THE EFFECT OF TEMPERATURE ON ETHYLENE EVOLUTION FROM ETHEPHON TREATED SOUR CHERRY LEAVES (PRUNUS CERASUS L., CV MONTMORENCY) AND FROM BUFFERED ETHEPHON SOLUTIONS

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ABSTRACT

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by

William Charles Olien

The effect of temperature on ethylene evolution from ethephon treated sour cherry (Prunus cerasus L., cv Montmorency) leaves and from buffered ethephon solutions was evaluated. Uniform, fully expanded leaves were treated in the field with 125 μg of ethephon, 2-(chlor-ethyl)phosphonic acid, applied in 5 drops (5 μl each) to the upper leaf surface. After 24 hours, leaves were detached and ethylene evolution was measured after incubation at temperatures between 10 and 34°C. Ethylene evolution was monitored from ethephon solutions of the same dose (125 μg) buffered between pH 2.6 and 7.0 with citrate-dibasic sodium phosphate buffer. The rate of ethylene evolution from treated leaves increased markedly with temperature, with an $E_a$ of about 30 Kcal mole$^{-1}$ ($Q_{10}$:10-20°C of about 7). A comparable temperature effect was observed for buffered ethephon solutions, regardless of pH. Endogenous ethylene is generally evolved over 0 to 30°C with...
an $E_a$ in the range of 14 Kcal mole$^{-1}$ ($Q_{10}: 10\text{°C}-20\text{°C}$ of about 2). The rate of ethylene evolution from ethephon treated leaves was found to be completely and quickly reversible with a reversal in temperature. The rate of ethylene evolution from buffered ethephon solutions at 20$\text{°C}$ increased exponentially with an increase in pH and was not associated with the pK$_a$ values apparent from the titration curve for ethephon.
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by

William Charles Olien

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INTRODUCTION

Cherry Harvest

The cherry industry is important to the economy of Michigan. Michigan is first in the nation in sour cherry production and fourth in sweet cherry production (Michigan Agricultural Statistics, 1975). In 1974, the total cherry crop was valued at roughly 45 million dollars.

Harvest is one of the most expensive operations in cherry production (Childers, 1973). Because of this, shake-and-catch machine harvest was developed in the early 1960's, and the practice was rapidly adopted by the growers (O'Brien, 1969). This resulted in a tremendous increase in efficiency and economy of the sour cherry harvest operation (Childers, 1973). The shaking force required to harvest mature sweet cherries was too great, however, and resulted in a high percentage of bruised fruit and severe damage to the tree (Whittenberger and LaBelle, 1969). For this reason, mechanical harvest was practical only for sour cherry. Damage to sour cherry trees was also apparent. The average life of a sour cherry tree has declined from 30-40 years to 25 years since the advent of shake-and-catch harvest
In addition to tree damage, other limitations are associated with the use of mechanical harvesters. The population of fruit on a tree does not mature uniformly, and machine harvest is not selective for mature fruit. The grower has limited control over fruit maturity and so has a limited capacity to program harvest.

More efficient transfer of the shaking force through the tree, and less bruising of fruit were attained with pruning practices more appropriate to machine harvest (Childers, 1973). The problems of programming harvest, variation in fruit maturity, and the high fruit removal force requiring a shaking energy damaging to the tree still remained (Bukovac, et al., 1971b; Wittenbach and Bukovac, 1972b). Because of strong varietal preferences of the market, and the long period of time required to develop new cultivars, plant breeding does not offer a solution to these problems at this time (Probsting, 1973). The use of plant hormones has provided an alternative solution.

Cherry Fruit Abscission

Removal of mature fruit from the tree is dependent on the development of an abscission layer between the fruit and the pedicel (Bukovac, 1971). Ethylene plays a critical role in the abscission of both sweet and sour cherry (Wittenbach and Bukovac, 1974).
A two-phase abscission process is visualized for sour cherry, similar to that proposed for leaf abscission in bean (Wittenbach and Bukovac, 1973). The development of the sour cherry abscission layer cannot be induced by ethylene much before stage III (final swell) of fruit growth. Induction of the layer probably occurs between Stages II (pit hardening) and III. Ethylene may shorten the duration of the induction phase, possibly affecting IAA levels by stimulation of peroxidase activity, but must be present in the developmental phase for abscission to occur (Wittenbach and Bukovac, 1974). The effect of ethylene is thought to be direct in the abscission layer development. Ethylene appears to promote the activity of hydrolytic enzymes in the cell wall (Bukovac, et al., 1971b), and has been associated with both the synthesis and secretion of cellulase (Abeles, 1973; Wittenbach and Bukovac, 1973).

Abscission in sweet cherry is also accelerated by ethylene in stage III of fruit development (Wittenbach and Bukovac, 1973). The effect seems to be associated more with fruit ripening, however, than with abscission layer formation (Wittenbach and Bukovac, 1972b, 1973). During pericarp expansion and fruit ripening mechanical forces result in cell wall shearing and separation (Bukovac, et al., 1971b; Wittenbach and Bukovac, 1972b), forming an abscission layer as defined by Esau (1965) and modified by Wittenbach and Bukovac (1972a). Cell wall
changes in pectins, non-cellulosic polysaccharides, and cellulose were not noted in sweet cherry (Wittenbach and Bukovac, 1972a, 1972b, 1973).

**Ethephon in Cherry Fruit Abscission**

Because of the important role ethylene plays in the abscission process, means were sought to deliver a controlled dose of ethylene to the abscission zone at the time desired. Direct application of ethylene gas under orchard conditions would be impractical. Many chemicals such as auxin, ABA, cycloheximide, ascorbic acid, and ethylene itself, induce endogenous ethylene evolution (Cooper, et al., 1968; Bukovac, et al., 1969). Other compounds, such as betahydroxyethylhydrazine and ethephon, 2-(chloroethyl)phosphonic acid, release ethylene on degradation (Warner and Leopold, 1967; Morgan, 1972). Of these compounds, ethephon has been most useful (Bukovac, 1971; Morgan, 1972; Abeles, 1973). The chemical structure of this compound is as follows:

\[
\begin{align*}
\text{ClCH}_2 \text{-CH}_2 \text{-P-OH} \\
\text{OH} \\
\text{OH}
\end{align*}
\]

The mechanism of ethephon degradation originally proposed by Maynard and Swan (1963) and later supported by Yang (1969) involves a nucleophilic attack of water or hydroxide ion on the phosphorus moiety with
the concerted elimination of chloride, releasing ethephon as a product. Ethephon is probably nonenzymatically degraded in plant tissue (Dennis, et al., 1970). Rate of ethephon degradation and the corresponding release of ethylene from aqueous solution is strongly affected by pH (Edgerton and Blanpied, 1968; Anonymous, 1969; Bukovac, et al., 1969; Warner and Leopold, 1969). Ethephon appears stable below pH 3.0 or 3.5 and degrades at an increasingly rapid rate with an increase in pH.

Initial testing of ethephon for promotion of cherry fruit abscission indicated that fruit removal force was decreased and that fruit maturity was advanced and more uniform within three days after application of a 500 to 4000 ppm dilute spray (Bukovac, et al., 1969). Maximum effect was noted after six days. In addition, a delay in anthesis the following spring was observed with the higher concentrations (2000 and 4000 ppm).

Ethephon was cleared for commercial cherry production in 1973 and has made mechanical harvest of sweet cherries practical for the first time. Ethephon must be applied in stage III of fruit growth because of the nature of the fruit ripening and abscission processes. Current recommendations for use may be found in the 1976 Fruit Pesticide Handbook and on the current product label. Generally, a 200 ppm dilute spray applied 7 to 10 days before the projected harvest date is
recommended for sour cherry. A dilute application of from 250 to 400 ppm applied 7 to 14 days before harvest effectively loosens sweet cherries.

The development of ethephon as a fruit abscission compound was an important advance in the mechanical harvest of cherries. However, phytotoxicity problems have been noted (Bukovac, et al., 1969) at higher rates (2000 and 4000 ppm) on sweet and sour cherry and plum. These included leaf yellowing, leaf abscission, terminal dieback, gummosis, and enlarged lenticels on current season's wood. In general, these effects were most severe on trees of low vigor and on weak wood in the central part of the tree. Similar effects have been reported by Anderson (1969) and Wilde and Edgerton (1975) for sour cherry, by Bradley, et al. (1969) for apricot, and by Buchanan and Biggs (1969), Proebsting and Mills (1969), Rom and Scott (1971), Stembridge and Gambrell (1971), and Daniell and Wilkinson (1972) for peach.

Phytotoxicity varies among species (Bukovac, et al., 1969). We compared the sensitivity of five tree fruit species in a study of bud expansion, gummosis, internal browning, and ethylene evolution by holding the bases of one-year-old shoots, collected April, 1975 shortly before bud swell, in 0, 50, 100, and 200 ppm ethephon solutions (Appendix 5). Species could be ranked from least to most sensitive as follows: apple (Malus domestica Bork., cv. McIntosh), sour

Phytotoxicity also varies from year to year (Wilde and Edgerton, 1975). Field observation has indicated that temperature is an important factor in the performance of ethephon (1976 Fruit Pesticide Handbook). In the summer of 1973, growers in Michigan experienced severe gummosis on ethephon treated cherry trees, and part of this was attributed to temperatures of 90 to 95°F for several days after ethephon application (Bukovac, personal communication). Gummosis in tulip bulbs is affected by temperature before and during exposure to ethylene (Kamerbeck, et al., 1971). The recommended temperature range for ethephon application is 15.5°C (60°F) to 29.4°C (85°F). The effect of temperature is, of course, continuous through this range. Temperature appears to be important not only during the absorption period, but also for several days thereafter, as most of the compound degrades. While earlier application may be substituted for higher concentration when using ethephon as an aid to cherry harvest (1976 Fruit Pesticide Handbook), temperature must also be considered in determining the dose for a desired response.
Temperature Effect on Rate of Ethylene Evolution

The dependence of reaction rate on temperature is often studied in terms of the Arrhenius equation. The derivation and limitations of this equation are presented in Appendix I. Briefly, the equation describes a line where \( I \), the integration constant, and \( \frac{E_a}{R} \), the slope, are constant parameters over the temperature range investigated.

Equation 1 \[ \ln k = I - \frac{E_a}{R} \frac{1}{T} \]

\( k \) = rate constant for the forward or reverse reaction

\( E_a \) = activation energy for the forward or reverse reaction

\( R \) = ideal gas constant

\( T \) = absolute temperature

The dependence of reaction rate on temperature over a linear portion of such a plot is expressed by \( E_a \), which is directly proportional to the slope. The energy of activation for a single reaction is the energy limit below which reactants can not be converted to products. Individual reactant molecules are distributed over a range of energy values. As heat energy is put into the system, the temperature (mean translational kinetic energy) increases and the portion of reactant molecules with energy above the activation limit increases.
This plot (Equation 1) may be used for homogenous gas reactions, reactions in solution, and heterogeneous processes. Thus, the slope and \( E_a \) may actually reflect several simultaneous reactions. To emphasize this, I will refer to the activation energy derived from the regression of an Arrhenius plot as "\( E_a \)."

Another method of expressing the effect of temperature on reaction rate is \( Q_{10} \). \( Q_{10} \) is defined as the ratio of the reaction rate at \( T + 10^\circ C \) over the reaction rate at \( T^\circ C \). This ratio and various equations used to estimate this ratio are based on \( T \) and not \( T^{-1} \). For this reason, even for a linear Arrhenius plot, the value of \( Q_{10} \) depends on the temperature interval selected. For an arbitrary linear Arrhenius plot (Figure 1), \( E_a \) is constant regardless of temperature, but \( Q_{10} \) is constant only for a given temperature interval. Since \( Q_{10} \) is attempting to indicate the slope of this plot, the predicted rates from the regression equation (Equation 1) are better estimates to use in calculating \( Q_{10} \) than the rates obtained experimentally. Since \( Q_{10} \) is often used in the literature, I will also give the \( Q_{10} \) value derived for a given temperature interval from the regression equation of the Arrhenius plot as \( Q_{10:T_1-T_2^\circ C} \). Experiments can then be compared by reference to either the defined \( Q_{10} \) or \( E_a \).
FIG. 1.--The relationship of $Q_{10}$ and $E_a$.

Note that $Q_{10}$ varies with the temperature interval selected for an arbitrary linear Arrhenius plot.
$Q_{10} : 40-50^\circ C = 5.17$

$Q_{10} : 30-40^\circ C = 5.92$

$Q_{10} : 20-30^\circ C = 7.00$

$Q_{10} : 10-20^\circ C = 7.78$

$E_a = 33.72 \text{ Kcal/mole}$

**Figure 1**
Few investigators have examined the effect of temperature on the rate of endogenous ethylene evolution in higher plants. Only two publications imply an analysis of the data in mentioning $Q_{10}$ (Hansen, 1945; Burg and Thimann, 1959), and a value for $Q_{10}$ is given only by Burg and Thimann (1959). Data for the effect of temperature on endogenous ethylene production, or approximations where necessary were obtained from five papers (Hansen, 1942, 1945; Biale, et al., 1954; Burg and Thimann, 1959; Lougheed and Franklin, 1972) and two unpublished sources (D. C. Coston; M. Saltveit). The experiments involved both fruit (pear, apple, cherimoya, and tomato) and vegetative tissue (subapical etiolated pea sections and tomato foliage). Values calculated for $Q_{10}$:10-20°C varied from 1.49 to 4.37 with a mean of $2.53 \pm 0.78$; values for $E_a$ ranged from 6.5 to 17.4 with a mean of $14.64 \pm 4.83$ Kcal mole$^{-1}$ (Table 1). This information cannot be taken as a critical evaluation of the effect of temperature on endogenous ethylene evolution, but it does indicate that the general trend is in the expected $Q_{10}$ range of 2.

Maximum rates of ethylene production are obtained between 20 and 30°C (Hansen, 1942; Burg and Thimann, 1959; Lougheed and Franklin, 1972). Ethylene evolution was inhibited above this temperature and was completely inhibited at 40°C (Hansen, 1942; Burg and Thimann, 1959). This inhibition is, to some extent, reversible (Burg and
Table 1. Effect of temperature on endogenous ethylene evolution.  

<table>
<thead>
<tr>
<th>Plant, Tissue</th>
<th>$Q_{10}$ (10-20°C)</th>
<th>&quot;E_a&quot; (Kcal mole⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pear, fruit</td>
<td>1.95</td>
<td>11.0</td>
<td>Hansen, 1942</td>
</tr>
<tr>
<td>Cherimoya, fruit</td>
<td>1.49</td>
<td>6.5</td>
<td>Biale, et al., 1954</td>
</tr>
<tr>
<td>Apple, fruit</td>
<td>2.86</td>
<td>17.4</td>
<td>Hansen, 1945</td>
</tr>
<tr>
<td>Apple, fruit</td>
<td>4.37</td>
<td>24.4</td>
<td>Hansen, 1945</td>
</tr>
<tr>
<td>Apple, fruit</td>
<td>2.82</td>
<td>17.1</td>
<td>Hansen, 1945</td>
</tr>
<tr>
<td>Apple, fruit</td>
<td>2.24</td>
<td>13.4</td>
<td>Burg &amp; Thimann, 1959</td>
</tr>
<tr>
<td>Apple, fruit</td>
<td>2.65</td>
<td>16.0</td>
<td>Coston, 1976 (unpublished)</td>
</tr>
<tr>
<td>Tomato, fruit</td>
<td>2.75</td>
<td>16.6</td>
<td>Lougheed &amp; Franklin, 1972</td>
</tr>
<tr>
<td>Tomato, foliage</td>
<td>2.08</td>
<td>12.0</td>
<td>Lougheed &amp; Franklin, 1972</td>
</tr>
<tr>
<td>Peas, subapical section</td>
<td>2.09</td>
<td>11.8</td>
<td>Saltveit, 1976 (unpublished)</td>
</tr>
<tr>
<td>mean</td>
<td>$2.53 \pm 0.78$</td>
<td>$14.6 \pm 4.8$</td>
<td></td>
</tr>
</tbody>
</table>

1 Calculations were made from the reported rates of endogenous ethylene evolution at various temperatures.
Thimann, 1959; Burg, 1962). The lower temperature limit for endogenous ethylene evolution has not been investigated, but Hansen (1942, 1945) showed ethylene evolution in pear and apple at 0°C. Ethylene evolution from Bosc pear was enhanced at room temperature by storage for the preceding week at 5 or 10°C (Sfakiotakis and Dilley, 1971). The effect was not as pronounced when pretreated at 0°C. Freeze induced ethylene was detected in tender orange leaves subsequently monitored at room temperature (Young and Meredith, 1971).

Little work has been done on the effect of temperature on the rate of ethephon degradation. Lougheed and Franklin (1972) studied this relationship using ethephon treated tomato fruit and foliage under both air and N₂ atmospheres. Calculated values based on their data are summarized in Table 2. Ethylene was measured 24 (fruit) or 4 (foliage) hours after ethephon application.

A number of complicating factors limit interpretation of these data. 1) A uniform temperature was not maintained during the absorption period. 2) Control and treated foliage were held in the same growth chambers, leading the authors to suggest that ethylene from the treated foliage may have stimulated ethylene production by the control foliage. 3) Selection of fruit at the same physiological state with respect to the climacteric was reportedly very difficult. 4) The treatment was replicated only two times. 5) Fruit held
Table 2. Effect of temperature on rate of ethylene evolution from control and ethephon treated tomato tissue.¹

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>$Q_{10}$ (10-20°C)</th>
<th>&quot;E_a&quot; (Kcal mole⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>Control, air</td>
<td>2.75</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Ethephon, air</td>
<td>3.00</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>Δ ethephon &amp; control², air</td>
<td>14.73</td>
<td>44.2</td>
</tr>
<tr>
<td></td>
<td>Ethephon, $N_2$</td>
<td>2.14</td>
<td>12.5</td>
</tr>
<tr>
<td>Foliage</td>
<td>Control, air</td>
<td>2.08</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Ethephon, air</td>
<td>13.87</td>
<td>43.2</td>
</tr>
<tr>
<td></td>
<td>Δ ethephon &amp; control, air</td>
<td>15.56</td>
<td>45.1</td>
</tr>
<tr>
<td></td>
<td>Ethephon, $N_2$</td>
<td>Treatment not performed</td>
<td></td>
</tr>
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</table>

¹Data recalculated from Lougheed and Franklin (1972).

²The rate of ethylene evolution from control tissue was subtracted from the rate for ethephon treated tissue at each temperature and the difference was analyzed as an Arrhenius plot.
in N₂ may not be comparable to those held in air. Temperature responses similar to those estimated for endogenous ethylene production (Table 1) were observed for control and ethephon treated fruit under both N₂ and air (Table 2). The ethylene evolved under N₂ was assumed to result only from ethephon degradation (Lougheed and Franklin, 1970). The difference between the ethylene evolved from control and ethephon treated fruit in air at 10 and 20°C (0.01 and 0.62 µl Kg⁻¹ hr⁻¹) represents the ethylene evolution resulting from ethephon degradation and any additional endogenous ethylene induced by the ethephon. However this difference is actually smaller than the value observed for treated fruit under N₂ at 10 and 21°C (0.51 and 1.19 µl Kg⁻¹ hr⁻¹) and raises the question of adequacy of the experimental method. Unfortunately, a similar comparison cannot be made for tomato foliage because ethephon treated foliage was not examined under N₂.

Recognizing the above limitations, some comparison of the values in Table 2 are of interest. Because the amount of ethephon absorbed was not controlled, probably only the relative magnitude of the values in Table 2 is meaningful. The Q₁₀:10-20°C for endogenous ethylene evolution from tomato fruits and foliage in air is between 2 and 3 (Table 1). This might be assumed to hold for both natural and induced ethylene evolution since the same mechanism of synthesis has been reported for both (Abeles and Abeles, 1972). Therefore, the
high $Q_{10}$ values reported in Table 2 must be due to ethephon degradation.

The $Q_{10}$ values for both control fruit and foliage were typical for endogenous ethylene evolution (Table 1). The values obtained by taking the difference of the rates of ethylene evolution for control and ethephon treated fruit in air at each temperature for both fruit and foliage have $Q_{10}$ values suggesting that most of the ethylene evolved was derived from ethephon degradation. This also appears to be true for ethephon-treated foliage in air, but ethephon treated fruit in air has a $Q_{10}$ in the range of endogenous production. Ethephon degradation must, therefore, be contributing little to the total ethylene evolved. This may be due to poor absorption of the ethephon through the thick fruit cuticle, characterized as 2-6 μm thick (Bukovac, et al., 1971a). Unfortunately, ethephon treated foliage was not studied under N$_2$ so that it might be compared with ethephon treated fruit under similar conditions. No explanation is offered for the apparent contradiction of the low $E_a$ for ethephon treated fruit under N$_2$ and the much greater $E_a$ for theΔ ethephon treated and control fruit in air.
Summary and Statement of Problem

Ethephon has become indispensible to the efficiency, economy, and programing of cherry harvest. It has made mechanical harvest of sweet cherries practical for the first time. Ethylene is involved in fruit ripening, fruit and leaf abscission, lenticel swelling, gummosis, and terminal dieback. Many environmental factors affect endogenous ethylene production as well as the sensitivity of the tree to ethylene. Temperature affects both ethylene biosynthesis and the degradation of ethephon to ethylene. Field observation has indicated that intensity of certain undesirable (e.g., gummosis) effects of ethephon may be associated with high temperature. Little detailed work has been done on the effect of temperature on the rate of ethylene evolution from ethephon or from ethephon treated tissue. This study focuses on the effect of temperature on ethylene evolution from ethephon treated leaf and shoot tissues and from solutions of ethephon in an effort to better understand the limitations of ethephon as an agricultural chemical.
METHODS AND MATERIALS

General Procedure

The rate of ethylene evolution from leaf and shoot tissues and buffered ethephon solutions was studied in sealed glass test tubes positioned vertically in controlled temperature water baths maintained at ± 0.5 degrees of the desired temperature. The tubes were sealed with rubber serum caps at two minute intervals so that the atmosphere could be sampled at a uniform time interval from the start of ethylene accumulation. One ml samples of the atmosphere in the tube were assayed for ethylene by gas chromatography using a Packard gas chromatograph (series 7300). A1O3 was used as the solid phase and N2 as the gas phase (80 cc min⁻¹) in a glass column (120 x 0.2 cm, i.d.). The injection port temperature was maintained at 130°C, the column at 50°C, and the flame (H2 and air) ionizing detector at 180°C. Data were quantified by comparing peak heights to those of ethylene standards of known concentration. Ethylene was measured either as total accumulation over a designated time period or as a time course with periodic determinations over a designated time interval. In time course studies, one ml of air was injected
into the sample tube so that the pressure in the tube would not be affected by repeated sampling. Mean rate data for ethylene evolution were transformed (log) to obtain homogeneous variance between treatments for statistical analysis. Individual values, rather than means, were used for all analyses of Arrhenius plots.

**Experiments with Leaves of Sour Cherry**

Ethephon (125 μg) was applied as 5 droplets (5000 ppm, w/v) of 5 μl each onto the upper surface of uniform damage-free leaves borne on the median portion of current season's shoots of vigorous sour cherry (*Prunus cerasus* L., cv. Montmorency) trees growing at the Michigan State University Horticulture Research Center. This dose was approximately equivalent to a 400 ppm dilute spray if 0.3 ml of spray solution were retained by a leaf. The droplets were delivered with a 50 μl syringe along the length of the leaf within one cm either side of the midvein and directly over secondary veins. Generally, after a 24 hour absorption period, the shoots with treated leaves were collected and transported to the laboratory. Leaves with about 1.5 cm petiole attached were positioned with the petiole in 2 ml of water in 75 ml test tubes. Each tube contained 2 overlapping leaves with the adaxial leaf surface oriented toward
the outside. The average gas space in the tube was 72 ml. Temperature was maintained by holding tubes in water baths. Ethylene was accumulated for no more than one hour at a time. If tubes were maintained in the water baths and not sealed, a porous stainless steel pad was inserted in the tube to just above the leaves, making sure of good wall contact. This pad acted as a thermostat, allowing good air exchange but preventing a temperature gradient from developing in the tube (Olien, 1971). Since treated leaves may evolve ethylene endogenously, as well as by ethephon degradation, the rate of ethylene evolution was corrected for leaf fresh weight and expressed as pmole g\(^{-1}\) time\(^{-1}\) assuming the ideal gas law. The average leaf fresh weight was 0.8 ± 0.16 g.

A. Effect of temperature on rate of ethylene evolution from ethephon treated sour cherry leaves.

In the first experiment, leaves were collected 20 hours after ethephon application and placed at 10, 18, 26, or 34°C for 4 hours. Ethylene was accumulated over the fourth hour of incubation and the final ethylene concentration was determined for 12 replications.

The rate of ethylene evolution was more critically determined in a second experiment measuring accumulation periodically during the initial hour of incubation at 18 or 33°C with 8 replications. Leaves were collected 22 hours after ethephon application.
B. **Effect of change in temperature on rate of ethylene evolution from ethephon treated leaves.**

Leaves in the first experiment were collected from the field 20 hours after ethephon application and incubated at 10, 18, 26, or 34°C for an initial 4 hours and then transferred to 26°C for an additional 4 hours. Final ethylene accumulation was determined over the fourth hour after transfer with 12 replications.

In a second experiment, leaves were collected 22 hours after ethephon treatment. Ethylene evolution was determined by time course over the initial hour of 4 sequential 2-hour time courses beginning at 22, 24, 26, and 28 hours after ethephon application. Temperature treatments were as follows: 1) constant 18°C, 2) constant 33°C, 3) alternating 18/33°C for periods of 2 hours, 4) alternating 33/18°C for periods of 2 hours. The effect of temperature on ethylene evolution could then be compared over all four temperature treatments for each time course. These treatments were replicated 4 times.

C. **Effect of temperature on rate of ethylene evolution from ethephon treated leaves over several days.**

Leaves were collected 24 hours after ethephon treatment and held at 18 or 33°C for 70 hours. The average field temperature during
the 24 hour preincubation period was 20°C. The rate of ethylene evolution was determined at varying intervals from 2 to 20 hours. The rate over the initial 24 hours was estimated from the Arrhenius plot in Figure 5, Line a. The experiment was replicated 2 times. A first approximation of reaction order, assuming all the ethylene evolved resulted from ethephon degradation, was determined for leaves after initiation of the 33°C treatment, by testing goodness of linear fit for the following plots:

<table>
<thead>
<tr>
<th>reaction order</th>
<th>equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>zero</td>
<td>(C = C_0 - kt)</td>
</tr>
<tr>
<td>first</td>
<td>(\ln C = \ln C_0 - kt)</td>
</tr>
<tr>
<td>second</td>
<td>(C^{-1} = C_0^{-1} + kt)</td>
</tr>
<tr>
<td>third</td>
<td>(C^{-2} = C_0^{-2} + 2kt)</td>
</tr>
</tbody>
</table>

These are the integral form of the equations defining reaction order. Here \(C\) = concentration of ethephon, \(C_0\) = initial concentration of ethephon, \(k\) = rate constant, and \(t\) = time.

**Effect of Temperature and Previous Ethephon Treatment on Rate of Ethylene Evolution from Quiescent Sweet Cherry Shoots**

First year shoots were collected before bud swell on April 25, 1976 from control and ethephon treated (300 ppm at 250 gal. acre\(^{-1}\))
sweet cherry trees (*Prunus avium* L., cv. Napoleon) in an orchard near Traverse City, Michigan used in a study on fruit abscission in July, 1975. The rate of ethylene evolution was determined for three replications of both control and ethephon treated shoots at 20, 30, and 40°C. The terminal 13 cm of the shoot was sealed in a 27 ml test tube for 9 hours with samples taken at 1, 3, and 9 hours. The rate of ethylene accumulation was expressed as pmoles g⁻¹ min⁻¹.

**Experiments with Ethephon Solutions**

Ethephon solutions, buffered with 1 ml of citrate (0.1 M) - dibasic sodium phosphate (0.2 M) buffer, were prepared in 27 ml test tubes. Buffer of the appropriate pH was equilibrated in the tubes at the desired temperature for 10 minutes, after which 25 μl of ethephon at 5000 ppm (125 μg), as was applied to leaves, was introduced into each tube with a 50 μl syringe and the tube was sealed. The data were collected as time course measurements, except for the titration study.
A. Effect of temperature on the rate of ethylene evolution from ethephon solutions buffered at pH 6.0.

Solutions of ethephon buffered at pH 6.0, were monitored at 10, 20, 30, and 40°C by the methods outlined above, except that 0.17 ml of 0.01N KOH was added to neutralize the ethephon present. This was later found to be unnecessary. The concentration of ethephon was 104.6 ppm (7.24 x 10^{-4} M) and the gas space over this solution was 26.1 ml. Two of nine replications were performed with sterile solutions to determine the possible effect of microorganisms. In addition, one replication each was examined at pH 5.0 and 7.0. Rate of ethylene evolution was expressed as pmole min^{-1} assuming the ideal gas law.

B. Effect of temperature and pH on the rate of ethylene evolution from buffered ethephon solutions.

Ethephon solutions were buffered at pH 3.0, 3.5, 4.0, 4.5, and 5.0 and held at 20, 30, 40, and 50°C. No KOH was added to the solutions. The concentration of ethephon in each tube was 122.0 ppm (8.44 x 10^{-4} M) and the gas space over this solution was 26.3 ml. The experiment was replicated once.
C. Effect of pH on rate of ethylene evolution from buffered solutions held at 20°C.

Rate of ethylene evolution from ethephon (125 µg) solutions buffered over a range of pH values in 75 ml tubes was examined at 20°C in two experiments. In the first experiment, 5 ml buffer was used in each tube and the rate of ethylene evolution was determined from the final accumulation over 43.6 hours for one replicate each at pH 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, and 5.0. In the second experiment, 1 ml buffer was used in each tube, and the rate of ethylene evolution was determined from the final accumulation over 45.25 hours for one replicate each at pH 4.0, 5.0, 6.0, and 7.0.

D. Titration curve for ethephon.

A titration curve for 100 ml of a 0.01 N ethephon solution titrated with 0.1N NaOH was determined with two replications. The first replication was titrated in 0.2 ml steps. The second replication was titrated in 1.0 ml steps and required less time for the titration to end point. The pH was monitored with a Beckman pH meter (Model Century SS) with a reference and a glass electrode. NaOH was delivered from a 100 ml burette and the solution was gently agitated with a magnetic stirrer at 22°C.
E. Effect of ethephon concentration on rate of ethylene evolution.

Ethephon solutions of 24.4, 12.2, 2.44, 1.22, and 0.244 ppm buffered at pH 6.0 were prepared in 1 ml buffer. The rate of ethylene evolution was determined by time course at 20°C with one replication. A first approximation of reaction order was then obtained by the degree of linear fit for the differential form of the equations defining reaction order in terms of concentration and rate.

<table>
<thead>
<tr>
<th>order</th>
<th>equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>zero</td>
<td>rate = -k[ethephon]^0</td>
</tr>
<tr>
<td>first</td>
<td>rate = -k[ethephon]^1</td>
</tr>
<tr>
<td>second</td>
<td>rate = -k[ethephon]^2</td>
</tr>
<tr>
<td>third</td>
<td>rate = -k[ethephon]^3</td>
</tr>
</tbody>
</table>
RESULTS

Effect of Temperature on Rate of Ethylene Evolution from Ethephon Treated Sour Cherry Leaves

The rate of ethylene evolution increased markedly from sour cherry leaves treated with 125 μg ethephon. The first experiment (Table 3) indicated an $E_a$ of about 30 Kcal mole$^{-1}$ ($Q_{10:10-20^\circ C} = 6.3$). The coefficient of determination for this linear regression was 86.2%, but the error in the slope was only 5.9% of the slope value, indicating greater variability between leaves at a given temperature than the response across temperatures. An analysis of the overall Arrhenius plot for the second experiment (Table 4) gave results similar to the first experiment, with an $E_a$ of about 32 Kcal mole$^{-1}$ ($Q_{10:10-20^\circ C} = 7.1$). The coefficient of determination was 92.4%, and the error in the slope was 7.7% of the slope value. The results of these two experiments can be compared visually in Figure 2.
Table 3. Effect of temperature on rate of ethylene evolution from ethephon treated leaves, first experiment.1

Ethylene accumulation was determined over the fourth hour of temperature treatment, 24 hours after ethephon application.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ethylene evolution (pmole g(^{-1}) min(^{-1}))(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.88(^{a}) ± 1.30</td>
</tr>
<tr>
<td>18</td>
<td>24.3(^{b}) ± 23.3</td>
</tr>
<tr>
<td>26</td>
<td>83.2(^{c}) ± 47.1</td>
</tr>
<tr>
<td>34</td>
<td>179(^{d}) ± 82</td>
</tr>
</tbody>
</table>

\(^{1}\)An "E" of 30.1 ± 1.78 Kcal mole\(^{-1}\) and Q\(_{10}\): 10-20°C = 6.26 was indicated by the overall Arrhenius plot of 48 observations with an error in the slope of 5.9% and \(r^2 = 86.2\%\).

\(^{2}\)Data are means of 12 replications.

\(^{3}\)Mean separation at P = 0.05 by Duncan's Multiple Range Test on the log transformed data.
Table 4. Effect of temperature on rate of ethylene evolution from ethephon treated leaves, second experiment.¹

Evolution rate was determined over a one hour time course 22 hours after ethephon application with an average $r^2$ of 98.4%.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ethylene evolution (pmole g$^{-1}$ min$^{-1}$)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>24.9a$^3$ ± 9.2</td>
</tr>
<tr>
<td>33</td>
<td>385b ± 187</td>
</tr>
</tbody>
</table>

¹An "E" of 32.1 ± 2.45 Kcal mole$^{-1}$ and $Q_{10:10-20°C} = 7.05$ was indicated by the overall Arrhenius plot of 16 observations with an error in the slope of 7.7% and $r^2 = 92.4%$.

²Data are means of 8 replications.

³Mean separation at $P = 0.05$ by a t test on the log transformed data.
FIG. 2.--Temperature effect on rate of ethylene evolution from buffered ethephon solutions, control tissues, and ethephon treated tissues.

Line a: Ethephon treated leaves, first experiment (Table 3)
Line b: Ethephon treated leaves, second experiment (Table 4)
Line c: Ethephon buffered at pH 6.0 (Table 9)
Line d: Endogenous production from control sweet cherry shoot tissue (Table 8).
In Rate (In p mole min⁻¹)

Temperature

- Ethephon treated leaves
- Ethephon buffered at pH 6
- Endogenous, shoot tissue

Figure 2
Effect of Change in Temperature on Rate of Ethylene Evolution from Etaphon Treated Leaves

The initial incubation temperature did not significantly (P = 0.05) affect rate of ethylene evolution during the fourth hour after transfer to 26°C (Table 5).

Table 5. Rate of ethylene evolution at 26°C from etaphon treated leaves as affected by preliminary temperature treatment.

Leaves were collected 24 hours after etaphon application and exposed to 10, 18, 26, and 34°C for a 4 hour period. At the end of this time, all leaves were transferred to 26°C and final ethylene accumulation was measured over the fourth hour after transfer.

<table>
<thead>
<tr>
<th>Temperature treatment (°C)</th>
<th>Ethylene evolution (pmole g⁻¹ min⁻¹)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 → 26</td>
<td>87.0a² ± 34.0</td>
</tr>
<tr>
<td>18 → 26</td>
<td>73.4a ± 38.9</td>
</tr>
<tr>
<td>26 → 26</td>
<td>72.0a ± 46.9</td>
</tr>
<tr>
<td>34 → 26</td>
<td>55.6a ± 23.6</td>
</tr>
</tbody>
</table>

¹Data are means of 12 replications.

²Mean separation at P = 0.05 by Duncan's Multiple Range Test on the log transformed data.
The rate of ethylene evolution was independent of previous temperature exposure over three sequential temperature reversals between 18 and 33°C, as compared to controls at 18 and 33°C (Table 6). Furthermore, rate of evolution changed in less than 10 minutes following the change in temperature. All time course regressions indicated a good linear fit (average $r^2 = 98.4\%$).

**Effect of Temperature on Rate of Ethylene Evolution from Ethephon Treated Leaves Over Several Days**

The rate of ethylene evolution from leaves held at 18°C remained fairly stable throughout the experiment (Table 7). Total estimated ethylene evolution during 94 hours accounts for only 29% of the total possible had all the ethephon applied been degraded. The rate of evolution from leaves held at 33°C declined with time, falling to the range for leaves held at 18°C after 30 hours (Table 7). Total estimated ethylene evolution accounted for 46% of that possible from the ethephon applied. The data for leaves at 33°C fit all but the zero order reaction plots with $P = 0.01$. Only the second and third order plots are linear with $P = 0.001$, and the correlation coefficient was higher for the third order ($r = 0.973$) than for the second order plot ($r = 0.962$).
Table 6. Effect of constant vs alternating temperatures on rate of ethylene evolution from ethephon treated sour cherry leaves.

Rates were determined from the regression of time course measurements collected over the first hour of temperature incubation with an average $r^2$ of 98.4%. Temperature reversals were made 10 minutes preceding each time course.

<table>
<thead>
<tr>
<th>Time Course</th>
<th>Time (hr)</th>
<th>Ethylene Evolution (pmoles g$^{-1}$ min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Constant 18°C</td>
</tr>
<tr>
<td>1</td>
<td>22 - 23</td>
<td>0.375a + 0.0154</td>
</tr>
<tr>
<td>2</td>
<td>24 - 25</td>
<td>0.380a + 0.133</td>
</tr>
<tr>
<td>3</td>
<td>26 - 27</td>
<td>0.380a + 0.084</td>
</tr>
<tr>
<td>4</td>
<td>28 - 29</td>
<td>0.343a + 0.107</td>
</tr>
</tbody>
</table>

1. Time from ethephon application.

2. Data are means of four replications.

3. Mean separation at $P = 0.05$ by Duncan's Multiple Range Test on the log transformed data.
Table 7. Effect of temperature and time on ethylene evolution from ethephon treated leaves.

Leaves collected 24 hours after field application of ethephon and maintained after that time at 18 or 33°C. The average temperature during the 24 hour preincubation period was 20°C.

<table>
<thead>
<tr>
<th>Time after Treatment (hours)</th>
<th>Temp.(°C)</th>
<th>Mean¹ rate C₂H₄ ev. (pmole g⁻¹ min⁻¹)</th>
<th>Total C₂H₄ ev. (n mole g⁻¹)</th>
<th>% of total possible from ethephon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 24</td>
<td>20</td>
<td>42.1</td>
<td>60.7</td>
<td>7</td>
</tr>
<tr>
<td>24 - 34</td>
<td>33</td>
<td>232</td>
<td>198</td>
<td>23</td>
</tr>
<tr>
<td>34 - 44</td>
<td>33</td>
<td>133</td>
<td>277</td>
<td>32</td>
</tr>
<tr>
<td>44 - 54</td>
<td>33</td>
<td>79.7</td>
<td>325</td>
<td>38</td>
</tr>
<tr>
<td>54 - 64</td>
<td>33</td>
<td>45.1</td>
<td>352</td>
<td>41</td>
</tr>
<tr>
<td>64 - 74</td>
<td>33</td>
<td>33.2</td>
<td>372</td>
<td>43</td>
</tr>
<tr>
<td>74 - 94</td>
<td>33</td>
<td>24.6</td>
<td>401</td>
<td>46</td>
</tr>
</tbody>
</table>

| 0 - 24                      | 20        | 42.1                                   | 60.7                       | 7                                     |
| 24 - 29                     | 18        | 31.4                                   | 70.6                       | 8                                     |
| 29 - 34                     | 18        | 38.4                                   | 82.1                       | 9                                     |
| 34 - 54                     | 18        | 44.7                                   | 136                        | 16                                    |
| 54 - 94                     | 18        | 47.5                                   | 250                        | 29                                    |

¹Data are means of 2 replications.
Effect of Temperature and Previous Ethephon Treatment on Rate of Ethylene Evolution from Quiescent Sweet Cherry Shoots

Both control and ethephon treated shoot tissue (Table 8) had an "Ea" in the range common for endogenous ethylene evolution (Table 1). "Ea" was about 9 Kcal mole\(^{-1}\) (Q\(_{10}:10\text{°C}-20\text{°C} = 1.75\)) for controls and about 14 Kcal mole\(^{-1}\) (Q\(_{10}:10\text{°C}-20\text{°C} = 2.41\)) for ethephon treated shoots (Table 8, Figure 2). These values were significantly different from each other at P = 0.025.

Effect of Temperature on the Rate of Ethylene Evolution from Ethephon Solutions Buffered at pH 6.0

The effect of temperature on ethylene evolution from ethephon buffered at pH 6.0 was similar to that observed for leaves treated with the same dose (Table 9, Figure 2). No deviation was observed with the sterile solutions from the nonsterilized replications. The overall "Ea" was 32 Kcal mole\(^{-1}\) (Q\(_{10}:10\text{°C}-20\text{°C} = 7.0\)). The coefficient of determination for this regression was 98.9% and the error in the slope was 1.8% of the slope value. The Arrhenius slopes for the single replication at pH 5.0 and pH 7.0 were not significantly different from each other or from the overall Arrhenius slope for pH 6.0 even at P = 0.50.
Table 8. Effect of temperature on rate of ethylene evolution from 'Napoleon' sweet cherry shoot tissue collected before bud swell.

Napoleon sweet cherry trees sprayed dilute with 300 ppm ethephon in July, 1975. Quiescent control and treated first year shoot tissue collected April, 1976.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ethylene evolution (pmole g⁻¹ min⁻¹)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ppm²</td>
</tr>
<tr>
<td>20</td>
<td>0.156 ± 0.002</td>
</tr>
<tr>
<td>30</td>
<td>0.305 ± 0.025</td>
</tr>
<tr>
<td>40</td>
<td>0.433 ± 0.093</td>
</tr>
</tbody>
</table>

¹Data are means of 3 replications.

²An "Eₐ" of 9.18 ± 1.13 Kcal mole⁻¹ and Q₁₀:10-20°C = 1.75 was indicated by the overall regression for control tissue of 9 observations with an error in the slope of 12.3% and r² = 90.5%.

³An "Eₐ" of 14.4 ± 1.59 Kcal mole⁻¹ and Q₁₀:10-20°C = 2.41 was indicated by the overall regression for treated tissue of 9 observations with an error in the slope of 11.0% and r² = 92.1%.
Table 9. Effect of temperature on rate of ethylene evolution from ethephon solutions buffered at pH 6.0.¹

Rate of evolution from $7.24 \times 10^{-4}$ M ethephon solutions (125 µg ethephon) buffered at pH 6.0 determined by time course with an average $r^2$ of 98.9%.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ethylene evolution (pmole min⁻¹)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>$3.85a^3 \pm 0.73$</td>
</tr>
<tr>
<td>20</td>
<td>$16.5b \pm 2.07$</td>
</tr>
<tr>
<td>30</td>
<td>$132c \pm 6$</td>
</tr>
<tr>
<td>40</td>
<td>$817d \pm 68$</td>
</tr>
</tbody>
</table>

¹An $E_a$ of $31.9 \pm 0.58$ Kcal mole⁻¹ and $Q_{10}:10-20^\circ C = 6.99$ was indicated by the overall Arrhenius plot of 36 observations with an error in the slope of 1.8% and $r^2 = 98.9%$.

²Data are means of 9 replications.

³Mean separation at $P = 0.05$ by Duncan's Multiple Range Test on the log transformed data.
The Effect of Temperature and pH on the Rate of Ethylene Evolution from Buffered Ethephon Solutions

The energy of activation does not appear to be affected by pH. The Arrhenius slopes for pH 3.0, 3.5, 4.0, 4.5, and 5.0 were not significantly different even at $P = 0.20$ (Table 10). Furthermore, the slope of the Arrhenius plot over all five pH values ($E_a = 35\text{ Kcal mole}^{-1}$, $Q_{10} = 8.5$) was not significantly different from the overall Arrhenius slope for pH 6.0 at $P = 0.50$.

Effect of pH on Rate of Ethylene Evolution from Buffered Ethephon Solutions Held at 20°C.

The rate of ethylene evolution from buffered ethephon solutions increases exponentially with increase in pH (Figure 3). Degradation of ethephon at 20°C was not detectable below pH 3.0.

Titration Curve for Ethephon

Two pKa's are evident for ethephon (Figure 4) at about pH 2.5 and 7.0. The end point for the titration with the glass electrode used was at pH 11. The pKa's for phosphorous acid are at pH 2.15 and 4.7 (25°C), and for phosphoric acid are at pH 1.96, 7.12,
Table 10. Effect of temperature and pH on the rate of ethylene evolution from buffered ethephon solutions.\(^1\)\(^2\)

Rate of evolution from 8.44 x 10\(^{-4}\) M buffered ethephon solutions (125 µg ethephon) determined by time course with an average \(r^2\) of 99.4%.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ethylene evolution (pmole min(^{-1}))(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 3.0</td>
</tr>
<tr>
<td>20</td>
<td>0.0211(^1)</td>
</tr>
<tr>
<td>30</td>
<td>0.179</td>
</tr>
<tr>
<td>40</td>
<td>0.876</td>
</tr>
<tr>
<td>50</td>
<td>8.32</td>
</tr>
</tbody>
</table>

\(^1\)An "E" of 35.0 ± 6.07 Kcal mole\(^{-1}\) and \(Q_{10}\):10-20°C = 8.45 was indicated by the overall Arrhenius plot of 20 observations with an error in the slope of 17.3% and \(r^2 = 64.9\%\).

\(^2\)The Arrhenius slopes for each pH are not significantly different at \(P = 0.20\).

\(^3\)Data are of one replication.
and 12.32 (18°C) (The Handbook of Physics and Chemistry, 1953). The rate of ethylene evolution does not show a dependence on proton dissociation from ethephon (Figures 3, 4).

The Effect of Ethephon Concentration on Rate of Ethylene Evolution

The rate of ethylene evolution from ethephon solutions buffered at pH 6.0 increased with ethephon concentration (Table 11). The data significantly fit a linear plot for a first order reaction at P = 0.001, for a second order reaction at P = 0.01, and for a third order reaction at P = 0.05. The linear correlation for a zero order reaction plot was not significant at P = 0.50.

Table 11. Effect of ethephon concentration on rate of ethylene evolution.

Ethephon buffered at pH 6.0 and held at 20°C. Rates determined by time courses with an average $r^2$ of 91.5%. Preliminary experiment consisting of one replication.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Ethylene evolution (pmole min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.4</td>
<td>2.24</td>
</tr>
<tr>
<td>12.2</td>
<td>1.03</td>
</tr>
<tr>
<td>2.44</td>
<td>0.211</td>
</tr>
<tr>
<td>1.22</td>
<td>0.0936</td>
</tr>
<tr>
<td>0.244</td>
<td>0.0399</td>
</tr>
</tbody>
</table>
FIG. 3.—The effect of pH on rate of ethylene evolution from buffered solutions of ethephon held at 20°C.
Figure 3
FIG. 4.--Titration curve for 100 ml 0.01N ethephon titrated with 0.1N NaOH.
Figure 46
DISCUSSION

The effect of temperature on rate of ethylene evolution from ethephon and from ethephon treated sour cherry leaves was much greater than expected. It is a general rule for a reaction with activation limits that the rate of reaction approximately doubles for a 10°C rise in temperature ($Q_{10} = 2$). A $Q_{10}$ of 2 calculated between 10 and 20°C is equivalent to an $E_a$ of 11.4 Kcal mole$^{-1}$. Ethephon solutions and ethephon treated leaves evolved ethylene with an $E_a$ of about 30 Kcal mole$^{-1}$ (Figure 2), while nontreated sweet cherry shoot tissue collected before bud swell (Figure 2) evolved ethylene with an $E_a$ of 9.2 Kcal mole$^{-1}$. Published data indicate that endogenous ethylene was evolved within the expected $E_a$ range regardless of species or tissue (Table 1). Why the rate of ethylene evolution from buffered ethephon solutions and from ethephon treated leaves is so responsive to temperature is not explained by this study. The response is not due to pH differences (Table 10) nor to differential uptake of the compound by leaves. A defined dose of ethephon was applied to each leaf and was followed with an absorption period in the field.
With the present data, we can predict what the limits on $E_a$ might be for ethephon treated tissue (Figure 5). The ethylene evolved from ethephon treated tissue may arise from degradation of ethephon, endogenous biosynthesis, or both. If all the ethylene were endogenously produced, we could expect an $E_a$ of $14.6 \pm 4.8$ Kcal mole$^{-1}$ (Figure 5, line b). If all of the ethylene were derived from ethephon degradation, we could expect an $E_a$ of $31.6 \pm 0.9$ Kcal mole$^{-1}$ (Figure 5, line a). If half the ethylene came from one source and half from the other, the $E_a$ would be expected to fall half way between these limits, at 23 Kcal mole$^{-1}$. The Arrhenius slopes of the data from two leaf experiments (Tables 3, 4), the experiment with ethephon buffered at pH 6.0 (Table 9), and the experiment examining the effect of pH on $E_a$ (Table 10) were found not to differ significantly. Therefore, essentially all of the ethylene evolved from leaves treated with ethephon, in the manner specified, must be derived from the degradation of ethephon. This conclusion is supported by the estimated values for total ethylene evolution during 94 hours from ethephon treated leaves (Table 7). The total ethylene accumulated after 24 hours at 20°C and an additional 70 hours at 33°C accounted for only 46% of the total ethylene possible if 100% of the ethephon applied had degraded. The total ethylene accumulated after 24 hours at 20°C and an additional 70 hours at 18°C accounted for only 29% of the total possible.
FIG. 5.—Estimated limits on the Arrhenius slope for rate of ethylene evolution from ethephon treated tissue.

Line a: Ethephon degradation (estimate of upper limit on Arrhenius slope for ethephon treated tissue). Overall regression of data from Tables 3, 4, 9. 
\[ \hat{Y} = 50.5 - 15900X \quad r^2 = 0.920 \]

Line b: Endogenous ethylene evolution (estimate of lower limit on Arrhenius slope for ethephon treated tissue).

From Table 1.

Slope = 7350 \pm 2420 deg

Intercept with line a is arbitrary.
In Rate (in pmole min⁻¹)

Ethephon Degradation

Endogenous Ethylene Evolution

Temperature

Figure 5
The opposite situation to that of the treated leaves might explain the data for control and treated sweet cherry shoots (Table 9) collected 9 months after ethephon treatment. The $E_a$ for control shoots was about 9 Kcal mole$^{-1}$ and the $E_a$ for the treated shoots was about 14 Kcal mole$^{-1}$, and the slopes were significantly different at $P = 0.025$. These data suggest that enough ethephon remained to affect the ethylene evolution response to temperature.

The best estimate of the upper limit for the $E_a$ of ethephon treated tissue (Figure 5, line a) was obtained by combining the data from the two leaf experiments (Tables 3, 4) and the experiment with ethephon buffered at pH 6.0 (Table 9). The experiment examining the effect of pH on $E_a$ (Table 10) was excluded because of excessive error. The overall regression of these 100 observations gives an $E_a$ of $31.6 \pm 0.9$ Kcal mole$^{-1}$ ($Q_{10}: 10-20^\circ C = 6.9$) with a $r^2$ of 92.0%.

The best estimate of the lower limit on $E_a$ (Figure 5, line b) for ethephon treated tissue where all the ethylene evolved is endogenously produced was derived from the recalculated rate data surveyed from the literature (Table 1). Some of this is not given in units that can be converted to pmole g$^{-1}$ min$^{-1}$. For this reason the plot was arbitrarily made to pass through the same point at 10°C as was determined for line a, to aid in visually comparing slopes.

The rate of ethylene evolution from ethephon treated leaves shifted rapidly and reversibly with change in temperature. The first
experiment (Table 5) indicated that the effect of temperature was reversible, but not how fast or how often. A second experiment (Table 6) demonstrated that the effect of temperature was completely reversible over several temperature reversals between 18 and 33°C. The change in rate occurred within 10 minutes. The \( r^2 \) values calculated after each temperature reversal were very high and similar to the \( r^2 \) values for the control leaves (Table 6).

The rate of ethephon degradation increases exponentially with increase in pH (Figure 3). This increase is independent of the regions of proton dissociation apparent from the titration curve for ethephon (Figure 4). This would indicate that, at least at pH 3.0 and above, rate is not controlled by the degree of proton dissociation. Degradation of ethephon at 20°C was not detectable below pH 3.0.

A first approximation of reaction order for the degradation of ethephon indicated a first order reaction in solution but some higher order reaction when applied to leaves. The data, especially that from leaves, are preliminary, but these results could be explained by additional factors becoming limiting in leaves. Limiting factors in leaves but not in solution might be availability of the nucleophyllic attacker referred to by Yang (1969) or the binding of ethephon with plant components. There is evidence that ethephon can
bind to sucrose (Lavee and Martin, 1971) and with sweet cherry fruit cell wall components (Edgerton and Hatch, 1972). Whether or not the reaction order for ethephon degradation differs between solutions and treated leaves, the effect of temperature on ethylene evolution has been shown to be the same in both cases for a dose of 125 µg ethephon.
CONCLUSIONS

The rate of ethephon degradation increases markedly with temperature for leaves treated with 125 μg ethephon and for solutions of ethephon at pH 3 and above. This temperature dependence can be expressed quantitatively by an "E_a" of about 30 Kcal mole⁻¹ (Q_{10:10-20°C} = 6.9). Why this value is so high is not known, but the fact itself is very important to the use of ethephon in agriculture. The degree that the rate of ethylene evolution is affected by temperature must depend on the ratio between ethylene derived from ethephon degradation and that produced endogenously. This ratio will depend on many factors, including the dose of ethephon applied and the capacity of the tissue for biosynthesis of ethylene. Absorption of ethephon may also be an important factor, and this is probably controlled by temperature, moisture, cuticle characteristics, etc. For these reasons, the temperature at the time of ethephon application in the orchard and for several days afterward is an important factor in determining response. This may, in part, explain the widespread ethephon induced phytotoxicity which was associated with the high temperatures of the 1973 cherry harvest season.
In many cases, the temperature effect on ethephon treated tissue does not appear to limit the usefulness of the compound. In cases where temperature may limit utility, the does applied must be adjusted in accordance with the predicted temperature over some number of days from ethephon application. Temperatures that are too low or too high may prevent the use of ethephon. Although not economically practical at this time, a potential method of avoiding the effects of high temperatures on sour cherry might be the use of evaporative cooling. This should be effective because of the rapid decline in the rate of ethylene evolution from ethephon treated tissue with a decline in temperature. Where temperature is a chronic problem, the use of some other existing compound or the development of a new one might be necessary, so that the effect of temperature on treated tissue is not so great.
APPENDIX A

THE ARRHENIUS EQUATION

For an isothermal reversible reaction that involves no radiant or electrical work, the change in Gibbs free energy is expressed by:

$$\Delta G = \Delta H - T \Delta S$$

- $\Delta G$ = Gibbs free energy
- $\Delta H$ = enthalpy
- $T$ = absolute temperature
- $\Delta S$ = entropy

The standard Gibbs free energy is defined by the activity of the reactants being equal to the activity of the product, so:

$$\Delta G = \Delta G^\circ = -RT \ln K$$

- $\Delta G^\circ$ = standard Gibbs free energy
- $R$ = ideal gas constant
- $K = \frac{\text{activity of products}}{\text{activity of reactants}}$

Over temperature intervals that are small in terms of absolute temperature, $H$ and $S$ are assumed to be constant. This approximation can generally be made for reactions involving no electrical or radiant work terms and not involving phase changes. When the last equation is differentiated with respect to temperature, we obtain:

$$\frac{d \ln K}{dT} = \frac{\Delta H}{R} T^{-2}$$
This equation can be partitioned into the forward and reverse reactions.

\[ \Delta H = E_a^f - E_a^r \]

\[ E_a = \text{activation energy for the forward or reverse reaction} \]

\[ \ln k = \ln \frac{k_f}{k_r} \]

\[ k = \text{rate constant for the forward or reverse reaction} \]

\[ \frac{d \ln k_f}{dT} - \frac{d \ln k_r}{dT} = \frac{E_a^f}{RT^2} - \frac{E_a^r}{RT^2} \]

\[ \frac{d}{dT} \ln K = \frac{E_a}{RT^2} \]

Integrating over indefinite temperature limits gives the Arrhenius equation.

\[ \ln K = I - \frac{E_a}{RT} - 1 \]

\[ I = \text{integration constant} \]

Arrhenius arrived at this equation empirically. Glasstone (1946) states that, "The Arrhenius equation is widely applicable not only to homogenous gas reactions, but also to reactions in solution and to heterogeneous processes. It frequently fails, however, for chain reactions."
APPENDIX B

DETERMINATION OF ETHEPHON DOSE PER LEAF
FOR ADEQUATE ETHYLENE EVOLUTION

A. Procedure

Five or 20 drops of ethephon solutions (0, 1000, or 5000 ppm) were applied to leaves in the field. Nontreated leaves were also included in the test. Five leaf samples were collected 25 hours after treatment and ethylene was accumulated in 265 ml jars fitted with serum caps. The experiment was repeated two times.

B. Results

<table>
<thead>
<tr>
<th>Chemical (ppm)</th>
<th>Drops per Leaf</th>
<th>Mean Ethylene Evolution (nl g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no treatment</td>
<td>0</td>
<td>0.747</td>
</tr>
<tr>
<td>0₁</td>
<td>5</td>
<td>0.853</td>
</tr>
<tr>
<td>0₁</td>
<td>20</td>
<td>1.02</td>
</tr>
<tr>
<td>1000</td>
<td>5</td>
<td>6.62</td>
</tr>
<tr>
<td>1000</td>
<td>20</td>
<td>12.5</td>
</tr>
<tr>
<td>5000</td>
<td>5</td>
<td>40.1</td>
</tr>
<tr>
<td>5000</td>
<td>20</td>
<td>421.</td>
</tr>
</tbody>
</table>

₁Rep I only.

C. Conclusions

Ethephon will be applied as a 5000 ppm solution in 5 drops (125 μg) per leaf. This is a convenient dose to apply and gives
an easily measured response at 24 hours. This dose is equivalent to a 400 ppm dilute spray if 0.3 ml is retained by the leaf.
APPENDIX C
ETHEPHON LEAF PHYTOTOXICITY

A. Procedure

Five drops of 0, 1000, 5000, and 10000 ppm ethephon, and pure (2.40 x 10^8 ppm) Ethrel (Amchem Products, Ambler, Penn.) were placed on detached sour cherry leaves arranged on the lab bench. Each treatment was replicated with five leaves. Observations for leaf injury were made over several days.

B. Results

No caustic effects were noted until the second day. Severe phytotoxicity was noted only for pure Ethrel, and none at the other treatments.

C. Conclusions

Five drops of a 5000 ppm ethephon solution should not be caustic to sour cherry leaves.
APPENDIX D

DETERMINATION OF THE AMOUNT OF ETHYLENE SOLUBLE IN
THE WATER PRESENT DURING ETHYLENE ACCUMULATION

Abeles (1973) gives the absorption coefficient of ethylene at
1 atm in water at 25°C as $\alpha = 0.108$ (v/v).

Ethylene was accumulated in tubes containing 2 ml of water and
two leaves with an average combined weight of 1.6 g. Assuming these
leaves are 75% water, then we have a total of 3.2 ml water present.
This means that at 25°C, 0.3456 ml of ethylene at 1 atm would be solu-
ble in the water present. Assuming the ideal gas law, this is equal
to $1.413 \times 10^{-5}$ moles ethylene.

A concentration of 0.1 ppm ethylene is near the lower range of
what can be measured. The partial pressure of ethylene at this con-
centration is $10^{-7}$ atm in a tube with total pressure of 1 atm. At
this pressure, $1.413 \times 10^{-12}$ moles are soluble in the water present at
25°C. There are $2.945 \times 10^{-10}$ moles of ethylene present at 25°C and
0.1 ppm in a gas space of 72 ml.

This means that for a tube with 3.2 ml of water and 72 ml gas
space at 25°C, 1 atm total pressure, and 0.1 ppm ethylene, 0.48% of
the ethylene is soluble in the water present. For this reason,
solubility of the gas can be considered a negligible error in the ethylene evolution studies made with this system.
APPENDIX E

RELATIVE SENSITIVITY OF QUIESCENT SHOOT TISSUE TO ETHEPHON AMONG SEVERAL TREE FRUIT SPECIES

A. Procedure

First year wood was collected at the MSU Horticulture Farm from apple (*Malus domestica* Bork., cv. McIntosh), sour cherry (*Prunus cerasus* L., cv. Montmorency), sweet cherry (*Prunus avium* L., cv. Windsor), plum (*Prunus domestica* L., cv. Stanley), and peach (*Prunus persica* L., cv. Redhaven). Napoleon sweet cherry twigs from Oregon were also available. These twigs were more vigorous than those collected at the MSU Hort Farm. The terminal 23 cm were placed in 50 ml solutions of 0, 50, 100, and 200 ppm ethephon on April 10, 1975. Visual gumming and bud expansion ratings were made 7 days later and at termination of the experiment (at 11 days). Also at termination, browning of the wood was rated, increase in mean bud fresh weight over the initial mean bud weight was determined, and the nl/g ethylene accumulated over one hour for the terminal 13 cm was determined at room temperature. All treatments were replicated three times.
B. Results

Peach was extremely sensitive to ethephon. All peach shoots except the control were killed very shortly after being placed in any concentration of ethephon and became dehydrated. They did not gum and showed little internal browning. No bud expansion or increase in bud weight occurred except in the controls. Ethylene was evolved at the control level at all concentrations of ethephon.

Napoleon sweet cherry showed a more marked inhibition of bud expansion, increase in bud fresh weight, and browning than any of the other tree fruit wood tested. Less gumming was observed, however, than occurred for either Windsor sweet cherry or Stanley plum.

The remaining species having similar vigor and remaining alive through the experiment can be described together. Apple, Windsor sweet cherry, sour cherry, and plum all showed similar inhibition of bud weight and expansion with an increase in ethephon concentration. Little additional effect was observed above 50 ppm. Gumming was most severe in plum, less so in Windsor sweet cherry, and absent in sour cherry and apple. No gumming was observed below 100 ppm.

The ethylene accumulation data were most useful for ranking the varieties in sensitivity to ethephon. Generally, these fell into two groups. The sweet cherries and plum were very responsive to ethephon. The response began to fall off above 100 ppm. Sour cherry, peach, and
apple were relatively unresponsive. Peach was killed at 50 ppm and so was not included in this comparison. The slopes of the ethephon concentration vs. ethylene evolution plots were determined and compared. Schematically, the results of this comparison can be shown as follows:

(Apple) (Sour Cherry) (Win. Sw. Ch.) (Plum) (Nap. Sw. Ch.)

The lines indicate slopes that were not significantly different at $P = 0.05$.

C. Conclusions

Summarizing all of the above observations, the five tree fruit species tested might be ranked from lowest to highest ethephon sensitivity as follows: McIntosh apple, Montmorency sour cherry, Windsor sweet cherry, Stanley plum, and Redhaven peach. It is not known where Napoleon sweet cherry would fall if it had been collected from the same area as the other wood.
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LIST OF REFERENCES


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