Analysis of Reduced Photosynthesis in the Apple Leaf under Sink-limited Conditions Due to Girdling

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On the 6th day after girdling, the photosynthesis rate of leaves on the 3-year-old stem, 2-year-old stem and new shoot of a branch of a field-grown 4-year-old apple tree decreased. The starch content in the leaves, bark and wood significantly increased, especially in shoot leaves. In the girdled branch, concomitant with the photosynthesis reduction, on the 6th day after girdling, the stomatal conductance of leaves on the 3-year-old stem, 2-year-old stem and new shoot declined. Under this condition, a significant correlation between the photosynthesis rate and stomatal conductance could be recognized. On the other hand, with girdling, the activity of ribulose-1,5-bisphosphate carboxylase (RuBPcase) per leaf area decreased on the basis of the decrease in RuBPcase activity per unit enzyme protein. The initial activity of RuBPcase per leaf area in shoot leaves decreased. Moreover, the activity of RuBPcase per leaf area significantly correlated with the photosynthesis rate. Based on these results, it is suggested that the decrease in the photosynthesis rate of the sink-limited branch of a young apple tree 6 days after girdling is mainly caused by closing of the stomatal aperture and reduction of RuBPcase activity per leaf area.

Key Words: apple tree, photosynthesis rate, ribulose-1,5-bisphosphate carboxylase (RuBPcase) activity, starch content, stomatal conductance.

Introduction

The photosynthesis capacity of leaves is known to vary due to differences in the variety, rootstock, shoot and leaf age of apple trees (Barden, 1978; Schechter et al., 1991; Wang et al., 1999). In mature apple trees, the leaf is roughly classified into two groups, shoot leaf (on long shoots) and spur leaf (on spurs). Moreover, these two groups are distinctly different not only in size and weight, but also in structure and function. A fully grown shoot leaf has a more highly-advanced palisade parenchyma, higher chlorophyll content and better light absorption efficiency than the spur leaf, and in addition, stoma density on the epidermis of the abaxial side of the shoot leaf is higher, and the intercellular space is larger; therefore, the photosynthesis rate of shoot leaves is high (Ghosh, 1973; Schechter et al., 1992). On one hand, in mature apple trees, the source organ is made up of these two kinds of leaves; on the other hand, the sink organ, including the long photoassimilation transport system, developing the leaf, stalk, root, and so on, exists concurrently. Consequently, the photosynthesis source-sink relationship in perennial woody fruit trees is much more complicated than in annual plants.

Surgical treatment, such as girdling and scoring, is effective for inducing flower bud formation and fruit setting, and increasing yield and fruit quality. It was found that surgical treatment did not have a significant influence on leaf photosynthesis for moderate fruit bearing (Di Vaio et al., 2001; Iglesias et al., 2002; Nii, 1992; Schechter et al., 1994). On the other hand, for extremely low fruit bearing or no fruit, surgical treatment induced both starch accumulation and photosynthetic reduction in leaves of the apple (Nii, 1989; Schechter et al., 1994), peach (Jordan and Habib, 1996; Nii, 1992), nectarine (Di Vaio et al., 2001), grape (Caspari et al., 1998; Roper and Williams, 1989), citrus (Iglesias et al., 2002; Li et al., 2003; Schaffer et al., 1986), avocado (Davie et al., 1995; Schaffer et al., 1987), and kiwifruit (Piller et al., 1998).

As for the decrease in the photosynthesis rate with girdling in grapes, it is theorized that it originated from reduced carboxylation efficiency (Roper and Williams, 1989). In addition, due to girdling, excessive starch accumulates in the leaves of the evergreen avocado tree; consequently, the photosynthesis rate declines, namely,
feedback inhibition of photosynthesis occurs following girdling (Schaffer et al., 1987). Furthermore, from study of the apple, a deciduous fruit tree, it is reported that when the branch of a no-fruit mature tree is girdled, the photosynthesis rate decreases, starch accumulates in the leaves, stomatal conductance simultaneously declines, and intercellular CO₂ increases (Schechter et al., 1994); however, in these studies, the activity and content of ribulose-1,5-bisphosphate carboxylase (RuBPcase) protein were not examined. Therefore, study concerning the regulation mechanism of photosynthesis inhibition due to surgical treatment of mature apple trees is still needed.

In this experiment, a branch of 4-year-old apple trees was girdled in the field and its influence on photosynthesis was examined. In the leaves of the sink-limited branch due to girdling, the corresponding relationships between the decrease in the photosynthesis rate and stomatal opening, RuBPcase activity, protein content of that enzyme, soluble sugar and starch contents were examined.

Materials and Methods

Plant material and treatment

Two similar 3-year-old branches of a 4-year-old, field-grown ‘Fuji’ apple tree were chosen. With regard to each of these two branches, 4 spur leaves on the 3-year-old stem, 4 spur leaves on the 2-year-old stem and 4 shoot leaves were randomly selected, and all of these leaves were fully grown. The photosynthesis rate, stomatal conductance and intercellular CO₂ in the above leaves were simultaneously measured on 19 September, 2002. Immediately after the measurement, one branch was girdled (girdling), while the other was not (control). A ring of bark (about 1 cm in width) around the branch at the base was removed using a sharp knife. On the 6th day following girdling treatment, 25 September, the above photosynthesis parameters in the same leaves as those on 19 September were measured again. Thereafter, the leaves, wood and bark of the girdled and control branches were collected.

Measurement of photosynthesis rate, stomatal conductance and intercellular CO₂

The photosynthesis parameters were measured using a portable photosynthesis measurement system (CIRAS-1, Koito Industrial Corporation, Tokyo, Japan). The measurement was carried out under the following conditions: photosynthetically active radiation on the leaf surface, 1 mmol·m⁻²·s⁻¹; CO₂ inside leaf chamber, 350 µL·L⁻¹; air flow rate, 200 mL·min⁻¹; temperature, 25°C; and relative humidity, 60%.

Measurement of leaf carbohydrate content

After measurement of the photosynthesis parameters, leaf discs (1.79 cm² per leaf disc) on each side of the main vein were punctured using a leaf puncher and immediately dropped into liquid nitrogen, and stored at −80°C until use. The leaf extract from the frozen leaf discs and the supernatant from the extract were prepared as described by Sawada et al. (2001). The supernatants of the soluble sugar and starch were completely dehydrated using a Centrifugal Dehydrator (EYELA, CVE-100D TYPE, Tokyo Science Equipment Corporation, Tokyo, Japan), and then re-dissolved with ultrapure water. HPLC analysis solutions for sucrose, sorbitol, glucose, fructose and starch were prepared by the method of Sawada et al. (2001). Starch concentration was expressed in glucose units.

Assays of RuBPcase activity

The procedures for preparation of the extract from leaf discs stored at −80°C and of the supernatant from the extract were those of Makino et al. (1988). RuBPcase activity in the supernatant was assayed at 25°C in a medium containing 100 mM Bicine at pH 8.2, 5 mM MgCl₂, 20 mM NaHCO₃, 5 mM creatin phosphate, 1 mM ATP-2Na, 0.1 mM NADH, 0.3 mM RuBP, 10 units of phosphocreatine kinase, 10 units of glyceraldehyde 3-phosphate dehydrogenase, and 10 units of phosphoglycerate kinase, as described by Sawada et al. (2001). Enzymatic activities were revised for the decrease in absorbance at 340 nm in the control assay medium prepared without ribulose bisphosphate. The “initial” activity which showed at RuBPcase activity almost existed in leaves was measured after the addition of 50 µL of the supernatant to 1950 µL of the assay medium. RuBPcase was activated for 20 min at 0°C in 10 mM MgCl₂ and 10 mM NaHCO₃ after preparation of the supernatant, and its activity was also measured to determine “total” activity, which is completely activated RuBPcase activity, although an unknown inhibitor is binding. The “maximum” activity, which shows the activity after removing the inhibitor, was measured after first incubating the supernatant with an equal volume of 0.5 M Na₂SO₄ for 30 min at 0°C prior to spin-desalting using a Sephadex G-25 superfine column (Amersham Pharmacia Biotech, AB, Uppsala, Sweden) equilibrated with extraction buffer (Helmerhorst and Stokes, 1980) and pre-incubation with activation medium.

Determinations of RuBPcase and soluble protein contents

The amount of protein in the supernatant was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard. The method of determining the RuBPcase amount was that of Makino et al. (1986) using purified RuBPcase as the standard.

Results

Photosynthesis parameters

In the control, the leaf photosynthesis rates of the new shoot and the spurs on the 3-year-old stem on 25 September were almost the same as when measured on
September, but for the spur on the 2-year-old stem, the photosynthesis rate on the last day was a little higher than on the first day (Table 1); however, in girdling, the photosynthesis rate in the shoot leaf and spur leaves on the 2-year-old stem and 3-year-old stem on the 6th day following girdling (25 September) decreased 43%, 52%, and 46% in comparison to the values before girdling, respectively.

Similar to the change in the photosynthesis rate, stomatal conductance in the leaves of the girdled branch decreased 49%, 62%, and 57% in comparison to the value before girdling, respectively. The intercellular CO$_2$ of the shoot leaf and spur leaves did not obviously vary after girdling.

On the 6th day after girdling, there was a significant correlation between the photosynthesis rate and stomatal conductance in the leaves on the new shoot and spurs of the girdled and control branches (Fig. 1), and the coefficient of correlation was 0.9942 ($n = 6$, $P < 0.001$); however, no correlation existed between intercellular CO$_2$ and the photosynthesis rate.

### Carbohydrate content

The sorbitol content was highest among the non-structural carbohydrates not only in the shoot leaf, but also in the spur leaves of the control, followed by starch (Table 2). Also, the sorbitol and sucrose contents of the shoot leaf were higher than those of the spur leaves, but the starch content was lower. The leaf starch contents of the girdled branch were higher than that of the control branch. In particular, in the shoot leaf, it was approximately 3 times that of the control. On the other hand, the sorbitol content of the girdled branch was a little higher than that of the control, and the sucrose content was not so different from the control. The glucose and fructose contents were low and stable (data not shown).

Similar to the leaf, the sorbitol content in the bark was also highest in the non-structural carbohydrate in the control; however, the starch content in the bark was much lower than the sorbitol and sucrose contents. The

![Fig. 1. Relationship between photosynthesis rate and stomatal conductance of spur leaves on 2-year-old and 3-year-old stems and shoot leaves of girdled and control branches of a young apple tree.](image)

### Table 1. Influences of girdling on photosynthesis rate, stomatal conductance and intercellular CO$_2$ of shoot leaves on the new shoot, and spur leaves on 2-year-old and 3-year-old stems of girdled and control branches of a young apple tree.

<table>
<thead>
<tr>
<th>Type of stem</th>
<th>Treatment</th>
<th>Photosynthesis rate ($\mu$mol·m$^{-2}$·s$^{-1}$)</th>
<th>Sept. 19</th>
<th>Sept. 25</th>
<th>Sept. 19</th>
<th>Sept. 25</th>
<th>Sept. 19</th>
<th>Sept. 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>New shoot</td>
<td>Control</td>
<td>14.1 ± 0.9</td>
<td>13.9 ± 1.3</td>
<td>327 ± 17</td>
<td>374 ± 29</td>
<td>216 ± 8</td>
<td>225 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>10.3 ± 2.0</td>
<td>5.9 ± 0.2</td>
<td>295 ± 17</td>
<td>121 ± 9</td>
<td>236 ± 15</td>
<td>223 ± 6</td>
<td></td>
</tr>
<tr>
<td>Spur on 2-year-old stem</td>
<td>Control</td>
<td>6.5 ± 0.5</td>
<td>9.3 ± 0.3</td>
<td>172 ± 12</td>
<td>198 ± 13</td>
<td>241 ± 8</td>
<td>223 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>9.2 ± 0.9</td>
<td>4.4 ± 0.3</td>
<td>200 ± 25</td>
<td>75 ± 4</td>
<td>220 ± 8</td>
<td>212 ± 3</td>
<td></td>
</tr>
<tr>
<td>Spur on 3-year-old stem</td>
<td>Control</td>
<td>6.4 ± 0.7</td>
<td>7.5 ± 0.7</td>
<td>148 ± 11</td>
<td>158 ± 19</td>
<td>237 ± 6</td>
<td>222 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>8.1 ± 0.8</td>
<td>4.4 ± 0.6</td>
<td>155 ± 15</td>
<td>67 ± 5</td>
<td>215 ± 2</td>
<td>204 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

Average ± SE ($n = 4$).

### Table 2. Influences of girdling on carbohydrate content of shoot leaf (spur leaf), bark and wood of the new shoot, 2-year-old and 3-year-old stems of girdled and control branches of a young apple tree.

<table>
<thead>
<tr>
<th>Type of stem</th>
<th>Treatment</th>
<th>Leaf (g·m$^{-2}$)</th>
<th>Bark (mg·g$^{-1}$DW)</th>
<th>Wood (mg·g$^{-1}$DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sorbitol</td>
<td>Sucrose</td>
<td>Starch</td>
</tr>
<tr>
<td>New shoot</td>
<td>Control</td>
<td>8.33 ± 0.31</td>
<td>2.23 ± 0.01</td>
<td>3.27 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>10.26 ± 0.23</td>
<td>1.24 ± 0.03</td>
<td>9.40 ± 0.35</td>
</tr>
<tr>
<td>2-year-old stem</td>
<td>Control</td>
<td>7.36 ± 0.09</td>
<td>1.58 ± 0.02</td>
<td>4.96 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>8.59 ± 0.14</td>
<td>1.17 ± 0.02</td>
<td>7.41 ± 0.15</td>
</tr>
<tr>
<td>3-year-old stem</td>
<td>Control</td>
<td>6.44 ± 0.12</td>
<td>1.49 ± 0.11</td>
<td>3.41 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>7.77 ± 0.07</td>
<td>1.36 ± 0.01</td>
<td>6.79 ± 0.05</td>
</tr>
</tbody>
</table>

Average ± SE ($n = 4$).
ratio of the sorbitol content to the sucrose content in the bark was much higher than that in the leaves. On the other hand, the sorbitol and sucrose contents in the bark of the shoot and 2-year-old stem of the girdled branch were lower than that of the control, but the starch content was slightly higher than that of the control. In addition, the starch content in the wood of the girdled branch was significantly higher than that of the control. In contrast, there were no evident differences in the starch, sorbitol and sucrose contents in the bark of the 3-year-old stem between the girdled and control branches.

The starch content in the wood of the control was much higher than the sorbitol and sucrose contents, different from the leaf and bark. In addition, the starch content in the wood of the girdled branch significantly increased in comparison to the control. In particular, in the shoot, it was approximately 3 times that of the control, while no obvious difference in the sorbitol and sucrose contents could be recognized between the girdled and control branches.

**RuBPcase activity and content**

In the girdled branch, the initial and total activity of RuBPcase per unit enzyme protein in the shoot leaf were lower than in the control, while, the maximum activity was almost the same as in the control (Table 3). With the same treatment, the activities of RuBPcase per unit enzyme protein in the spur leaves of the 2-year-old and 3-year-old stems varied almost in the same way as in the shoot leaf.

Both the soluble protein and RuBPcase contents of the new shoot leaf were obviously higher than the spur leaves of the 2-year-old and 3-year-old stems in the control (Table 5). In addition, the soluble protein and RuBPcase contents in both the shoot leaf and spur leaves of the girdled branch were not distinctively different from the control.

The initial and total activity of RuBPcase per leaf area of the new shoot leaf in the girdled branch were lower than in the control, but the maximum activity was almost the same as the control (Table 5). RuBPcase activities in the spur leaves of the 2-year old and 3-year old stems were generally lower than in the new shoot. All activities of RuBPcase in the spur leaves of the 3-year-old stem of the girdled branch were slightly lower than that of the control. Although the initial and total activity of RuBPcase in the spur leaves of the 2-year-old stem with girdling were a little lower than that of the control,

**Table 3.** Influences of girdling on RuBPcase activity per unit enzyme protein in spur leaves on 2-year-old and 3-year-old stems and shoot leaves of girdled and control branches of a young apple tree.

<table>
<thead>
<tr>
<th>Type of stem</th>
<th>Treatment</th>
<th>Initial activity (mmol CO₂ g⁻¹ RuBPcase min⁻¹)</th>
<th>Total activity (mmol CO₂ g⁻¹ RuBPcase min⁻¹)</th>
<th>Maximum activity (µmol CO₂ m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New shoot</td>
<td>Control</td>
<td>0.75±0.03</td>
<td>0.95±0.04</td>
<td>0.96±0.03</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>0.49±0.03</td>
<td>0.71±0.03</td>
<td>0.90±0.03</td>
</tr>
<tr>
<td>2-year-old</td>
<td>Control</td>
<td>0.73±0.03</td>
<td>0.91±0.02</td>
<td>0.94±0.04</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>0.66±0.03</td>
<td>0.83±0.05</td>
<td>1.01±0.03</td>
</tr>
<tr>
<td>3-year-old</td>
<td>Control</td>
<td>0.89±0.02</td>
<td>1.04±0.02</td>
<td>1.07±0.04</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>0.71±0.02</td>
<td>0.89±0.02</td>
<td>1.00±0.02</td>
</tr>
</tbody>
</table>

Average± SE (n=4).

**Table 4.** Influences of girdling on soluble protein and RuBPcase contents in spur leaves on 2-year-old and 3-year-old stems and shoot leaves of girdled and control branches of a young apple tree.

<table>
<thead>
<tr>
<th>Type of stem</th>
<th>Treatment</th>
<th>Soluble protein (g·m⁻²)</th>
<th>RuBPcase (g·m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New shoot</td>
<td>Control</td>
<td>4.48±0.34</td>
<td>2.34±0.15</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>5.15±0.54</td>
<td>2.31±0.12</td>
</tr>
<tr>
<td>2-year-old</td>
<td>Control</td>
<td>3.37±0.61</td>
<td>1.39±0.26</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>3.52±0.05</td>
<td>1.42±0.06</td>
</tr>
<tr>
<td>3-year-old</td>
<td>Control</td>
<td>2.97±0.12</td>
<td>1.14±0.06</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>2.76±0.10</td>
<td>1.09±0.06</td>
</tr>
</tbody>
</table>

Average± SE (n=4).

**Table 5.** Influences of girdling on RuBPcase activity per leaf area in spur leaves on 2-year-old and 3-year-old stems and shoot leaves of girdled and control branches of a young apple tree.

<table>
<thead>
<tr>
<th>Type of stem</th>
<th>Treatment</th>
<th>Initial activity (µmol CO₂ m⁻² s⁻¹)</th>
<th>Total activity (µmol CO₂ m⁻² s⁻¹)</th>
<th>Maximum activity (µmol CO₂ m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New shoot</td>
<td>Control</td>
<td>29.4±1.2</td>
<td>37.2±1.4</td>
<td>37.5±1.3</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>18.9±1.3</td>
<td>27.4±1.1</td>
<td>34.8±1.3</td>
</tr>
<tr>
<td>2-year-old</td>
<td>Control</td>
<td>17.0±0.6</td>
<td>21.2±0.5</td>
<td>21.7±0.8</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>15.6±0.8</td>
<td>19.7±1.2</td>
<td>24.0±0.6</td>
</tr>
<tr>
<td>3-year-old</td>
<td>Control</td>
<td>16.8±0.5</td>
<td>19.8±0.5</td>
<td>20.3±0.7</td>
</tr>
<tr>
<td></td>
<td>Girdled</td>
<td>12.5±0.4</td>
<td>15.6±0.4</td>
<td>17.5±0.4</td>
</tr>
</tbody>
</table>

Average± SE (n=4).
maximum activity was higher in the leaves of the girdled stem.

On the 6th day after girdling, there was a significant positive correlation ($y = 0.5582x - 2.6877$) between the leaf photosynthesis rate and the initial activity of RuBPcase per leaf area (Fig. 2), and the coefficient of correlation was 0.8943 ($n = 6$, $P < 0.01$).

**Discussion**

With girdling treatment, which obstructs transportation of the photoassimilate from the leaves into sink organs such as the root, the photosynthesis rate in both the shoot leaf and spur leaf decreased, and the leaf starch content significantly increased. The starch content of the bark and wood also simultaneously increased. These results showed that girdling induced sink-limited conditions and reduced the photosynthesis rate.

**Carbohydrate accumulation in sink-limited conditions**

With girdling treatment, starch from the photosynthate significantly accumulated not only in the leaves, but also in the bark and wood (branch) in this experiment; however, the soluble sugar contents in these organs did not obviously change. This was reported even in the girdling experiment of a 3-year-old peach tree (Jordan and Habib, 1996). In addition, in the sink-limited leaf by steam girdling of the petiole in a peach tree, it was reported that although sorbitol similarly accumulates like starch, the sucrose content does not change (Moing et al., 1994); however, in this experiment, the increase in the leaf sorbitol content was much less than that of the starch (Table 2). Besides, even in the bark and wood, the sorbitol content did not obviously change; therefore, in the sink-limited apple tree in this experiment, sorbitol was not considered to have the same reserves as starch.

**Relationship between the photosynthesis rate and starch content**

Regarding non-fruit trees, starch accumulated in the leaves, bark and wood (branch) of peach, avocado and citrus trees following girdling treatment (Davie et al., 1995; Jordan and Habib, 1996; Li et al., 2003). In the same treatment, the utilization of a photoassimilate becomes so saturated in the sink organ above the girdle (mainly, wood) that starch also accumulates in the bark, which is the transport system. Consequently, the export of soluble sugar in the transport substance from the leaves is heavily obstructed, so the excessive photoassimilate is in the form of starch and accumulates in the leaves. From this result, as Schaffer et al. (1987) reported from the result of a girdling experiment in avocado, it is thought that feedback inhibition of photosynthesis occurred, although the mechanism is still unknown.

**Photosynthesis rate and opening of stomata, intercellular CO$_2$**

The photosynthesis rate in leaves significantly decreased after girdling, and opening of the stomata also concomitantly fell by a significant extent. Moreover, the photosynthesis rate in the shoot and spur leaves was correctly correlated with stomatal conductance on the 6th day after girdling (Fig. 1), but the intercellular CO$_2$ did not vary. On the other hand, in sink-limited young apple trees with 3 days of continuous light, intercellular CO$_2$ fell due to closing of the stomata with almost no RuBPcase activity change, so that stomata closing directly caused photosynthesis decline (Cheng et al., 2004). Consequently, in the sink-limited branch with girdling treatment, it is reasonable that closing of the stomatal aperture caused the decreased photosynthesis rate.

With girdling treatment, the change in intercellular CO$_2$ was not like the photosynthesis rate and stomatal conductance, that is to say, it was not obviously changed after treatment. A similar observation has been seen in sink-limited navel orange trees following fruit thinning (Syvertsen et al., 2003) and peach trees with fruit removal (Li et al., 2007). Furthermore, it was also reported that when girdling is performed in mature apple trees, stomatal conductance significantly decreases, although intercellular CO$_2$ increases in contrast (Schechter et al., 1994a, b). In other words, as differences in the treatment method and period, the width of the girdling, the amount of fruit thinning and so on, although stomatal conductance significantly decreases under sink-limited conditions, intercellular CO$_2$ differs following stomatal closure. Based on the results of this experiment, it is suggested that, according to the girdling treatment, accompanying closure of the stomatal aperture under sink-limited conditions, other factor(s), for example, the decrease in RuBPcase content or activity, also significantly depressed the basic reaction in photosyn-
thesis metabolism, and because of this, intercellular CO₂ did not change or increased.

**Relationship between photosynthesis rate and RuBPcase activity**

Due to the girdling treatment in this experiment, RuBPcase activity per leaf area decreased due to the decrease in RuBPcase activity per unit enzyme. Moreover, on the 6th day after treatment, a significant correlation was recognized between the photosynthesis rate and initial activity of RuBPcase per leaf area in the shoot and spur leaves (Fig. 2). Based on this result, it was suggested that photosynthesis reduction with girdling was related to the decrease in RuBPcase activity per leaf area. A similar result was also observed in grapevines under low night temperature (Bertamini et al., 2005). The activity and contents of RuBPcase in spur leaves on 2-year-old and 3-year-old stems were lower than in shoot leaves without treatment, and this might relate to the lower effect of girdling on the photosynthesis rate and RuBPcase activity.

In this experiment, the difference in the maximum activity and total activity of RuBPcase per unit enzyme on the 6th day after girdling was greater than the control (Table 3). Simultaneously, the difference in total activity and initial activity was almost identical to the control. It is suggested that in the leaves of the sink-limited branch, either the inhibitor(s) strongly binding to RuBPcase or decrease in the activity of RuBPcase activase leads to the decrease in RuBPcase activity per unit enzyme.

The reduction of RuBPcase activity by binding an inhibitor has been reported in some plants. As for the inhibitors of RuBPcase activity, carboxyarabinitol-1-phosphate (CA1P), xylulose-1,5-bisphosphate (XuBP), 3-ketorabinitol-1,5-bisphosphate (KABP), RuBP of sugar phosphoric acid have already been reported (Gutteridge et al., 1986; Jordan and Chollet, 1983; Moore et al., 1991; Zhu and Jensen, 1991); however, inhibitors of RuBPcase in fruit trees have not been reported.

In addition, it is known that sugar phosphoric acid inhibitors could be dissociated from RuBPcase by RuBPcase activase (Portis, 1992; von Caemmerer and Quick, 2000), and there is a theory that the first cause of the decreased photosynthesis rate under the sink limit is not an inhibitor(s), but the decreased activity of RuBPcase activase. For tomatoes under high CO₂ conditions, the mRNA level of RuBPcase activase decreases, leading to reduced activase content. Furthermore, the activity of the activase decreases, and finally, RuBPcase activity is decreased, which inhibits the photosynthesis rate (van Oosten et al., 1994). Consequently, as already described, it could be considered that with the girdling treatment in this experiment, binding of the inhibitor(s) or decreased activase activity decreased RuBPcase activity, but further research is necessary.

In conclusion, it is suggested that the decreased photosynthesis rate in the branch of a young apple tree under sink-limited conditions 6 days after girdling treatment was mostly due to stomata closing and decreased RuBPcase activity per leaf area. In this research, however, the photosynthesis rate and related parameters of the leaves were compared between controls and girdled stem using one tree. Thus, tree factors were not considered, and further work will be needed to study the effect of tree conditions such as tree vigor and the nutrient state.

**Literature Cited**


Makino, A., T. Mac and K. Ohira. 1986. Colorimetric measurement of protein stained with Coomassie Brilliant Blue R on sodium