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Biology of Mimulus guttatus in Thermal Areas in Yellowstone National Park, Wyoming

Barbara Rice
Utah State University

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BIOLOGY OF *MIMULUS GUTTATUS* IN THERMAL AREAS

IN

YELLOWSTONE NATIONAL PARK, WYOMING

by

Barbara Rice

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

BOTANY

(Ecology)

Approved:

Major Professor

Committee Member

Committee Member

Committee Member

Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

1973
ACKNOWLEDGEMENTS

Various aspects of the research reported in this dissertation were aided by the assistance of Utah State faculty members and friends. Dr. Frank Salisbury, Dr. Ray Lynn, and Dr. Martyn Caldwell loaned valuable equipment for the field studies. Dr. Ivan Palmblad assisted in the difficult task of obtaining field data during the winter, and offered encouragement during all phases of the research.

Dr. Klaus Raschke generously allowed the use of his laboratory at Michigan State University for the gas exchange work, and Dr. Bert Drake assisted in interpreting the results obtained there.

Robin Fitz was a cheerful and extremely helpful companion on several occasions in the field. The programs and methods for statistical analysis of the data were provided by Mark Westoby, who also helped by discussing the results and ideas contained in this work.

Finally, the co-operation of the National Park Service in permitting research within Yellowstone Park is greatly appreciated.

Barbara Rice
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ABSTRACT

Biology of *Mimulus guttatus* in Thermal Areas

in Yellowstone National Park, Wyoming

by

Barbara Rice, Doctor of Philosophy

Utah State University, 1973

Major Professor: Dr. Ivan G. Palmblad
Department: Botany, and the Ecology Center

*Mimulus guttatus* populations in Yellowstone Park were studied in relation to their thermal habitat. Thermal areas have an extra-solar heat source, and are not snow covered in winter. Snow depth on adjacent nonthermal soils may exceed 1 meter. The hypothesis that these populations have formed a distinct high temperature tolerant race was tested.

Thermal populations form two categories, based on habitat type: plants on sinter and plants in boggy areas. Plants growing on sinter are dwarfed, with an average height of $5.6 \pm 2.2$ cm. They are facultative annuals which germinate in the fall, overwinter as rosettes, and flower the following spring. Plants in boggy areas average $27.7 \pm 4.3$ cm in height, are perennials which colonize open areas by vegetative reproduction, and which also overwinter as rosettes. Plants removed from sinter and placed in conditions with ample water assume the
morphological characteristics and perennial habit of plants from boggy sites. Summer leaf temperatures of healthy plants in thermal areas seldom exceed 36 C, but root temperatures may reach 40 - 45 C. The upper limit of root temperature in the field is about 45 C. Winter leaf temperatures ranged from 5 - 20 C and root temperatures from 10 - 29 C.

Seeds from thermal populations, germinated at four temperature combinations, show a negative correlation between germination success and increasing temperature of treatment. Germination was optimal at 10/5 C (day/night temperatures). In the laboratory, seed was obtained from artificial cross-pollination between Mimulus populations growing on sinter and in boggy areas. At 20/10 C germination success varied among populations from 62 % to 81 %. Seed from crosses between plants of thermal and nonthermal populations had germination success from 92 % to 100 %, varying with population.

Photosynthesis rates for nonthermal Mimulus at 8, 25, and 35 C showed a maximum at 25 C. Plants grown at low temperatures, but pretreated for 24 hours at 25 C had higher photosynthetic rates at 25 C and 35 C than plants pretreated at 10 C, indicating an ability to adapt quickly to temperature changes.

Plants from the thermal areas do not have high leaf temperatures, nor do they exhibit tolerance to high temperatures during germination or during photosynthesis. No significant reproductive barriers were found between thermal and nonthermal populations; the only criteria examined were seed set of cross-pollinated plants and germinability of the F₁
seed. It is concluded that *Mimulus guttatus* in the Yellowstone thermal areas is probably not a high-temperature tolerant race. Its success in high temperature areas is derived from the ability to utilize more than one life cycle and growth form strategy in response to stress.

(88 pages)
INTRODUCTION

The Nature of the Problem

*Mimulus guttatus* DC., the Yellow Monkeyflower, occurs in moist habitats over a wide geographical range in western North America. The species may be either annual or perennial, but in either case seed germination and seedling establishment require large amounts of water. The combination of extensive distribution and a specialized water requirement has created thousands of discrete small populations, each existing in slightly different environmental conditions from the others, and all spatially isolated to some degree. Such a situation is ideal for the development of new races or species as a result of divergence and reproductive isolation. Of all the subtly varied habitats in which *M. guttatus* is found, one is strikingly different from the rest. This is the high temperature habitat of the thermal springs and creeks in Yellowstone National Park, where the plants are rooted in soils as much as 30°C warmer than normal soils. Normal areas of the earth's surface have an upward nonsolar heat flux of 1.5 µcal cm\(^{-2}\) sec\(^{-1}\), whereas thermal areas, or areas with anomalous heat flux, have an upward heat flux an order of magnitude or more greater (Elder 1965).

Has *Mimulus guttatus* evolved a high temperature-resistant population in response to the thermal environment in Yellowstone Park? This
is the fundamental question that formed the basis for the research discussed in this thesis.

**Research Objectives**

In seeking the answer to the question posed, three research objectives were formulated.

First, a frame of reference was provided for consideration of adaptations and selective pressures (stresses) by the cataloguing of the physical features of the thermal habitats. This included quantitative information regarding temperature relationships in the soil-plant-air continuum.

The second objective was to ascertain whether or not *Mimulus* had developed adaptations to high temperatures. Adaptation (resistance) in the juvenile and mature stages of the plant's life cycle were tested by comparing the responses of thermal and nonthermal *Mimulus* populations to various temperature conditions. Seed germination and seedling establishment were chosen to test juvenile response, while photosynthesis was the process chosen for the mature stage. The photosynthetic response was considered in relation to responses reported in the literature for high temperature resistant species, many of which are xerophytes. *Mimulus* is a mesophyte.

The third objective was to establish the degree of reproductive isolation among thermal populations and between thermal and nonthermal populations. This was investigated by cross-pollination experiments. The second and third objectives had much in common, i.e., the search
for genetically fixed adaptations which would characterize a thermal race of *Mimulus guttatus*.

**Thesis Organization**

The research on thermal *Mimulus* populations was performed both in the field and the laboratory. The work is presented in three chapters, which allows greater clarity in the discussion of results. A summary at the end of all three chapters presents a final discussion and conclusions.

Chapter I introduces the general subject of heat resistance and considers the nature of the temperature environment and the seasonal stresses imposed upon *M. guttatus* in Yellowstone Park. Chapter 2 contains data on the photosynthetic response of *M. guttatus* to temperature and compares it to *Xanthium strumarium*, a species whose physiological responses are well documented (Drake *et al.* 1970; Meidner and Mansfield 1968; Salisbury and Ross 1969). Chapter 3 deals with germination response to temperature and the pollination biology of thermal and nonthermal populations.
CHAPTER I

TEMPERATURE RELATIONSHIPS IN POPULATIONS OF MIMULUS GUTTATUS IN YELLOWSTONE PARK

Review of Literature

The genus *Mimulus* is large and widely distributed, with about 150 species in the Northern and Southern Hemispheres. Grant (1924) reviewed the taxonomy of the genus. Two sections are of interest to this study. The section *Simiolus*, in which *Mimulus guttatus* DC. is placed, has been studied extensively by Vickery and his co-workers (Lindsay and Vickery 1967; Vickery 1959, 1964, 1966, 1967). *Mimulus cardinalis* Dougl. and *M. lewesii* Pursh. in the *Erythranthe* Section have been investigated biosystematically, genetically, and physiologically by Hiesey, Nobs, and Bjorkman (1971). *Mimulus cardinalis* is the only member of the genus whose races have been intensively investigated physiologically.

*Mimulus guttatus* is an extremely variable and polymorphic species, a herbaceous annual or perennial which roots at the nodes, and grows in moist sites. *Mimulus guttatus* usually occupies a cool habitat, but in Yellowstone National Park, Wyoming, it is commonly found along the edges of hot springs and streams (Rice 1971). There are reports (Grant 1924) that *M. guttatus* was often found, over much of its range, in the vicinity of thermal waters before human activity
altered the vegetation near such areas. It now occurs around hot springs in Alaska and the Northwest Territories, as well as in Yellowstone.

When considering the biology of plant species under thermal stress, it is not unusual to use the temperature of aerial parts, usually leaves, as a measure of the stress. For example, plants that grow in desert environments are frequently subjected to high air temperatures, plus high radiation loads. Those whose leaves reach high temperatures but that are not permanently damaged, are generally considered heat tolerant. A listing of measured leaf temperatures for various desert species is shown in Table 1. Heat tolerance for high temperature tolerant species may be evaluated by determination of the thermal death point, usually of leaf tissue (Alexandrov 1964; Lange 1959). The thermal death temperature is often somewhat above the actual leaf temperatures found in the field, but there is generally good correlation between field and laboratory resistance data.

Heat resistance in plants is a matter of either tolerating or avoiding the stress. A plant can control leaf temperature to some extent, primarily by balancing incoming radiation energy by a transpirational energy loss, but also as a function of leaf shape and wind speed (Drake et al. 1970; Gates 1963). Alpine species may have temperatures 20° C above air temperature (Salisbury and Spomer 1964), and certain desert species avoid heat stress by keeping leaf temperature below air temperature (Lange 1959). These latter desert
Table 1. Some high leaf temperatures recorded under natural conditions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Highest temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhamnus alaternus</td>
<td>52.2</td>
<td>Konis 1949</td>
</tr>
<tr>
<td>Arbutus andrachne</td>
<td>49.6</td>
<td>Konis 1949</td>
</tr>
<tr>
<td>Tidestromia oblongifolia</td>
<td>49.5</td>
<td>Bjorkman et al. 1971</td>
</tr>
<tr>
<td>Quercus calliprinos</td>
<td>47</td>
<td>Konis 1949</td>
</tr>
<tr>
<td>Quercus macrocarpa</td>
<td>46</td>
<td>Gates 1963</td>
</tr>
<tr>
<td>Fouquieria splendens</td>
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<td>Drake 1970</td>
</tr>
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</table>
plants have been termed "undertemperature" plants by Lange; although he does not specifically say so, it seems reasonable to infer that these plants had sufficient water supplies to maintain a high rate of transpiration. Those desert species that have leaf temperatures above air temperatures he called "overtemperature" plants. These have small sclerophyllous leaves and may often be drought tolerant as well. Succulents may be quite heat tolerant (Sapper 1935), but Lange and Schwemmle (1960) found them less so than the hard-leaved species.

The heat tolerance of roots is less well documented. *Pinus radiata* seedlings grown at root temperatures of 25 C had greater root growth and higher phosphate content than seedlings grown at 15 C (Bowen 1970). Petinov and Rasmaev (1961) state that the protein synthesis system is more heat stable in roots than in leaves of the same species, but do not make it clear whether the roots were actually subjected to the highest temperature used in their experiments. Kuiper (1964) found that *Phaseolus* roots grown at 24 C in sand culture had water uptake of 0.1 gm hr\(^{-1}\) at 12 C and 2.0 gm hr\(^{-1}\) at 38 C. Gur, Bravdo and Mizrahi (1972) have studied the effects of supraoptimal root temperatures (25 - 40 C) upon several physiological processes of apple plants. The optimal temperature for root growth was near 25 C, while O\(_2\) consumption and CO\(_2\) evolution reached a maximum at 35 C. They claim that as root temperatures increased, leaf temperatures also increased and therefore the resistance of the leaf to
the passage of water vapor decreased. Temperatures above 30°C reduced both root and shoot growth; temperatures above 35°C damaged leaves.

There are several theories on the mechanism of heat tolerance, the most convincing of which suppose that protein denaturation and aggregation are the immediate cause of heat damage to plants (Alexandrov 1964; Henckel 1964; Koffler et al. 1957; Levitt 1972). Tolerance has to be due either to thermostability of proteins, or to an increased rate of protein resynthesis (Levitt 1972). Alexandrov, Lomagin and Feldman (1970) propose that proteins are thermostable in heat-tolerant species because of reduced protein flexibility, thus attaining more stable structural bonds. Thermostability of proteins is not only a genetic property, but is dependent to some extent both upon the organism and the conditions to which it has been previously exposed (Feldman 1968; Kamentseva 1969; McNaughton 1966). Phillips and McWilliam (1971) suggest that the critical factor for tolerance in some species, especially those lacking apparent photorespiration, is an increased rate of protein synthesis. It is possible that imbalance in reaction rates among cell biochemical processes causes heat death (Langridge 1963) or that composition of lipids in the cell membranes is adversely affected by high temperatures (Henckel 1964; Kleinschmidt and McMahon 1970). Levitt (1972) has constructed a general tolerance hypothesis that is intended to explain response or tolerance to several stresses, including heat, cold, and water stress. This hypothesis is dependent upon the idea that protein denaturation and aggregation are
the result of changes in molecular bonding within and between sulfhydryl and disulfide groups of the protein molecules. He explains the process as follows:

1) Protein molecules approach each other as the protoplasm becomes dehydrated.

2) When close enough, chemical combination between S atoms of adjacent protein molecules may occur by oxidation of two SH groups (\[2RSH + \frac{1}{2} O_2 \rightarrow RSSR + H_2O\]), or by SH ↔ SS interchange (\[R_1 - S + R_3SH \leftrightarrow HSR_1R_2SSR_3\]).

3) The protein molecules are conformationally changed by aggregation, leading to injury or death.

To resist the stress, the plant must prevent the changes by maintaining the protein SH groups in the reduced state by means of a specific reducing system; or, as in the case of heat tolerant plants, SH and SS groups may be protected within the native molecules by highly hydrophobic proteins which prevent denaturation. Protein aggregation may be repaired if the plant has a mechanism capable of splitting intermolecular SS bonds reductively (\[SS \rightarrowSH\]), and thus returning the molecules to the natural state.

The fact that in Yellowstone Mimulus guttatus grows in a thermal habitat suggested that these populations might be unique, within the species, in possessing adaptations to extreme high temperatures. Such adaptations could be sufficient to distinguish the thermal populations
as a separate ecological race. The concept of ecological races has been
developed to explain the genetics of species that occupy a wide range
of habitat. Several workers have examined the genetics of ecological
races, using heritable morphological or physiological characters for
classification. Both transplant studies and crosses are used for
determination of differences between races. Cooper (1963) has found that
with regard to flowering, growing season, and seed dormancy, populations
of forage species collected on a north-south transect in Europe respond
differently to winter cold or summer drought. Also, performance of a
given transplant clone in one environment was not an indication of
behavior in another; different sets of genes were operative in different
environments. A similar pattern of response has been described for
climatic races in two species in California (Clausen and Hiesey 1958).
Hiesey et al. (1971) studied the interfertile combinations of two species,
*Mimulus cardinalis* and *M. lewesi*. Parental forms that could not survive
in a given environment produced hybrids that were quite vigorous. Other
workers have verified that species that contain a number of races tend
to be genetically plastic, heterozygous, and outbreeding (Cooper 1963;
Heslop-Harrison 1966; Hiesey 1953; Mooney and Billings 1961).

The concept of the ecological race as the subset of the species which
reflects local climate was largely the result of the work of Turesson
(1922, 1925, 1931). Although any one species may contain a number of
climatic races, these rarely cover the entire range of environmental
extremes tolerated by plants. *Oxyria digyna*, for example, shows great
differences between Arctic and Sierran races in respect to temperature response (Mooney and Billings 1961) but it does not have a true high temperature race.

The genetics of Mimulus guttatus have been investigated at length by Vickery (1959, 1964, 1966, 1967). He has demonstrated that the species consists of a number of widely distributed populations, which are often spatially isolated from each other, and which tend to have inter-population crossing barriers that are constantly shifting in response to varying environmental selection pressures. The species is a model of a rapidly evolving entity, and seemed likely to contain climatic races. However, in considering the possibility of a high temperature tolerant race it should be noted that M. guttatus is a mesophyte. In the literature on heat resistance (Drake 1970; Gates 1963; Lange 1959) those species that are mesophytic and also live in high temperature environments appear to avoid heat rather than to tolerate it.

**Methods and Materials**

A brief description of the Yellowstone study sites is given in Rice (1971) and in Table 2. More complete information on habitat characteristics of particular sites is given with the experimental results in the relevant sections.

Spot soil temperatures were recorded for 25 populations in Yellowstone Park, and for several populations in other states. These were taken with a Yellow Springs Instrument Company thermistor and soil probe, or with soldered copper-constantan thermocouples (30 gauge)
Table 2. Habitat conditions and life form of plant populations studied and mentioned by name in text. Site abbreviations are those used for cross-pollination studies.

<table>
<thead>
<tr>
<th>Location</th>
<th>Site Abbreviation</th>
<th>Heat Flux</th>
<th>Habitat Type</th>
<th>Water Source</th>
<th>Winter Snow Conditions</th>
<th>Predominant Life Form</th>
</tr>
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<tr>
<td>Yellowstone Park, Wyo., 45° N</td>
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<td></td>
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</tr>
<tr>
<td>White Creek</td>
<td>WC</td>
<td>present</td>
<td>sinter</td>
<td>heat flow, rain</td>
<td>no snow cover</td>
<td>annual</td>
</tr>
<tr>
<td>Tangled Creek</td>
<td>TC</td>
<td>present</td>
<td>boggy</td>
<td>creek</td>
<td>no snow cover</td>
<td>perennial</td>
</tr>
<tr>
<td>Nez Perce Creek</td>
<td>NP</td>
<td>present</td>
<td>boggy</td>
<td>creek</td>
<td>no snow cover</td>
<td>perennial</td>
</tr>
<tr>
<td>Obsidian Creek</td>
<td>OC</td>
<td>present</td>
<td>boggy</td>
<td>creek, springs</td>
<td>no snow cover</td>
<td>perennial</td>
</tr>
<tr>
<td>Madison Junction</td>
<td>MJ</td>
<td>present</td>
<td>boggy</td>
<td>creek</td>
<td>no snow cover</td>
<td>perennial</td>
</tr>
<tr>
<td>Black Sand Basin</td>
<td>BSB</td>
<td>present, low</td>
<td>boggy</td>
<td>creek</td>
<td>not known</td>
<td>perennial</td>
</tr>
<tr>
<td>Logan Canyon, Utah 41° 45' N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring Hollow</td>
<td>LC</td>
<td>absent</td>
<td>boggy</td>
<td>creek</td>
<td>snow cover</td>
<td>annual or perennial</td>
</tr>
</tbody>
</table>
and a Leeds and Northrup potentiometer. Ideally all thermal populations would have been tested at the same time on the same day. As this was impossible, readings were made on consecutive days in July, between 0800 and 1800 hours. Populations in thermal areas heavily used by park visitors were not studied to avoid attracting visitors into dangerous areas.

Soil, leaf, and air temperatures taken over 24-hr periods were recorded about twice each hour on a Cole-Parmer strip chart recorder. Copper-constantan thermocouple lead wire (30 gauge) soldered to copper discs 3.5 cm in diameter and coated with fiberglass were used for soil readings. Leaf and air temperatures were taken with 30 gauge copper-constantan soldered thermocouples that were also fiberglass coated. All thermocouples were connected to an automatic switch box which was powered by a 6 volt DC motor. The switch box, batteries, strip chart recorder, and a 0°C ice bath temperature reference were placed in an insulated ice chest for protection. Soil temperatures were taken at 1 cm below the surface (root depth), and air temperatures at 1 cm, 6 cm, and 25 cm above soil surface. Air temperatures were made with unshielded thermocouples, as it was decided to accept a radiation error rather than to shield the sensors and thereby create a moisture trap for the steam rising from the thermal features. On August 9-10, 1970, the error was evaluated at the Tangled Creek site; 16% of the readings taken while the sensors were irradiated showed an error, averaging +3°C. Leaf thermocouples were threaded through the leaf and the lower surface temperature recorded. Leaves were usually within 2 cm of the soil surface.
Leaf and air temperatures recorded during the winter were taken with 30 gauge copper-constantan thermocouples and a Leeds and Northrup potentiometer; some leaf temperatures were taken with a Barnes Engineering Radiation Thermometer.

To test heat tolerance in thermal populations, two populations were selected that had soil/root temperatures exceeding 30°C. Roots and leaves were treated separately at each temperature. Each leaf and root treatment consisted of 5 single plant replicates. Plants used for leaf treatments were cut off below the first basal node; all roots were removed. Plants used for root treatments had all leaves removed and were cut off above the first basal node. The treated plant part was totally immersed in a water bath at the desired temperature for 10 minutes. Water temperatures were obtained by mixing water from thermal creeks with ice water in an insulated thermos. Water temperature decreased about 1°C during treatment. Immediately after treatment the plants were potted in water saturated soil and placed in a greenhouse. Recovery of turgor and normal coloration were the criteria for "survival"; plants that met these requirements after 2 days proved to be capable of vegetative reproduction. This determination of survival was chosen instead of various cellular methods, since the question of importance is whether *Mimulus* is capable of recolonizing areas after exposure to high temperatures.

Preliminary tests showed that *Mimulus* could tolerate temperatures below 45°C for over an hour, so the plants were tested at 1°C increments from 45°C to 50°C.
Results

General habitat description

Two types of thermal habitat predominate in Yellowstone, which have been designated as "sinter" and "boggy". Sinter (Figure 1) is the decomposition product of opaline silica that is deposited by thermal waters; the sinter particles are variable in size, but usually less than 1 cm in diameter. The particles form a very loose substrate that is only about 1 - 2 cm deep over much of the sinter areas. These areas are from a few square meters to several hectares in extent. Beneath the decomposed sinter lies amorphous opaline silica, which is essentially impermeable to herbaceous plant roots. There is frequently moisture, sufficient to support algal growth (Synechococcus sp.), a few millimeters below the surface. The Mimulus plants on sinter are dwarfed and occur in small populations with low plant densities. Boggy habitat occurs on the edges of stream banks and around springs (Figure 2). The soil is a mixture of mud and decaying organic matter; it is always water-saturated. Fog, or steam, rising from the thermal waters may blanket the plants during the night or cooler daylight hours. Mimulus plants in boggy areas are larger than plants on sinter and usually grow in a solid mat.

Soil temperatures for both types of thermal population were analyzed together and compared with those from nonthermal populations outside Yellowstone. The average soil temperature for thermal populations at a depth of 1 - 2 cm was higher (28 C) than the average
Figure 1. The sinter thermal habitat at White Creek, Yellowstone Park. June 9, 1970. The *Mimulus guttatus* plants shown are about 4 cm in height.
Figure 2. The boggy thermal habitat at Obsidian Creek, Yellowstone Park. July 15, 1970.
temperature for nonthermal populations (14 C) \( F_s = 151; \) df = 1.30; \( P < .001 \). Soil temperatures on sinter areas tended to be lower than temperatures on boggy thermal sites. There are no *M. guttatus* populations on cool moist sinter, although the species occupies cool moist creek bank sites in the same geographical area. This problem is discussed in connection with the subject of winter temperatures in thermal areas.

**Daily and seasonal temperature variation in two thermal habitats**

Soil, leaf, and air temperatures were recorded for populations in the sinter and boggy thermal habitats. One 24 hr period was monitored for each site in each of the month of July, August, and September. The August readings were compared to data obtained for a nonthermal *Mimulus* population in Logan Canyon in August, the warmest month for these populations. The nonthermal site was not monitored for a full twenty-four hours, but maximum and minimum temperatures were recorded.

The sinter site is occasionally subject to high winds, and steam rising from a nearby pool may blanket the population during the night. Figures 3 and 4 show the warmest and coolest 24 hr periods recorded for the White Creek sinter site (August and September). Here air, soil and leaf temperatures are very closely related, and all parts of the plant are subjected to the same intensity of temperature fluctuations. In spite of the heat available from the soil and the effect of steam from the pool, night temperatures are low. On September 21, 1971, the leaf
Figure 3. Daily air, leaf, and soil temperature cycle for a *Mimulus* population on sinter at White Creek, Yellowstone Park. August 12 – 13, 1971. The line joining the air temperature points was not drawn.

Figure 4. Daily air, leaf, and soil temperature cycle for a *Mimulus* population on sinter at White Creek, Yellowstone Park. September 20 – 21, 1971.
Figure 3.

Figure 4.
temperature during the night was at or below freezing for at least eight hours. Although the leaf monitored appeared senescent (yellowish) and was less than 1 cm in width, it was the largest, healthiest one available in the population at the time. August temperatures caused the greatest temperature flux in leaves, varying 34 C within 6 hours. During the time when the leaves are not in the sun, root temperatures are slightly above leaf temperature. During the growing season this situation is probably true for most terrestrial plants for at least part of the day, because of the time lag between soil and air temperatures.

The data for the Obsidian Creek boggy site (Figures 5 & 6) show a striking difference between boggy and sinter habitats: in water-saturated soils roots were never recorded at a lower temperature than leaves. Root temperatures exceeded leaf temperatures by 26 C at the time of maximum temperature divergence. This "inverted" temperature relationship has no apparent detrimental effects upon the plants. The 36 C leaf maximum is not extreme when compared to temperatures tolerated by desert species (Table 1) although the latter are hard-leaved xerophytes. Comparison of leaf and root temperatures of boggy, sinter, and nonthermal plants (Figure 7) in August indicates that leaf conditions in thermal and nonthermal populations are similar, but that thermal roots at any given time are warmer than nonthermal roots. Heat tolerance in thermal populations

Roughly speaking, Mimulus roots appear to have a thermal death point about 2 C higher than leaves (Figure 8). To test for significant
Figure 5. Daily soil, leaf and air temperature cycle for a *Mimulus* population in a boggy thermal area at Obsidian Creek, Yellowstone Park. August 9-10, 1971.

Figure 6. Daily soil, leaf and air temperature cycle for a *Mimulus* population in a boggy thermal area at Obsidian Creek, Yellowstone Park. September 23 - 24, 1971.
Figure 5.

Figure 6.
Figure 7. Maximum and minimum air, soil, and leaf temperatures in a daily temperature cycle for a nonthermal *Mimulus guttatus* population. Logan Canyon, Utah; August 20 – 21, 1970.
Figure 8. Number of plants surviving (n = 5) two days after 10 minutes immersion in water bath. All plants from populations with root temperatures exceeding 30 C.
differences between root and leaf responses, the temperatures which killed 50% of the treated samples were determined (LD$_{50}$). A horizontal line was extended from the 50% point (2.5) on the Y axis of Figure 8. At the points where the survival curves crossed this line, vertical lines were drawn to the X, or temperature, axis. The LD$_{50}$ temperatures thus determined were 45.8 and 46.6 for leaves and 48.6 and 49.6 for roots. The temperatures which roots survived were significantly higher than those for leaves ($F_s = 26.47$; df = 1, 2; $P < .05$).

**Winter temperatures and seedling survival on sinter**

At the end of the first summer of field work, two significant observations had been made regarding the dwarf plants growing in sinter: 1) there were never mature populations on nonthermal sinter, even if there seemed to be sufficient water for growth, and 2) *Mimulus* seeds germinated in the fall on sinter and therefore must survive the winter on the exposed sites. This suggested that soil temperature might be a factor limiting survival on nonthermal sinter areas, and two trips were made during winter to observe the thermal habitats.

On March 6, 1971, temperature data were obtained from Madison Junction, a thermal creek supporting a large *Mimulus* population during winter (Figure 9). Temperatures taken at 1500 hours were considered to approximate a maximum, and temperatures at 0600 hours a minimum. The sky was overcast during the morning readings, and clear with a strong wind blowing during the afternoon. There was a strong temperature gradient from the thermal soil up to about 24 cm,
Figure 9. Aspect of a boggy thermal habitat in winter. Madison Junction, Yellowstone Park. March 6, 1971. The *Mimulus* plants form a narrow border extending about 15 cm back from the creek edge.
after which temperature stabilized (Figure 10). Any living plant
material extending more than a centimeter above the soil surface would
have been frozen. However, the rosette leaves of *Mimulus* are in contact
with the soil surface, and are quite wrinkled, so that leaf temperatures
are comparable to those of summer. It is not known whether the leaf
wrinkling has any adaptive significance, but the character is not usually
present during the summer. It is possible that wrinkling decreases
radiative energy loss from the leaf during winter. Leaves were greener
than when plants are flowering (April - August), and reached 4 cm in
length. Extreme cold, which precludes growth during this season in
the nonthermal sites, is avoided by the ability to remain a rosette and
below the height (1 cm) at which air temperatures were freezing.

On February 12, 1972, the White Creek sinter areas were visited.
Temperature data were taken and seedling plots established. Seedlings
had been observed on the sinter in October, and it had been determined
that all parent plants of the previous growing season were dead. At
the time of the observations the sky was overcast and a strong wind
was blowing the sinter particles about slightly. The sinter was largely
free of snow (Figure 11). Seedlings ranged from the two-leaf
stage to 3 - 4 leaf pairs, the largest of which were about 4 cm wide.
As at Madison Junction, all plants were rosettes with leaves closely
pressed to the soil. The extreme leaf wrinkling was also present.
Dependence of plants on heat flux for survival was illustrated by a
bit of soil, carrying plants, which had been separated from the sub-
Figure 10. Winter soil-plant-air temperature relationships for a thermal boggy and a thermal sinter *Mimulus* population. Readings at 0600 hrs were taken before sunrise; at 1500 hrs leaves were fully illuminated.
28

Figure 11. Aspect of a sinter thermal habitat in winter. White Creek, Yellowstone Park. February 12, 1972. *Mimulus* seedlings are present on the open areas as rosettes that do not exceed 1 cm in height.
strate by buffalo trampling, and was solidly frozen. Sinter sites classified as nonthermal had soil temperatures at or below 0 °C at this time. Any seedlings establishing on these sites in October could not possibly have survived through February.

During winter there are two well defined microsites available for *Mimulus* on sinter: flat exposed relatively dry areas, and moss hummocks. The latter are more mesic due to retention of snow melt, and seeds germinating on such sites can produce a few vegetative plants by February. On the drier sites seedlings are more scattered and confined to the small rosette produced by the original seed.

To estimate seedling survival through the winter, four 51 cm x 68 cm plots were established and all visible seedlings counted in February, April, and June. The plots were laid out in an irregular line about 15 m long on the drier sinter. This allowed a more accurate estimate of density and mortality than would have been possible on the mesic moss hummocks, where vegetative reproduction complicated matters.

None of the seedlings on the survival plots survived through July (Table 3). However, of a total of 269 seedlings, 25 flowered and set seed before June. As the average number of seeds per capsule for sinter plants is 157 ± 29, even this small number of successful individuals could maintain the population from year to year. Also, although seeds tend to fall near the parent plants, they are light enough (one seed = ca. 0.000035 gm) to be scattered by wind over short
Table 3. Seedling survival on sinter. Mean of four plots with standard deviations.

<table>
<thead>
<tr>
<th></th>
<th>Number Counted</th>
<th>Number which matured seed</th>
<th>Soil Temperature (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Feb.</td>
<td>59 ± 30</td>
<td></td>
<td>11 ± 3</td>
</tr>
<tr>
<td>April</td>
<td>65 ± 31</td>
<td></td>
<td>20 ± 4</td>
</tr>
<tr>
<td>June</td>
<td>0.25 ± 0.5</td>
<td>6 ± 3</td>
<td>22 ± 4</td>
</tr>
</tbody>
</table>
distances. Seeds from adjacent more favorable microsites, which produce more flowering plants, could provide immigrants to the drier sites.

**Vascular plant species associated with *M. guttatus* in thermal areas**

*Mimulus guttatus* is not the sole inhabitant of the hotter thermal habitats. Because *Mimulus* can grow in waterlogged soils it is the dominant species immediately next to streams and some pools; in certain sites *Eleocharis pauciflora* (Lightf.) Link. (Spike-rush) may be co-dominant. Table 4 lists species of angiosperms collected with *M. guttatus* in the thermal areas. The soil/root temperatures recorded for each species were taken at the time of collection, which was about midday, and during the plant's flowering period. The temperature range is shown, unless the soil conditions were so uniform that one temperature prevailed over a large area (i.e., in a creek habitat). Four species attain root temperatures comparable to the maximum for *Mimulus*, and all of these reproduce successfully at these sites.

**Discussion**

The field investigations indicate that the temperature environment is extreme primarily with respect to soil temperatures. It was previously noted (Rice 1971) that the area occupied by a given population may change seasonally. That is, plants die in areas of the habitat that become extremely hot during the summer, and vegetatively recolonize the area when the temperature drops in the fall. Since germination is unsuccessful at high temperatures (see Chapter 3), seed would not
Table 4. Root/soil temperatures recorded for angiosperms which may grow in association with *M. guttatus* in Yellowstone Park. These are spot temperatures recorded between 1000 hrs and 1600 hrs on July 15 - 19, 1969, and are only intended to indicate the conditions at the time and location where taken.

<table>
<thead>
<tr>
<th>Species</th>
<th>Temperature (°C)</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panicum occidentale</td>
<td>18 - 45</td>
<td>meadow, dry soils</td>
</tr>
<tr>
<td>Agrostis scabra</td>
<td>28</td>
<td>meadow, creek banks</td>
</tr>
<tr>
<td>Eleocharis pauciflora</td>
<td>20 - 40</td>
<td>wet meadows, springs</td>
</tr>
<tr>
<td>Triglochin maritima</td>
<td>20</td>
<td>creeks, springs</td>
</tr>
<tr>
<td>Castilleja miniata</td>
<td>32</td>
<td>wet meadows</td>
</tr>
<tr>
<td>Prunella vulgaris</td>
<td>15 - 25</td>
<td>wet meadows</td>
</tr>
<tr>
<td>Nasturtium officinale</td>
<td>10</td>
<td>creeks</td>
</tr>
<tr>
<td>Antennaria rosea</td>
<td>28 - 45</td>
<td>meadows, sinter</td>
</tr>
<tr>
<td>Gnapthalium microcephalum</td>
<td>30</td>
<td>near creeks</td>
</tr>
<tr>
<td>Helianthus maximilianii</td>
<td>30 - 40</td>
<td>creek banks</td>
</tr>
<tr>
<td>Aster occidentalis</td>
<td>20 - 25</td>
<td>wet meadows</td>
</tr>
</tbody>
</table>
seem to be capable of colonizing areas already too hot for mature plants. Populations do not occupy habitats where root temperatures exceed 40 - 45 °C for long periods of time. Leaf temperatures seldom exceed 36 °C, and the continual temperature fluctuation undoubtedly modifies the effect of this high temperature. Thus, *Mimulus guttatus* avoids the extreme high temperatures of the thermal sites. Although the leaf system is close to the root system, high root temperatures per se apparently have little effect on increasing the leaf temperature. For air temperatures to reach lethal levels for leaves would require soil temperatures far above root tolerance levels. It thus seems probable that soil temperature becomes a limiting factor in plant growth and survival before leaf temperature does. Also, since the "thermal death point" determined for roots is close to the maximum soil root temperatures for living plants measured in the field, it seems reasonable to suggest that death is due to temperature (i.e., is thermal death), rather than to related processes of water and nutrient uptake, or transpiration rates.

Anomalous heat flux provides the only means by which plants (as opposed to seed) can survive through the winter, either near creeks or on sinter. The origin of this energy is subterranean magma, probably still molten. Near the surface, ground temperatures may be governed by a system such as diagrammed in Figure 12 for a sinter site; the
Figure 12. Generalized temperature-depth curve (after White 1969) for a sinter area, showing the mechanism by which both heat and moisture may be supplied to plants. Below the point $D_0$ heat flow is convective; above $D_0$ flow is both conductive and convective.
mechanism has only recently been proposed by White (1969). Water vapor mixed with gases rises from a boiling water table; most of the steam condenses below the soil surface, and its released heat of condensation is conducted upward. The sinter soil temperatures in the Mimulus root zone generally vary between 15 - 30 C, depending upon the initial position of the water table, composition of substrate through which the steam rises, and air temperature. As air temperature increases in summer the moisture layer in the loose sinter evaporates more quickly, which could create a moisture stress for the plants. This could explain the fact that most plants on sinter die by July, unless there have been considerable amounts of rain.

Table 5 is a summary of the life strategy options available to M. guttatus in the Yellowstone habitat types. The seeds require no afterripening (Vickery 1967), and germination is triggered as soon as there is sufficient moisture available. It is deduced that in the boggy sites seeds are capable of germinating immediately after dispersal. However, boggy sites are hottest when seeds are being shed, and the only open soil is too hot even for mature plants. Should the seeds fall on cooler soil, establishment would have to be accomplished in competition with established plants. It therefore seems probable that vegetative reproduction is more important than seed germination in the boggy sites. Elsewhere in the Intermountain area in nonthermal sites the populations may overwinter as rootstocks and so persist.
Table 5. Feasibility of life strategies which *M. guttatus* might use in Yellowstone Park habitats. Based on germination timing.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Possible Germination</th>
<th>Winter Survival</th>
<th>Flowering</th>
<th>Summer Survival</th>
<th>Feasible Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal Creek</td>
<td>Yes</td>
<td>Yes, rosette</td>
<td>Yes</td>
<td>Yes, rosette</td>
<td>Perennial</td>
</tr>
<tr>
<td>Spring</td>
<td>No (a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal Sinter</td>
<td>Yes</td>
<td>Yes, rosette</td>
<td>Yes</td>
<td>No (b)</td>
<td>Annual</td>
</tr>
<tr>
<td>Spring</td>
<td>No (c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cool Sinter</td>
<td>Yes (d)</td>
<td>Yes, seed</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. As no afterripening is necessary and these seeds are shed onto a moist substrate, germination occurs immediately, temperature permitting.

b. Water apparently becomes limiting after July.

c. Assumes temperatures adequate for germination in fall, plus adequate moisture provided by rain.

d. Assumes that temperatures in fall might be low enough to prevent or retard germination.
Sinter populations are dependent on anomalous heat flux for two reasons: 1) the heat provided permits seedlings to survive winter, and 2) the rising and condensing water vapor provides the necessary moisture to complete the life cycle. It has been observed above that seedlings on cool sinter would be killed by low temperatures. It is true, however, that seeds on nonthermal sites often do not germinate in fall even if on a saturated substrate. Laboratory tests with seeds from nonthermal populations from Logan Canyon confirmed that these seeds germinate very slowly if kept at low temperatures with little diurnal fluctuation (Table 6). The 8°C day/5°C night temperature regime followed conditions in the field for late September. There is no light requirement for germination.

Table 6. Germination success of *Mimulus* seed from nonthermal sites. Seeds were successively treated at low temperature with little diurnal fluctuation, low constant temperature, and constant room temperature (22°C). Means and standard deviations of four replications are given.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 days at 8/5°C; 24 hrs dark.</td>
<td>2.5 ± 2.50</td>
</tr>
<tr>
<td>14 days at 3°C; 24 hrs dark.</td>
<td>4.5 ± 5.25</td>
</tr>
<tr>
<td>12 days at 22°C; natural daylight.</td>
<td>86 ± 9.09</td>
</tr>
</tbody>
</table>

When the seeds are subsequently placed at room temperature (22°C = "spring" conditions) germination proceeds rapidly. It is
possible that a situation similar to this could occur on cool sinter, and seeds could germinate in spring instead of fall. If it did, however, the life cycle would have to be completed in the short period between the spring increase in air temperature and summer drought. Furthermore, field observations confirm that in Yellowstone *Mimulus* on sinter germinates in fall, after rains begin. Thus, winter survival requires a heat source. The period of winter establishment for the seedlings is necessary to enable the plants to bolt and flower as soon as spring air temperatures permit; the problem of water becoming limiting is avoided.

*Mimulus* populations on thermal areas have to cope with two stresses: high root temperature in boggy sites and seasonal drought on sinter. Thus, the species has adopted two strategies as optimal, and is an annual on sinter and a vegetatively reproducing perennial in boggy areas.

It is apparent that all of these strategies (annual, perennial, vegetative reproduction) have in common the avoidance of stresses. The strategies are known to occur in the species (Grant 1924) throughout its range. Considering the boggy areas only, where the development of a high temperature race was expected, heat tolerance does not seem to have evolved. This conclusion is based largely on the assumption that leaf temperatures of 36 C or less are not truly extreme. However, heat resistance may consist of either tolerance or avoidance (Levitt 1972). Therefore, *Mimulus guttatus* is heat resistant in that it persists in a habitat which becomes extremely hot, yet avoids heat stress by utilizing various life cycle and growth form strategies as circumstance demands.
CHAPTER 2

EFFECT OF TEMPERATURE ON PHOTOSYNTHESIS RATES IN THERMAL AND NONTHERMAL POPULATIONS OF MIMULUS GUTTATUS

Review of Literature

A species that is successful in an extreme environment must not only survive, but also grow and complete a life cycle. A number of criteria can be used to evaluate "success". Net photosynthesis (net CO$_2$ fixed = CO$_2$ uptake - CO$_2$ evolution) gives an indication of the ability of a plant to grow under given conditions. It is a necessary, though not in itself sufficient, condition for survival. Consequently, effects of temperature on net photosynthesis and/or productivity have been recorded and investigated by many workers for some years. It is now recognized that in many plants respiration is greater in the light than in the dark (Hew, Krotkov, and Canvin 1969; Osmond 1971; Zelitch and Day 1969). The component of respiration which occurs only in light, photorespiration, can often set an upper limit on the amount of carbon fixed at very high temperatures (Charles-Edwards and Charles-Edwards 1970).

Obviously, temperature conditions that are extreme for one set of individuals may be optimal for another set. Physiological investigations on ecotypes within species have clarified considerably the complexities of the adaptive phenomena. Three of the alternative adaptive strategies
that plants may use to combat extreme temperatures are as follows:

First, a species may adapt to a seasonal increase in temperature by producing summer leaves that are different from spring leaves both in morphology and physiology. When the photosynthetic and respiratory rates of spring and summer leaves of *Pulmonaria* were measured it was found that summer leaves exhibit the greater thermal stability (Kamentseva 1969).

The second alternative is for a species to have a large number of climatic races, each with a slightly different temperature optimum. Mooney and Billings (1961) showed large differences in optimal temperature for photosynthesis in latitudinal races of *Oxyria digyna*. Alaskan races exhibited maximum photosynthesis at 15°C to 19°C; California and Colorado races at 30°C to 34°C. Other workers (Belakoff 1930; Decker 1959; Sorokin and Meyers 1953) have found that ecological races have photosynthesis rates that correspond to the habitats in which they are found.

The third alternative strategy is illustrated by *Mimulus cardinalis*, which is of some interest in that it is the only well-studied *Mimulus*. Milner and Hiesey (1964), studying climatic (altitudinal) races of this species, determined light saturated photosynthesis rates at temperatures from 0°C to 40°C. Using light 20% above saturation they found that photosynthesis rates declined rapidly with time above 45°C, and at the highest temperature studied, CO₂ fixation was often negative. All six races studied show maximal photosynthesis at or near 30°C. The response differed between races as the temperature diverged from that
optimum. Thus, unlike the races of *Oxyria*, *M. cardinalis* has a common optimum temperature for a physiological process among the species' races even though they differed in performance at different temperatures. It is also of interest that Decker (1959) found that *M. cardinalis* clones from sea level, and *M. lewesii* clones from alpine regions in California both had a respiration rate in the light about 3.3 times greater than the rate in the dark.

It was decided to compare the photosynthesis rates of thermal and nonthermal *M. guttatus* populations at several temperatures. Clearly, *Mimulus* in the Yellowstone thermal areas is in a higher temperature environment than *Mimulus* in Logan Canyon (see Chapter 1). It might be expected therefore that if the mature plants of thermal populations were adapted to tolerate the higher temperatures, they would have higher rates of photosynthesis at high temperatures than Logan Canyon plants.

**Methods and Materials**

Mature *Mimulus guttatus* plants were collected on July 9, 1971, from Obsidian Creek, Yellowstone Park. This site supported a large population at soil temperatures of 27 C (night minimum) to 40 C (day maximum) on the date of collection. Leaf temperatures varied from 4 C to 35 C over the same 24 hour period. The leaves were less than 1 cm in width, but green and turgid. The plants were placed in an insulated container with the roots in water at 35 C and taken to Logan, where they were placed in a growth chamber at 35 C. Plants from a population in Spring Hollow, Logan Canyon, Utah, were collected on July 10, 1971,
for comparison with the thermal plants. These had root temperatures of 7°C minimum and 28°C maximum. Leaf width varied from 1 to 2 cm. On July 11, all the plants were transported in insulated containers, with root temperatures maintained near 35°C and 9°C for the thermal and nonthermal populations respectively, to the Atomic Energy Commission Plant Research Laboratory at Michigan State University. There they were kept in growth chambers with air temperatures of 35/25 for thermal plants and 10/5 for nonthermal plants until used in the experiments.

Three different photosynthetic responses were investigated: 1) thermal Mimulus rates at high temperature (36°C); 2) nonthermal Mimulus compared with Xanthium strumarium L. at three temperatures (8, 25, and 35°C); 3) nonthermal Mimulus rates at 8°C, 25°C, and 35°C, with two separate temperature pretreatments for the plants.

For each experiment single detached leaves were placed in sealed porometers and the porometers immersed in a water bath adjusted to the desired temperature. Porometers of 0.6 cm diameter were used for the thermal Mimulus leaves and of 2.5 cm diameter for nonthermal Mimulus and Xanthium leaves. Carbon dioxide at a concentration of $305 \pm 5 \times 10^{-1}$ was fed into the leaf chamber at a flow rate of 50 l hr$^{-1}$. Infrared gas analysers (Hartmann and Braun, URAS 2) measured the exchange of CO$_2$ (assimilation and dark respiration) and water vapor. Photosynthesis, respiration, and leaf resistances and temperatures were analyzed by computer from data recorded on magnetic tape (Cummins, Kende and Raschke 1971). The light source was a water jacketed
2500 watt Xenon arc lamp, with radiation intensity of approximately 1.1 cal cm\(^{-2}\) min\(^{-1}\). Results were calculated separately for each surface, but are reported as the average of both surfaces. All results are plotted against leaf temperature. Equations used to calculate net assimilation, transpiration, internal CO\(_2\) concentration, and diffusion resistances were taken from Gaastra (1959) and are given in Appendix I.

Results

Photosynthesis rates of *Mimulus guttatus* from a thermal habitat

Due to lack of plant material only one air temperature was tested, 36 C. Leaf temperature varied from 34 C to 35 C. Both dark respiration and photosynthesis were determined (Table 7). Internal CO\(_2\) was not limiting during the experiment although photosynthesis was extremely low. The fact that leaf conductance was low indicates that the stomates were nearly closed in the light as well as in the dark, which caused both the low transpiration rate and the high leaf temperature. The combination of low photosynthesis rate, high leaf resistance and high internal CO\(_2\) concentration indicates however, that the normal assimilation processes were essentially non-functional in the plants tested. Partial stomatal closure in itself may not completely limit photosynthesis, as there is evidence that gas exchange can take place in wilted leaves through stomata which are nearly closed (El-Sharawy and Hesketh 1964).

Nonthermal Mimulus compared with *Xanthium strumarium*

Because its physiological responses are well known, and because it could be considered representative of plants which exhibit photorespiration,
Table 7. Responses of thermal *Mimulus* leaves at 35 C. Means of four replications and standard deviations.

<table>
<thead>
<tr>
<th></th>
<th>CO₂ assimilation</th>
<th>CH₂O</th>
<th>CO₂</th>
<th>[CO₂]₁</th>
<th>ΣrH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.225</td>
<td>0.133</td>
<td>0.085</td>
<td>298.4</td>
<td>7.915</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 0.307</td>
<td>± 0.000</td>
<td>± 0.018</td>
<td>± 7.390</td>
<td>± 2.000</td>
</tr>
<tr>
<td><strong>Dark</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-2.900</td>
<td>0.192</td>
<td>0.122</td>
<td>349.2</td>
<td>6.640</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 0.302</td>
<td>± 0.132</td>
<td>± 0.080</td>
<td>± 17.38</td>
<td>± 2.850</td>
</tr>
</tbody>
</table>

CO₂ assimilation = rate in mg CO₂ dm⁻² hr⁻¹
CH₂O = conductance of water vapor in cm sec⁻¹
CO₂ = conductance of carbon dioxide in cm sec⁻¹
ΣrH₂O = resistance to water vapor diffusion in sec cm⁻¹
[CO₂]₁ = internal CO₂ concentration in \( \text{mol} \text{ l}^{-1} \)
Xanthium was used for comparison with Mimulus at three temperatures. At each temperature two Mimulus leaves were tested at the same time as one leaf each of two differently pretreated Xanthium plants. The Xanthium leaf labeled (10 C) in all charts and graphs was selected from a plant grown in a greenhouse at 25 ± 2 C and pretreated at 10/5 C for 72 hours prior to testing. The leaf labeled (25 C) was selected from a plant grown under the same greenhouse conditions, but not removed until immediately before testing.

Mimulus photosynthesis rates are lower than those of Xanthium at all temperatures, but the rates peak at the same temperature, 26 C (Figure 13). Mimulus CO₂ conductance is less than that of Xanthium (Figure 14), and as would be expected under such conditions, the Mimulus transpiration rate is low (Figure 15). However, mean internal CO₂ levels in Mimulus (Figure 16) are high enough that it is impossible that the stomates are the cause of the low level of photosynthesis. In fact, the low and relatively constant conductances can be considered evidence that stomatal conductance has little to do with the differences in $P_n$ observed at different temperatures. Xanthium has lower internal CO₂ concentrations, yet still produces a higher CO₂ fixation rate than Mimulus. Stomates of both species did respond to a higher leaf temperature by opening which indicates that the Mimulus stomatal apparatus was still functional even if not fully open.

In general, Mimulus dark respiration rates are lower than those of Xanthium (Figure 17). On the whole, Mimulus shows essentially the same
Figure 13. \( \text{CO}_2 \) assimilation in light. Nonthermal *Mimulus guttatus* and *Xanthium strumarium* pretreated at 10 C and 25 C.
Figure 13.
Figure 14. CO₂ conductance in light. Nonthermal *Mimulus guttatus* and *Xanthium strumarium* pretreated at 10 C and 25 C.
Figure 15. Transpiration rates in light. *Mimulus guttatus* and *Xanthium strumarium* pretreated at 10 C and 25 C.
Figure 15.
Figure 16. Mean internal CO$_2$ concentration in light. Nonthermal Mimulus guttatus and Xanthium strumarium pretreated at 10°C and 25°C.
Figure 17. Comparison of dark respiration in nonthermal *Mimulus guttatus* and *Xanthium strumarium* pretreated at 10 C and 25 C.
pattern of physiological response to temperature change as the Xanthium plants, only the magnitude of the response is less. This could be considered a normal species difference, lacking further evidence to the contrary.

Response of nonthermal Mimulus to two temperature pretreatments

In order to test the ability of a low temperature Mimulus clone to adapt to a higher temperature regime than the one from which it came, one nonthermal plant was treated at 10/5 C temperatures for 48 hours and another treated at 10/5 for 24 hours, then moved to a 25/20 temperature regime for 24 hours.

Figure 18 indicates that photosynthesis is low in both groups of Mimulus plants, compared to the rates attained by Xanthium in the previous experiment. The response of the two groups of pretreated plants to test temperature was analyzed by a two-way analysis of variance. Mimulus plants pretreated at high temperature have significantly higher rates of photosynthesis than the cold-treated plants ($F_s=24.78; \text{df} = 1,6; P < .01$). Observation of the graphed curves suggests that high-temperature pretreated plants photosynthesize best at 25 and 35 C, and cold-temperature plants best at 8 C. This suggestion seems to be confirmed by a significant interaction effect between pretreatment temperature and test temperature ($F_s=16.32; \text{df} = 2,6; P < .01$). Thus, M. guttatus plants taken from a typically low temperature habitat have the ability to adapt rapidly to an increased environmental temperature.
Figure 18. Comparison of photosynthesis rates between two nonthermal Mimulus plants, one treated at 10/5 C for 48 hours and the other treated at 10/5 C for 24 hours and 25/20 C for 24 hours.
Discussion

I tested the question: does *Mimulus guttatus* from thermal areas have higher rates of CO\textsubscript{2} fixation at high temperatures than *Mimulus* from nonthermal sites?

Thermal *Mimulus*, tested at 36 C, did not give conclusive evidence that these populations are physiologically adapted to high temperatures. The photosynthetic rates of *Mimulus* from nonthermal sites did not seem to be related to stomatal resistances, and internal CO\textsubscript{2} levels were high at all temperatures. This indicates that the stomates were probably not the factor which limited net photosynthesis in these tests.

However, assimilation rates for nonthermal *Mimulus* peak at 25 C and drop considerably at 35 C; maximum assimilation at or near 30 C has been recorded for several temperate species, including *Xanthium*, *Mimulus cardinalis* (Milner and Hiesey 1964), *Gossypium hirsutum*, and *Helianthus annuus* (El-Sharkawy and Hesketh 1964). Therefore, nonthermal *Mimulus* can probably be categorized with these species as having photosynthetic rates representative of temperate zone, mesophytic plants.

Nonthermal *Mimulus* pretreated at 10 C and 25 C gave good evidence that plants taken from a cold temperature environment could rapidly adapt to temperatures higher than experienced in the usual habitat. Thus, it is probably true that the species is capable of physiological adjustment to higher temperatures. This species capability could, in a habitat that functions as the Yellowstone thermal areas do (Chapter 1), replace
replace the need for a genetically fixed high temperature-tolerant race at the adult level. Consider the gradual temperature rise that occurs in the thermal habitat during the change from winter to summer conditions. It seem likely that since the plants are growing 12 months of the year, the populations adapt on a seasonal basis to increasing temperatures. As previously noted, the plants die out of areas where root temperatures exceed 45 C, and maximum leaf temperatures are neither high nor of long duration.

As mature *Mimulus* plants avoid high temperatures it is not surprising that a high-temperature-stable photosynthesis apparatus is not present: there is no need for one. Further testing of populations from more locations is necessary to determine whether or not genetically fixed eco-types with different photosynthetic temperature optima do occur. This data however, suggests that *Mimulus guttatus* may be an example of a species that has a temperature optimum common to the species over a wide latitude, and that therefore does not tend to develop physiological races.
CHAPTER 3

GERMINATION AND POLLINATION BIOLOGY OF THERMAL AND NONTHERMAL MIMULUS POPULATIONS

Review of Literature

Seed germination was considered as a possible heat limited phase in the Mimulus life cycle in the Yellowstone thermal areas. In general, seed germination can be viewed in either a proximate or ultimate sense: as a mechanism, and as an adaptative strategy.

The various germination mechanisms employed by plant species are complex. Yet whatever the means, the end is to insure the continuation of the population and the species. It is recognized that seed dormancy may be innate, induced by conditions affecting the parent, or environmentally imposed on the seed (Amen 1966; Harper et al. 1970). Triggering mechanisms are often subtle and may require combinations of stimuli. Some species (e.g. apple and pine) require afterripening, plus a light flash when moisture conditions are optimal, in order to break dormancy (Sauer and Struik 1964). Capon and Van Asdall (1967) found that high temperatures in the field before seed is shed may promote the maturation process, but that high temperature may not be of great importance to the germinating seed. Desert annuals respond to combinations of temperature and precipitation, with the total percentage of germination
correlated with the amount of precipitation up to an optimum, after which germination decreases (Juhren, Went, and Phillips 1956). Went (1949) also found that when the temperature was above 20 C summer annuals germinated whereas winter annuals would not, and when temperatures were below 20 C the reverse was the case. Amen (1966) believed that many cold tolerant species are dormant due to seed coat inhibition. Such seeds require scarification, stratification, afterripening, or various light conditions. However, some species will not germinate at temperatures lower than 10 - 15 C, regardless of seed coat treatment, implying a temperature imposed dormancy. In addition, some species appear to respond to their own population density, or to those of other species (Harper and Chancellor 1959; Harper and McNaughton 1962).

There are two aspects to germination strategy, 1) the seed must not break dormancy too soon, as it must operate to avoid adverse conditions during the growing period, 2) it must break dormancy successfully when the conditions become suitable for completing the life cycle. From this it follows that at least three generalizations may be made regarding seed germination. First, the seed should respond to definite single event stimuli in environments such as deserts, where good growing conditions are temporary and erratic (Juhren et al. 1956; Tevis 1958a, 1958b; Went 1949). Second, the triggers that break dormancy should be those occurring fairly regularly but only before good growing conditions, such as are found in arctic and alpine environments (Amen 1968; Billings and Mooney 1968). Third, variability is expected among seeds of any population when there is some risk of the germination
triggers being imperfect predictors of subsequent good conditions.

For example, an annual population that relied upon one strategy would run the risk of becoming extinct in a single bad growing season, if that happened to follow the germination trigger. As a result, germination polymorphism has developed in many colonizing plant species (Cavers and Harper 1966; Cook 1962). Polymorphism may occur within seeds of one plant, between plants in one population, or between populations in slightly different habitats.

Knowledge of germination and life cycles of *Mimulus* is based on nonthermal populations. There are two life cycles that the species may utilize: annual and perennial. For perennials, water is not limiting during the growing season, and thus would not be a relevant germination trigger. Photoperiod does not seem to be of importance, as the seed will germinate either in total darkness or on a 12 hr day (Vickery 1967; Rice, unpub. data). The annual form has been little studied, but there is evidence that annuals may maintain genes for the perennial life cycle. Thus, the stimulus of interest is temperature. Vickery (1967) examined differences in the temperature tolerance of germination between and within thirteen populations of the *Mimulus guttatus* complex. In *M. guttatus* he found some germination in 17 of 27 temperature combinations. The 27 combinations ranged from 4/-7 C to 50/35 C. Ten combinations were held in common among three geographically widely separated populations. The lowest combination producing germination was 9/4 C and the highest 50/25 C. Interestingly, there was not a strong correlation between the ability to germinate at a given temperature
and the habitat temperature of the population. It has been noted previously (Chapter 1) that at low temperatures, seed from nonthermal populations requires a temperature fluctuation for germination.

*Mimulus guttatus* in the Yellowstone thermal areas uses both the annual and perennial life cycle. The three generalizations discussed above give some idea of the germination strategies that might be expected in *Mimulus*. The first condition, that of temporary and erratic growing conditions, is not particularly applicable. The thermal annuals germinate in the fall, and water does not become limiting until late in the spring; the problem here is to control the growth form as a rosette until frost danger is past. The second rule has some relevance in that lowered temperatures, especially in boggy thermal sites, would be an indication of subsequent conditions with lowered heat stress. General rule three is again not necessarily applicable. With respect to water and temperature requirements, good growing conditions will not fail in the boggy areas nor in the fall-germinating sinter populations. The possibility of sinter plant seed all germinating in response to a cool, wet late-summer period followed by extremely hot, dry conditions may exist. However, even if all seedlings should perish it is probable under such conditions that some plants on sinter would persist as rosettes, and these would bloom the following year.

The research on *M. guttatus* in thermal areas was originally undertaken with the assumption that the species in Yellowstone was high-temperature tolerant, since it lived in a high temperature environment.
Thus, it was thought that the seeds would fall onto high temperature substrates. The general hypothesis was formulated that seeds of thermal populations would therefore require high temperatures for germination. The question investigated in the laboratory was whether seeds of thermal *M. guttatus* germinate best at higher or lower temperature.

On the basis of the high temperature tolerance supposition, the question was also raised whether the Yellowstone populations had diverged sufficiently from other populations (nonthermal and thermal) to become a distinct species or ecotype. The Yellowstone plants were diversified in their morphology and presumably in physiology as well as being geographically isolated. As Vickery (1964) indicates, however, the evolution of genetic isolating mechanisms may be either concurrent or independent of such diversifications. Therefore, the possibility of reproductive isolation was investigated by crossing thermal populations with themselves and with a nonthermal population from Logan Canyon, Utah. The criterion for success of crossing was the germinability of the \( F_1 \) seed. The hypothesis was that the populations would be isolated; the alternative was that suggested by Vickery (1964, 1967): outcrossing is more successful than incrossing in spatially separated populations, leading to a large shared gene pool retaining much plasticity.

**Methods and Materials**

**Temperature tolerances of germinating seeds**

Five populations were selected from within the boundaries of Yellowstone Park for testing temperature tolerances during germination. The
temperatures were selected on the basis of substrate temperatures taken during the summer of 1969 and were considered to cover the range of temperatures available to thermal and nonthermal populations in Yellowstone. Populations and habitat characteristics are described in Table 8. Seed capsules were gathered from within the populations and seeds mixed to give a bulk sample from which test material was taken.

Seeds were sown on sterilized greenhouse soil in 18 x 13 x 5 cm containers at a rate of 50 seeds per container. There were placed in growth chambers and continuously subirrigated by being kept in large pans of water. This kept the entire soil system water saturated and the relative humidity immediately surrounding the seeds at about 100%. Temperature combinations used were 10/5, 20/15, 30/25, and 40/35 C (day/night). The higher temperature in each combination was correlated with a 12 hour period of illumination with fluorescent light, and lower temperature with 12 hours of darkness. Soil temperatures were about 5 C below air temperature. Scherer-Gillette growth chambers were used for the three higher combinations and a Percival growth chamber for the lower combination. Relative humidity was maintained at maximum for each chamber and varied from 68 % to 80 %. Four replications of each population test were run simultaneously. Results were analyzed by analysis of variance (Sokal and Rohlf 1969). The tests were continued for 61 days, although there was little additional germination after 45 days.

Germination of seeds from cross-pollination experiments

To investigate the possibility of reproductive isolation between
Table 8. *Mimulus* populations selected from Yellowstone Park for germination testing at four temperature combinations. Soil temperature was taken at time of collection.

<table>
<thead>
<tr>
<th>Site</th>
<th>Habitat Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Thumb</td>
<td>Boggy shore of Yellowstone Lake. Perennials, 7 - 14 cm tall. $T_s = 45$ C. June 29, 1969.</td>
</tr>
<tr>
<td>Obsidian Creek</td>
<td>Boggy soil of creek bank. Perennials, 10 cm tall. $T_s = 37$ C. June 20, 1969.</td>
</tr>
<tr>
<td>White Creek, sinter</td>
<td>Sinter substrate. Annuals, 2 - 7 cm tall. $T_s = 31$ C. June 20, 1969.</td>
</tr>
</tbody>
</table>
populations inside and outside Yellowstone Park four populations were selected for cross-pollination. Population data are given in Table 2, Chapter 1. A small plug of vegetative material from each population was obtained, potted in greenhouse soil and cultured in the greenhouse. Pots were subirrigated. The original material from each population was eventually cloned 16 times: four replicates of each cross and four plants used in each replicate. The germination percentages of the four plants in each replication were averaged, and these figures then averaged to give a single value for each cross. These single values were used for the analysis of variance.

To initiate flowering, the plants were placed on a 16 hour photoperiod; this was maintained until the experiment was completed. Twenty flowers were pollinated on each plant of each reciprocal cross. The stigmas of *M. guttatus* are receptive a day or so before the anthers shed pollen (Clausen and Hiesey 1958). As the stamens are attached to the corolla it is an easy operation to remove both of these floral parts before artificially pollinating the flowers. The removal of the corolla prevents accidental pollination by insects or by selfing. When a receptive stigma surface is touched the lobes close, further discouraging accidental pollination. Seeds were harvested when mature, placed in manila coin envelopes and refrigerated at 0 °C. The number of seeds per capsule was counted for 5 capsules of each population.

For germination trials, seeds were sown on wet filter paper in petri plates at a rate of 50 seeds per plate. There were placed in the Percival
growth chamber with 12 hours fluorescent light and 12 hours darkness with a temperature fluctuation of 20/10 °C. Tests were terminated after 22 days.

Results

Temperature tolerances during germination

The number of seeds per capsule of these populations varied from 30 to 292 for sinter plants, with a mean of 157 ± 29 (S.E.). Plants from boggy sites had a seed number variation from 221 - 744 per capsule, and a mean of 573 ± 90 (S.E.). Although seed number varied between habitats, seed size was relatively constant. Other workers (Palmblad 1968; Harper et al. 1970) have found that seed weight does not vary significantly within species.

The percentages of germination achieved by seeds of the different populations tested for temperature tolerances were analyzed by a two-way analysis of variance. The population and treatment means are given in Table 9. There is a significant difference between populations in germination success ($F_s = 14.85; \text{df} = 4,60; P < .01$). This is not unexpected, since variability between populations of the same species in many parameters is well documented (Cook 1962; Heslop-Harrison 1966; McWilliams et al. 1968; Solbrig 1971; Lindsay and Vickery 1967). Of considerably greater interest is the significant increase in germination for all populations with decrease in temperature ($F_s = 224.69; \text{df} = 3,60; P < .001$). This result is in direct opposition to the original hypothesis that thermal M. guttatus populations would prefer high germination temperatures.
Table 9. Population and treatment means of percentage germination at four temperature combinations.

<table>
<thead>
<tr>
<th>Temperature Combination</th>
<th>Population Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White Creek sinter</td>
</tr>
<tr>
<td>10/5</td>
<td>90.0</td>
</tr>
<tr>
<td>20/15</td>
<td>73.0</td>
</tr>
<tr>
<td>30/25</td>
<td>56.0</td>
</tr>
<tr>
<td>40/35</td>
<td>0</td>
</tr>
<tr>
<td>Population Mean</td>
<td>54.8</td>
</tr>
</tbody>
</table>
There is significant interaction between temperature and population 
\( (F_s = 5.00; \text{df} = 12.60; P < .01) \). This indicates that response to tempera-
ture differs within each population; examination of Figure 19 shows the 
response of the population from the White Creek sinter area to temp-
erature is probably responsible for most of the interaction.

All population means were compared by a Student-Newman-Keuls 
test (a posteriori test) with the means arrayed in order of magnitude as 
follows: White Creek sinter \( (\bar{Y}_1) \); West Thumb \( (\bar{Y}_2) \); Obsidian Creek \( (\bar{Y}_3) \); 
White Creek boggy \( (\bar{Y}_4) \); Black Sand Basin \( (\bar{Y}_5) \). At \( P < .05 \) the results 
were: \( \bar{Y}_1 > \bar{Y}_2 = \bar{Y}_3 = \bar{Y}_4 > \bar{Y}_5 \).

Three trends may thus be seen in the data presented.

1) The population from the dryest habitat, sinter, has the best 
overall germination \( (\bar{Y}_1) \).

2) On the whole, thermal populations germinate better at all 
temperatures than the nonthermal population \( (\bar{Y}_5) \).

3) For all populations, thermal and nonthermal germination increase 
as the temperature decreases.

**Cross-pollination: Reproductive isolation of populations**

For the statistical analysis of these data the following hypotheses 
were stated:

1) There is significant variation between the germination success of 
seed from female parents from different sites (same male parent).

2) There is significant variation between the germination success 
of seed from one female parent (male parents from different sites).
Figure 19. Response of seed from five *Mimulus guttatus* populations to four temperature combinations during germination. All the populations grow in Yellowstone thermal areas.
Figure 19.

- • West Thumb
- •-• White Creek, sinter
- △-△ White Creek, boggy
- □-□ Obsidian Creek
- □-□ Black Sand Basin
These first two hypotheses were examined by a nested two-level analysis of variance (Anova), with origin of female parent being the first level and origin of male parent the second. The Anova table is shown in part below:

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>$F_s$</th>
<th>$P &gt;$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between female parent origins</td>
<td>3, 6</td>
<td>3940</td>
<td>1.243</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Within female parent origins,</td>
<td>6.65, 82</td>
<td>3171</td>
<td>10.73**</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>between male parent origins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thus, hypothesis 1 is rejected, and hypothesis 2 is accepted.

Following this preliminary analysis, the following hypotheses were tested:

3) Self-pollinated plants produce seed that germinates at a lower frequency than seed from plants that are cross-pollinated to other populations.

4) Seeds whose male and female parents were from sites close to each other germinate better than those whose parents originated far apart.

5) Seeds which are similar except that the origins of their male and female parents are reversed germinate equally well (comparison of reciprocal crosses).

For hypothesis 1 and 2 the design of a nested anova rather than that of a two-way anova was chosen, as in a two-way anova the "treatment" of interest (for example, self-pollination) would have been arranged...
in diagonals, rather than by rows or columns (see Table 10). For the same reason it was not possible to proceed to test hypotheses 3, 4, and 5 by dividing the variance orthogonally in the usual way. Therefore these hypotheses were tested by specific two-group, one-way anovas. This is legitimate if the total number of hypotheses tested is small; that is, when less than all possible comparisons are made (Sokal and Rohlf 1969).

Hypothesis 3 is confirmed; seeds from self-pollinated plants do not germinate as well as seeds from outcrossed plants ($F_s = 16.3; \text{df} = 1,60; P < .001$).

All the Yellowstone sites are close together compared to Logan Canyon. Hypothesis 4 can therefore be formulated: crosses between the Logan Canyon population and a Yellowstone population should germinate less well than crosses among Yellowstone populations (see Table 2, Chapter 1). If the germination success of a cross between a female A and a male B is represented (A x B), hypothesis 4 can be subdivided into:

H4a: \[ (WC \times LC) < (WC \times TC) \]

H4b: \[ (LC \times WC) = (LC \times NP) \]

The two groups in H4a are significantly different ($F_s = 9.4; \text{df} = 1,23; P < .01$), but as Table 10 shows, the difference is in the opposite direction to that hypothesized. The two groups in H4b are not significantly different ($F_s = 3.12; \text{df} = 1,31; P > .05$), and the hypothesis is accepted. Overall, hypothesis 4 is rejected, as there is some evidence that crosses between distant parents are more successful than crosses
Table 10. Mean germination percentages of seeds from cross-pollination between thermal and nonthermal *Mimulus* populations. ... indicates that cross-pollination was not attempted.

<table>
<thead>
<tr>
<th>Male Parent</th>
<th>Female Parent</th>
<th>White Creek</th>
<th>Logan Canyon</th>
<th>Nez Perce Creek</th>
<th>Tangled Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Creek</td>
<td>White Creek</td>
<td>75.6</td>
<td>99.6</td>
<td>....</td>
<td>61.5</td>
</tr>
<tr>
<td>Logan Canyon</td>
<td>Logan Canyon</td>
<td>94.1</td>
<td>83.3</td>
<td>92.0</td>
<td>....</td>
</tr>
<tr>
<td>Nez Perce Creek</td>
<td>Nez Perce Creek</td>
<td>....</td>
<td>97.0</td>
<td>11.0</td>
<td>....</td>
</tr>
<tr>
<td>Tangled Creek</td>
<td>Tangled Creek</td>
<td>80.8</td>
<td>....</td>
<td>....</td>
<td>68.3</td>
</tr>
</tbody>
</table>
between close parents.

Hypothesis 5 was subdivided into the following individual comparisons:

H5a: \[(WC \times LC) = (LC \times WC)\]
H5b: \[(WC \times TC) = (TC \times WC)\]
H5c: \[(LC \times NP) = (NP \times LC)\]

The results were:

H5a: rejected \(F_S = 10.23; \text{df} = 1,30; P < .01\).
H5b: accepted \(F_S = 1.9; \text{df} = 1,15; P > .05\).
H5c: accepted \(F_S = 3.4; \text{df} = 1,23; P > .05\).

The following conclusions can be drawn from the analysis of germinability of crosses:

1) Female parents from different sites do not differ significantly in the viability of the seeds they produce.

2) Seeds produced by cross-pollinating plants from different populations are significantly more viable than seed from self-pollinated plants.

3) There is some evidence that crosses are more viable the further apart the origins of their parents (see p. 69).

4) There is no consistent evidence that the contribution of the male parent to the viability of the seed is different from that of the female parent. The exception \((WC \times LC) < (LC \times WC)\) is unexplained.
Discussion

When seed from several populations in Yellowstone Park were germinated at different temperatures, there was a decrease in germination success as temperature increased. The "optimal" temperature combination, 10/5, is surprisingly low, especially since it was shown in Chapter 1 that temperatures only a few degrees lower can retard germination. In some respects, the germination characteristics of *Mimulus guttatus* are similar to those of many arctic and alpine plants. Germination is controlled by external environmental conditions, and alternating temperature conditions may be necessary (Billings and Mooney 1968; Sayers and Ward 1966; Wager 1938). These mechanisms enable alpine species to germinate in spring during early snowmelt and when substrate and air temperatures are increasing. In *Mimulus* the general trend toward better germination with lower temperature might be due to the fact that the species usually occupies a rather cool habitat, but one which would not inflict sudden freezing temperatures on seedlings. It is clear however, that there is no adaptation to high temperatures during the germination stage by thermal *Mimulus* populations.

It is probable that there is actually little selective pressure for high temperature tolerance during germination and establishment. The thermal plants growing on sinter germinate during fall, when lower soil temperatures are coincident with increased precipitation. Plants in boggy sites shed seed from late May to September; their seeds are the most liable to exposure to temperatures above 30 C. As these
Mimulus populations tend to cover all available ground rapidly by vegetative reproduction, seeds and seedlings would either be in intense competition with established individuals, or be on soil that was too hot even for adult plants. There would be little opportunity for a gradual selective process involving temperature tolerance when the seeds can not germinate or establish because of competitive factors.

The cross-pollination tests confirm the results of field data, physiological data, and temperature tolerance tests. In spite of geographical isolation and morphological divergences, there appear to be very few crossing barriers operating between thermal and non-thermal M. guttatus populations. It is true that crossing barriers are difficult to analyze unless very large numbers of combinations are used, or the barriers are complete. However, there may be two sorts of partial barriers operating between the investigated populations. The data that suggest these barriers are not extensive, and the possibility needs to be investigated further by cross-pollination studies using larger sample sizes and more populations. The types are described by Vickery (1958, 1964) as being 1) barriers that reduce set of the hybrid seeds, and 2) barriers that inhibit or prevent germination of hybrid seeds.

A barrier that reduces hybrid seed set appears to be operating in the combination NP x LC (Table 11). The expected seed set for the cross, based on the average of selfed seed set for both parents (Vickery 1964), is 106. The fact that seed set is reduced to 50 when plants from the
Table 11. Mean seed set per capsule and standard deviation for *Mimulus* interpopulation crosses, n = 20. Logan Canyon is a nonthermal population; all others are from Yellowstone Park thermal areas. .... indicates no cross attempted.

<table>
<thead>
<tr>
<th>Male Parent</th>
<th>Female Parent</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White Creek</td>
<td>Tangled Creek</td>
<td>Nez Perce Creek</td>
<td>Logan Canyon</td>
</tr>
<tr>
<td>White Creek</td>
<td>113 ± 34</td>
<td>178 ± 95</td>
<td>....</td>
<td>212 ± 171</td>
</tr>
<tr>
<td>Tangled Creek</td>
<td>238 ± 144</td>
<td>74 ± 36</td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>Nez Perce Creek</td>
<td>....</td>
<td>....</td>
<td>63 ± 17</td>
<td>237 ± 171</td>
</tr>
<tr>
<td>Logan Canyon</td>
<td>125 ± 81</td>
<td>....</td>
<td>50 ± 31</td>
<td>149 ± 10</td>
</tr>
</tbody>
</table>
Nez Perce (NP) population are used as female parents, and seed set is 237 when plants from Logan Canyon (LC) are the female parents suggests that Nez Perce plants limit seed set by producing fewer ovules.

The second type of barrier may be visible in the combination WC x LC. Examination of Table 3 indicates that in most cases inter-population hybrids are more viable than self-pollinated plants, and that effect of male and female parents are equal. However, when White Creek plants are used as the female parent in WC x LC, only 94% of the seeds are viable as opposed to 99.6% when plants from Logan Canyon are used as the female parent. This was the only combination tested with significant differences; P < .01. This indicates that there is a weak barrier affecting germination in this cross, but the mechanism is not explainable on the basis of the data available.

The conclusion is reached that the presence, absence or strength of crossing barriers which could affect germination characteristics in *M. guttatus* populations can not be predicted from visible diversity between populations or on the basis of habitat, and is consistant with the findings of Vickery (1958, 1964). If selection pressure is the most important factor in producing speciation, then it appears from the crossing experiments in this study that the thermal habitat has not applied sufficient pressure to result in a definite species adapted to high temperatures, during the germination stage of the life cycle. The lack of such pressure is discussed in relation to the temperature tolerance
tests. Thus, it must be concluded from both germination temperature
tolerance and cross-pollination tests, that *M. guttatus* in Yellowstone
thermal areas does not appear to be either high-temperature tolerant
or a separate species, as was originally suggested.
SUMMARY

There is no evidence from experiments carried out in this study that populations of *Mimulus guttatus* that grow in the thermal areas of Yellowstone National Park have evolved a high-temperature tolerant race. Neither seed germination nor photosynthetic rates are enhanced by increasing temperatures; both of these processes respond best to temperatures optimal for typical mesophytes. There is evidence, however, that plants from nonthermal habitats can adapt rapidly to temperatures higher than those in the habitat from which they were taken. In the thermal areas, root temperatures reach the highest temperatures (45°C), but leaf temperatures of plants in the field are not extreme and remain well below temperatures endured by species considered heat tolerant. Cross-pollination experiments between thermal and nonthermal populations and germination of the F1 seed, showed no significant reproductive barriers. It is possible that further tests involving analysis of hybrid vigor and fertility would reveal genetically based population differences. At present, it does not appear that temperature per se is acting as a strong selective pressure for genetic differentiation between populations living in different temperature microenvironments.

Although the Yellowstone populations are not heat tolerant, they may be considered heat resistant. Levitt (1972) has classified heat
resistant plants into two groups: those living in extreme environments and tolerating the stress, and those avoiding the stress. *Mimulus guttatus* falls into the latter group. Certain areas that the species colonized in winter have soil temperatures of about 40°C; these same areas may have soil temperatures reaching 75°C in summer. The plants remain on the sites until soil temperatures reach about 45°C, then die back. Since temperatures over an area as small as one square meter may be extremely variable, any site has a mosaic of temperature microsites available, some cooler than others. The plants can survive in the cooler sites until fall, when they again re-vegetate the areas abandoned during summer. This "migratory" ability is the result of the species being able to reproduce vegetatively by rooting at stem nodes. In a sense, this has conferred upon the plants an evolutionary choice of either avoiding the stress by pre-programmed mechanisms, or of evolving a tolerance mechanism at some stage of the life cycle. It seems clear that avoidance is the easier choice. On the sinter thermal areas, the plants can avoid both heat and water stress by adopting an annual life cycle, and surviving difficult periods as seed. This ability is also a typical response for the species over the rest of its range. It is of interest that any one plant may function as a perennial, annual, and may or may not reproduce vegetatively according to the conditions under which it is currently growing. Furthermore, no plant is irrevocably committed to any of these strategies but may change as conditions permit. This suggests that the species is genetically structured so that
a basic genotype is capable of a plastic response at the phenotypic or
developmental level. Adaptations can thus be made quickly and do not
need to go through a recombination process in order to respond to stress.

*Mimulus guttatus* has been able to survive in the high temperature
environment of thermal areas by utilizing a genetically programmed
phenological plasticity, which allows the plants to avoid, rather than
to tolerate, heat stress.
LITERATURE CITED


Hiesey, W.M. 1953. Comparative growth between and within climatic races of Achillea under controlled conditions. Evolution 7: 297-316.


Phillips, P.J., and J.R. McWilliam. 1971. Thermal responses of the primary carboxylating enzymes from C3 and C4 plants adapted to


APPENDIX
APPENDIX I

The equations described here are those used by Gaastra, Med. Landb. Wageningen 59 (13): 1-68. 1959.

1. Photosynthesis rate is determined by analyzing the CO₂ concentration in the air stream before and after passage over the leaf.

\[ P_{\text{actual}} = \frac{Q [C]_0 - [C]_e}{A} \times 10^{-2} \]

where \( Q \) = flow rate
\([C]_0\) = % CO₂ in incoming air
\([C]_e\) = % CO₂ in outgoing air
\( A \) = leaf area

As the rate of photosynthesis is also identified with the rate of CO₂ diffusion from the external air toward the chloroplast, the equation may also be expressed as

\[ P_n = \frac{[CO_2]_a - [CO_2]_i}{r'_a + r'_s} \]

where \( P_n \) = rate of photosynthesis (cm³ CO₂ cm⁻² sec⁻¹)
\([CO_2]_a\) = CO₂ concentration in external air
\([CO_2]_i\) = CO₂ concentration in intercellular spaces
\( r'_a \) = diffusion resistance of air (boundary layer) to CO₂
\( r'_s \) = diffusion resistance of stomates to CO₂
2. Internal CO₂ concentration is calculated by taking the difference between CO₂ concentration of air and the net CO₂ flux,

\[ [\text{CO}_2]_1 = [\text{CO}_2]_a - P_n \left( r_a + r_s \right) 1.56 \]

where \( r_a \) = diffusion resistance of air to water vapor

\( r_s \) = diffusion resistance of stomates to water vapor

1.56 = ratio of the square roots of the molecular weights of CO₂ and H₂O

3. Transpiration is measured by analyzing the difference in moisture of the air stream before and after passage over the leaf,

\[ V = \frac{[\text{H}_2\text{O}]_e - [\text{H}_2\text{O}]_o}{r_a + r_s} \]

where \( V \) = transpiration (mg H₂O dm⁻² hr⁻¹)

\([\text{H}_2\text{O}]_e\) = water vapor concentration in outlet air

\([\text{H}_2\text{O}]_o\) = water vapor concentration in inlet air

4. Diffusion resistance can be obtained from the rates of transpiration or photosynthesis and the concentration difference of CO₂ or H₂O vapor at the two ends of the path considered,

\[ (r_s + r_a)\text{H}_2\text{O} = \frac{[\text{H}_2\text{O}]_1 - [\text{H}_2\text{O}]_a}{V} \] (sec cm⁻¹)

\( V \) and \([\text{H}_2\text{O}]_a\) are measured while \([\text{H}_2\text{O}]_1\) is derived from leaf temperature. Air in the intercellular space is assumed to be saturated, therefore the \([\text{H}_2\text{O}]_1\) corresponds to the saturated H₂O vapor pressure at the measured leaf temperature (Gaastra 1959).
5. The conductance of water vapor or carbon dioxide into the leaf may be calculated as the reciprocal of the appropriate diffusion resistance,

\[
C_{\text{H}_2\text{O}} = \frac{1}{(r_a + r_s)_{\text{H}_2\text{O}}} \quad \text{(cm sec}^{-1}\text{)}
\]

\[
C_{\text{CO}_2} = \frac{1}{(r'_a + r'_s)_{\text{CO}_2}} \quad \text{(cm sec}^{-1}\text{)}
\]

Conductances can be compared directly with fluxes and potentials (difference between vapor concentration inside and outside the leaf) and were therefore used in graphs in preference to resistances. Thus, when conductivity for \( \text{CO}_2 \) increases \( P_n \) also increases (graphically) whereas when resistance increases \( P_n \) decreases.
VITA

Barbara Louise Rice

Candidate for the Degree of

DOCTOR OF PHILOSOPHY


Major Field: Ecology

Biographical Information:

Personal Data: Born at Dinuba, California, December 19, 1944, daughter of Edwin Rice and Eleanor Rice.

Education: Attended elementary school in Sultana, California; graduated from Dinuba High School in 1962; received Bachelor of Science degree from University of California at Davis, with a major in Biological Sciences, in 1966; received Master of Science degree in Ecology at Utah State University in 1971.

Professional Experience: 1970-1972, Research Assistant, Ecology Center at Utah State University; 1968-1970, Teaching Assistant, Botany Department, Utah State University; 1967-1968, Laboratory Technician, Department of Botany, University of California at Davis, (Paleobotany); 1963-1967, Laboratory Assistant, Department of Botany, University of California at Davis, (Taxonomy); summer of 1966, Research Associate, Department of Botany, University of California at Davis (Ecology).