. The results of studies of other self-incompatible and self-compatible species further substantiate this correlation. A large decrease in respiration rate following heat treatment may be a general response in self-incompatible *Oenotheras*. It is not yet possible, however, to decide whether the lowering of the respiration rate induced by the heat treatment is a major factor in the inactivation of incompatibility or is a symptom of the metabolic state of the stigma when it has lost the self-incompatibility mechanism.

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