Table 4. Resistance to CO\textsubscript{2} diffusion from the fruit\textsuperscript{2}.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Respiration rate (ml CO\textsubscript{2}/kg·hr)</th>
<th>Internal CO\textsubscript{2} concn (%)</th>
<th>Resistance to CO\textsubscript{2} diffusion\textsuperscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satsuma mandarin</td>
<td>21.0</td>
<td>6.8</td>
<td>0.32\textsuperscript{x}</td>
</tr>
<tr>
<td>Navel orange</td>
<td>20.3</td>
<td>4.2</td>
<td>0.21\textsuperscript{b}</td>
</tr>
<tr>
<td>Eureka lemon</td>
<td>16.2</td>
<td>3.2</td>
<td>0.20</td>
</tr>
<tr>
<td>Hassaku</td>
<td>21.5</td>
<td>2.7</td>
<td>0.13\textsuperscript{c}</td>
</tr>
<tr>
<td>Natsudaiai</td>
<td>23.2</td>
<td>2.8</td>
<td>0.12\textsuperscript{c}</td>
</tr>
<tr>
<td>Trovita orange</td>
<td>21.6</td>
<td>1.8</td>
<td>0.08\textsuperscript{d}</td>
</tr>
<tr>
<td>Valencia orange</td>
<td>23.9</td>
<td>1.8</td>
<td>0.08\textsuperscript{d}</td>
</tr>
</tbody>
</table>

\textsuperscript{2}Samples consisted of 7 fruit each with 3 replications.

\textsuperscript{3}Resistance to CO\textsubscript{2} diffusion = Internal CO\textsubscript{2} concn/Respiration rate.

\textsuperscript{x}Mean separation by Duncan’s multiple range test, 5% level.

The main site of resistance to gas diffusion from orange is in the flavedo, according to Ben-Yehoshua (1). Satsuma flavedo is not as thick as that of ‘Hassaku’ or ‘Natsudaiai’. Resistance to gas diffusion among citrus cultivars will be investigated in the future.

\textsuperscript{1}Received for publication May 13, 1977. Technical Paper No. 4554, Oregon Agricultural Experiment Station.

\textsuperscript{2}This research was supported in part by Contract No. CPA-130 from the Division of Ecological Effects Research of the Environmental Protection Agency.

\textsuperscript{3}Department of Statistics, Oregon State University, Corvallis, OR 97331.


**Response of Sweet Cherry Leaf Tissue to Hydrogen Fluoride Fumigation at Different Nitrogen Levels\textsuperscript{1}**

T. J. Facteau, S. Y. Wang, and K. E. Rowe\textsuperscript{3}

Mid-Columbia Experiment Station, Oregon State University, Hood River, OR 97031

Additional index words. air pollution, *Prunus avium*

**Abstract.** Foliar uptake of fluoride (F) resulting from hydrogen fluoride (HF) fumigations was linear with dose (concentrations of F in \( \mu \)g/m\(^3\) \times duration of exposure in hours) at F concentrations lower than 17.5 \( \mu \)g/m\(^3\). Above this level, duration of exposure was the only important factor and uptake was non-linear with time. Higher leaf N levels resulted in greater F uptake. Production of CO\textsubscript{2} was increased more by high F concentration for short periods than by low concentration for longer periods where leaf N was optimal or supraoptimal. Amino nitrogen (AN) levels increased more at low F concentration for longer periods than high concentration for shorter periods. The response patterns were similar at optimal and supraoptimal leaf N. Protein nitrogen (PN) decreased with increasing ln HF dose at optimal and very low leaf N levels. Changes in PN and AN were significantly correlated in leaf tissue with optimal, but not in tissues with deficient or supraoptimal N.

Our objectives were to investigate F accumulation rates and possible changes in CO\textsubscript{2} production, PN and AN in sweet cherry leaf tissue as related to fumigation with HF and leaf N content.

**Materials and Methods**

One-year-old ‘Napoleon’ and ‘Bing’ sweet cherry trees were planted in the spring of 1969 in 18.9 liter metal containers and grown in a lath house. Randomly selected normal trees were used all 4 years (1969–1972) for HF fumigation during July and August but different trees were used each year. Fumigations all 4 years were carried out in 2 mylar paneled chambers, 1.07 x 1.07 x 2.44 m, with an additional chamber as a control. Table 1 gives an outline of fumigation duration, F concn, and analyses conducted. The N application rates that resulted in differential N levels are also listed. Generally 2 trees of each cultivar in 1969 and 1970, and 2 ‘Napoleon’ trees of different N levels in 1971 and 1972 were fumigated at each fumigation.

---

**Literature Cited**


---

Table 1. Outline of fumigation duration, HF concn, and analysis of amino-N (AN), protein-N (PN), F, N, and CO₂ production in 'Napoleon' sweet cherry leaf tissue.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fertilizer (g/tree)</th>
<th>HF concn² (μgF/m³)</th>
<th>Time² (hr)</th>
<th>Analysis</th>
<th>CO₂ (mg/g fresh wt/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1969</td>
<td>20</td>
<td>1.0 - 27.3</td>
<td>4 - 48</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>1970</td>
<td>40</td>
<td>1.3 - 26.8</td>
<td>4 - 48</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>1971</td>
<td>0</td>
<td>0.4 - 26.7</td>
<td>4 - 48</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>1972</td>
<td>0</td>
<td>0.1 - 19.7</td>
<td>5 - 59</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>1972</td>
<td>40</td>
<td>0.1 - 19.7</td>
<td>5 - 59</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

²Actual concn in μgF/m³ for each year were: 1969, 1.0, 1.2, 1.6, 2.2, 2.3, 3.3, 3.9, 4.4, 4.8, 5.0, 10.0, 17.5, 21.2, and 27.3; 1970, 1.2, 24.5, and 26.8; 1971, 0.4, 0.5, 0.7, 1.0, 2.9, 6.9, 9.7, 13.7, 16.4, 17.7, and 26.7; 1972, 0.1, 0.3, 0.5, 0.7, 2.5, 5.0, 10.4, 11.9, 12.5, 13.1, 14.0, 19.7.

Actual fumigation durations in hours were: 1969 to 1971, 4, 8, 24, 48; and 1972, 5, 7, 20, 24, 26, 32, 48, 59.

duration and concn. HF was introduced into the incoming air stream by the method of Hill et al. (14) except that a heated, insulated box was used instead of a water bath. This system consisted of metering air through hydrofluoric acid then into the chambers. Air was forced from the bottom of the chambers through a Teflon film plenum at a rate to change the air twice per min. In 1969, air F concn were sampled with a Greenburg-Smith impinger by passing air through 0.001 n NaOH at 1 m³/hr. The other 3 years, air F levels were monitored by using NaOH impregnated filter papers (21), which were changed whenever leaf samples were taken, but never longer than 24 hr. Fluoride analyses were done all years by the semiautomated microdistillation method (10).

Leaf samples consisting of 3 to 5 leaves taken from below the terminal on each new shoot were analyzed for CO₂ production, PN, AN, F, and N levels. Subsamples consisting of 10 mature leaves were ground for 3 min in a Lourdes Omnimixer in 80% ETOH, boiled for 1 min, then filtered through Whatman No. 1 filter paper. The filtrates were adjusted to 100 ml with 80% ETOH, and aliquots taken for AN analysis. Protein N was measured in the dried alcohol-insoluble residue by the method of Lowry et al. (15). Amino N was measured according to Yemm and Cocking (25). Leaf F levels were determined according to Cralley et al. (10) using leaves washed in 0.05% (wt/vol) alconox and 0.05% (ethylene dinitrilo) tetraacetic acid, disodium salt. Total N was measured on a Technicon Auto-CO₂ analyzer (12). Carbon dioxide evolution rates were determined on a weighed 4-leaf subsample. Petioles were placed in water in small glass vials and sealed with florist’s putty. The vials were then placed in aluminum foil-covered pint Mason jars and air was passed through at 200 ml/min. After 5 hr equilibration at 20°C, CO₂ was monitored with a Beckman Model 215A infrared analyzer. All data were analyzed using linear regression techniques to express the effects of HF fumigations of varying concen for various durations. Measurements taken on unfumigated trees were not included in any of the regression analyses.

However, careful attention was paid to the agreement between the mean of unfumigated observations and the constant terms (predictions at 0 dose) of each of the regression equations.

Results and Discussion

F accumulation. Fumigation with HF resulted in increased leaf F content of both ‘Napoleon’ and ‘Bing’ sweet cherry leaf tissue. For concn less than 17.5 μgF/m³, the increase in leaf F content was found to be very highly significantly (P<0.1%)
related to dose (Table 2). At concn greater than 17.5 $\mu$gF/m$^3$, the response to fumigation did not appear to be a function of dose, but more a function of the duration of fumigation irrespective of the HF concn (Fig. 1). Effects of different concn (in the range 17.5 to 27.3 $\mu$gF/m$^3$) were statistically non-significant. The division at a concn of 17.5 is admittedly arbitrary, but all data do suggest some change in the effect of fumigation at or near this level.

The increase in leaf F level for each unit increase in dose was more than 3 times as large in 1970 as in 1969 (Table 2). At that point there seemed no obvious reason except that more fertilizer had been applied in 1970 than in 1969 (40 g and 20 g NH4NO3/tree, respectively). This was reflected in leaf N levels (Table 2). In 1971 and 1972 two N fertilization regimes (0 and 40 g) were superimposed on the fumigations. Results from these years are consistent with, but not as dramatic as, the difference between 1969 and 1970 (Table 2).

There were no visible symptoms of injury until 2 to 3 days after completion of the fumigations and then only on the high HF dose trees that received optimal or supraoptimal levels of N. Young, newly emerging leaves were severely burned at the tips. The no-N trees showed no symptoms and had already set a terminal bud. Brennan et al. (5) found that moderate levels of N, Ca, and P enhanced uptake of toxic amounts of F whereas low or deficient nutrient levels decreased uptake of F. Others reported that N had no effect on F uptake (2). Our data indicate that F uptake was enhanced by high leaf N and that low N sweet cherry leaves developed no tip burn on new leaves at the high HF doses used in this study.

Trees receiving N in 1972 had an average leaf N content of 4.7% (Table 2). A few weeks after fumigation were over, severe dieback of new shoots was found on all plus N trees, whether fumigated or not. Apparently NH4NO3, at the rates used to give 4.7% leaf N, was toxic.

Uptake of F by sweet cherry has been observed to be linear using total accumulated $\mu$gF/m$^3$ over a 100-day period (8). The specific linear relation to dose that we have found may not be appropriate for expressing accumulation under long-term orchard conditions, for reasons suggested by MacLean and Schneider (16). They found differences in F accumulation in timothy and red clover between continuous and intermittent fumigations with the same HF dose and suggested that factors such as growth dilution, weathering, non-continuous fumigations, and accumulation rates precluded extrapolation of experimentally derived data to field conditions. However, field responses under short-term fumigations would probably be similar to our experimentally derived relationship.

$CO_2$ production. Fumigations with HF increased $CO_2$ production from ‘Napoleon’ and ‘Bing’ sweet cherry tissue in all 4 years. The increase could be shown to be related to dose, but in 1969 and 1972 there were enough data to effectively examine the separate effects of concn and duration. Analysis shows that dose made up of a high concn for short duration caused a greater increase in $CO_2$ production than that same dose made up of a lower concn over longer duration. This is shown as a response surface (Fig. 2). This figure represents the average level of $CO_2$ production in the 2 years. $CO_2$ production was higher in 1969 than in 1972, but the form of the response to varying concn and durations was very similar, therefore the 2 years were combined in Fig. 2. Differences between years are also reflected in the constant terms of the prediction equations as follows:

1969 $CO_2$ production = 2.21 + 0.031 concn + 0.00028 dose
1972 $CO_2$ production = 1.49 + 0.031 concn + 0.00028 dose

The constant terms from the $CO_2$ production equations above and from the combined model in Fig. 2 compare favorably with the actual values obtained these years at 0 dose (Table 3). This suggests the form of the HF response is as described in Fig. 2 because the 0 dose values were not used in the analysis.

Table 3. Average fluoride, nitrogen, amino-N (AN), protein-N (PN), and $CO_2$ production levels of non-HF treated sweet cherry leaf tissue.

<table>
<thead>
<tr>
<th>Year</th>
<th>N fertilizer (g/tree)</th>
<th>Leaf N (%)</th>
<th>Amino-N (mg/g fresh wt)</th>
<th>Protein-N (mg/g dry wt)</th>
<th>$CO_2$ production (mg/g fresh wt per hr)</th>
<th>F content (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1969</td>
<td>20</td>
<td>2.1</td>
<td>5.3 ± 0.1$^y$</td>
<td>36.2 ± 0.6</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>1970</td>
<td>40</td>
<td>2.6</td>
<td>17.0 ± 0.2</td>
<td>98.1 ± 0.7</td>
<td>2.7 ± 0.1</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>1971</td>
<td>0</td>
<td>0.7 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.3</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>1972</td>
<td>0</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>43.3 ± 2.1</td>
<td>1.3 ± 0.1</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>1972</td>
<td>40</td>
<td>4.7 ± 0.2</td>
<td>14.7 ± 0.5</td>
<td>54.5 ± 1.9</td>
<td>1.5 ± 0.1</td>
<td>4.2 ± 0.3</td>
</tr>
</tbody>
</table>

$^y$Values for 1969 and 1970 include ‘Napoleon’ and ‘Bing’. Values for 1971 and 1972 are for ‘Napoleon’ only.

Fig. 3. Response plot of amino nitrogen levels in 'Napoleon' sweet cherry leaf tissue as affected by varying durations of exposure and concn of HF. a) 1969, mg AN/g fresh wt = 5.38 + 0.036 duration + 0.0018 dose HF, N = 20, r² = 0.67; b) 1972, mg AN/g fresh wt (plus N treatment) = 14.75 + 0.027 duration + 0.0067 dose HF, N = 49, r² = 0.36.

of the data yet the prediction values are close. The consistency between the 2 years suggests that the pattern of CO₂ production during HF fumigation was not affected by the range of leaf N levels in this study.

Fluoride has been shown both to reduce (18) and to increase respiration (2, 3, 19). Hill et al. (13) concluded that fluoride caused changes in respiration rates of plants only when there was visible damage. Others (2, 3, 19), however, found that bush beans and gladiolus had accelerated O₂ uptake when exposed to HF at concn below those causing visible foliar symptoms. Our data indicate that HF can cause an enhancement of CO₂ production from sweet cherry leaf tissue in the absence of any foliar symptoms. There is some evidence that levels of HF have been high enough to be in the range that could result in enhancement of CO₂ production (9) under actual sweet cherry growing conditions even considering that those reported levels may be only 50 to 60% gaseous F (11). The immediate or long-term responses of cherries to an increase of CO₂ production is not known. Measurements in 1969, one week after fumigations, indicated that CO₂ production was different from the non-fumigated trees at concn of 1.0, 3.3, and 3.9 μgF/m³, but averaged 21% greater where the F concn had been 17.5 and 21.4. These high HF levels, however, are not commonly found in ambient conditions (8, 9, 11).

**AN and PN.** Fumigation of HF resulted in changes in AN levels in sweet cherry leaf tissue. In general, AN level increased with increased doses of HF in 1969 and 1972. However, increases were greater at doses consisting of long duration at low concn than the same dose made up of shorter duration and higher concn. Statistically significant interactions were found between years and levels of fumigation. Results are shown in Fig. 3a for 1969 and Fig. 3b for 1972 (trees receiving 40 g NH₄NO₃). The general pattern of response is not particularly dissimilar and the interactions may be due in particular to other year-to-year differences. The range of durations was much longer in 1972 than in 1969 (Table 1) and the level of AN in unfumigated trees was nearly 3 times as high in 1972 as in 1969 (Table 3).

Increasing levels of fumigations resulted in changes in PN levels where leaf N was considered to be optimum (1969). The decrease in PN was found to be most linearly related when fumigation was expressed in the form:

\[ PN = 36.2 - 0.94 \ln (dose + 1), r^2 = 0.29, P<1\% \]

Where leaf N levels were supraoptimal (1972), the response to HF fumigation was not statistically significant, but PN did decrease as the dose increased. Where leaf N levels were low in 1972, PN significantly decreased as \( \ln (dose + 1) \) HF increased.

\[ PN = 46.6 - 2.00 \ln (dose + 1), r^2 = 0.32, P<1\% \]

Thus, it appears that HF fumigations did decrease PN over a wide range of leaf N levels and that leaf N levels influence the magnitude of the effect of the HF fumigations. The amounts of AN and PN were significantly correlated only where the leaf N values were optimum in 1969. The following equation expresses the decrease in AN as PN increases.

\[ AN = 18.1 - 0.34 PN, r^2 = 0.42, P<0.1\% \]

Amino-N values decreased as PN increased in the high-N trees in 1972, but no such trend was observed in the no-N trees that same year. The changes in AN and PN with HF suggest that F may either accelerate protein breakdown or decrease protein synthesis in sweet cherry leaf tissue. Fluoride has been postulated to possibly influence protein synthesis either by its action on DNA or by changing ratios of nucleotide species (7). It has also been suggested that growth rates are controlled by the rate of protein synthesis (20). Changes in PN levels might lead ultimately to changes in vegetative growth of sweet cherry. Elevated leaf F levels have been correlated with reduced terminal growth (11) but there is no direct evidence to show an involvement of F in sweet cherry growth.

We have no explanation for the different response of CO₂ production, AN and PN to varying times of exposure and pollutant concn. It is believed that high concn for short periods should have more of a general effect on plants than low concn for longer periods (22). Sweet cherry CO₂ production in response to F fumigation followed this pattern but changes in AN and PN did not.
Literature Cited


