EFFECT OF FLURIDONE ON SOME PHYSIOLOGICAL AND QUALITATIVE FEATURES OF RIPENING TOMATO FRUIT

JUSTYNA GÓRAJ-KONIARSKA1, MARIAN SANIEWSKI1*, RYSZARD KOSSON1, WIEŚŁAW WICZKOWSKI2 AND MARCIN HORBOWICZ3

1Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
2Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Department of Chemistry and Biodynamics of Food, Tuwima 10, 10-748 Olsztyn, Poland
3University of Life Sciences and Humanities, Faculty of Life Sciences, Chair of Botany and Plant Physiology, Prusa 12, 08-110 Siedlce, Poland

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In tomato fruits, chlorophyll, lycopene and β-carotene are mostly responsible for the color. During ripening of tomato fruits, the color of the pericarp changes from green to red as chlorophyll is degraded and carotenoids accumulate. These changes are associated with an increase in respiration and ethylene production. Carotenoid biosynthesis pathway in plants can be disturbed by herbicide fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4(1H)-pyridinone), which inhibits the activity of phytoene desaturase, an enzyme responsible for conversion of phytoene to phytofluene. Fluridone is also used as an inhibitor of biosynthesis of abscisic acid (ABA) and strigolactones, and it reduces chlorophyll production in plants. In our research we studied the effect of fluridone on some physiological parameters, such as color, firmness, ethylene production, lycopene and chlorophyll content during ripening of the tomato fruit. Tomato plants cv. Altadena (Syngenta) were cultivated in a greenhouse in controlled temperature and both immature and mature fruits were used for the experiments, performed between August and November 2016. Fluridone at concentrations of 0.1% and 1.0% in lanolin paste was applied as a 2-3 mm stripe from the top to the base of tomato fruits, and as a control a stripe of lanolin was applied in the same way on the opposite side of the fruits. Fluridone at a concentration of 1.0% greatly inhibited lycopene accumulation in the pericarp of tomato fruits from the treated side. The measurements of fruit firmness have shown no significant differences between firmness of the part of the tomato fruits treated with fluridone, and the non-treated ones. Tomato fruits treated with fluridone produced amounts of ethylene similar to those found in control tissues on the opposite side of the same fruit. Fluridone delayed chlorophyll degradation in tomato fruits. The metabolic significance of these findings is discussed with the role of carotenogenesis inhibition in tomato fruit ripening.

Keywords: fluridone, tomato, fruit, ripening, lycopene, ethylene, chlorophyll, firmness

INTRODUCTION

Ripening of tomato fruits is characterized by extremely fast changes in color, texture and taste, which are responsible for the fruit sensory quality. During ripening of tomato fruits, the color of the pericarp changes from green to red as chlorophyll is degraded and carotenoids accumulate. The color of the tomato fruit is determined by lycopene and β-carotene, which accumulate during the conversion of chloroplast into chromoplast (Bramley, 2002). During the process, the chlorophyll level is rapidly reduced, and there is lycopene accumulation and fruit softening (Giuliano et al., 1993; Alexander and Grierson, 2002; Pirrello et al., 2009; Barry and Giovaonni, 2007; Bramley, 2002; Seymour et al., 2013).

These changes are associated with an increase in respiration and ethylene production (Pech et al., 2012; Su et al., 2015). Ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC) level and ACC synthase activity are very low at
preclimacteric stages but they increase when the tomato fruit begins to ripen (Hoffman and Yang, 1980; Kende and Boller, 1981). Su et al. (1984) observed low activity of ACC synthase at the mature green stage which was followed by a marked increase at the breaker stage. The ACC level followed the same pattern as ACC synthase activity (Su et al., 1984). Hoffman and Yang (1980) suggested that preclimacteric tomato tissue lacks the capability not only for the synthesis of ACC but also the conversion of ACC to ethylene, and that ACC oxidase is also rapidly activated as the tomato fruit reaches the successive stages of maturity. In a fully ripe tomato fruit the level of ACC is high but ethylene production is reduced due to low ACC oxidase activity (Hoffman and Yang, 1980).

Carotenoid biosynthesis pathway is presented in Fig. 1. It is well known that fluridone (1-methyl-

![Carotenoid biosynthesis pathway](https://example.com/carotenoid_biosynthesis.png)

3-phenyl-5-[3-trifluoromethyl(phenyl)]-4(1H)-pyridinone, Fig. 2) interferes with the biosynthesis of carotenoids by inhibiting phytoene desaturase, which converts phytoene to phytofluene (Bartels and McCullough, 1972; Bartels and Watson, 1978; Rasmussen et al., 1997). Therefore, fluridone is also used as an inhibitor of abscisic acid (ABA) biosynthesis in studies relating to seed germination and dormancy (Schmitz et al., 2001; Le Page-Degrivry and Garello, 1992; Yoshioka et al., 1998; Quantrano et al., 1997; Xu and Bewley, 1995; Jullien et al., 2000; Worarad et al., 2016; Chen et al., 2016), bulb dormancy (Yamazaki et al., 1999), as well as growth and graviresponsiveness (Moore and Smith, 1984). Besides, fluridone inhibits the biosynthesis of strigolactones (López-Ráez et al., 2008; Jamil et al., 2010).

![Chemical structure of fluridone](image)

**Fig. 2.** Chemical structure of fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl(phenyl)]-4-(1H)-pyridinone).

Fluridone reduces chlorophyll production in plants and affects many other physiological and biochemical processes, as photosynthesis, biosynthesis of RNA and proteins (Drexler and Fletcher, 1981; Fletcher et al., 1984; Berard et al., 1978; Popova, 1998).

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Materials and Methods

Immature and mature green tomato fruits cv. Altadena F1 (Syngenta) were used for the experiments, done from the end of August until the beginning of November 2016. Tomato plants were cultivated in a greenhouse in controlled temperature, 18°C at night and 20°C during the day. Tomato fruits were treated with fluridone (Duchefa) at concentrations of 0.1% and 1.0% w/w in lanolin paste containing 30% of water. This was applied as 2–3 mm stripe from the top to the base of the fruit, and as a control a stripe of lanolin was applied in the same way on the opposite side of the fruit. Within experiments, the fruits were kept at ambient temperature (18–20°C) and natural daylight conditions. Color changes of the tomato fruits were observed and measured daily (untreated and treated sides of the fruits). During different stages of fruit ripening the firmness, ethylene production, lycopene and chlorophyll content were measured.

Determination of Surface Color of Fruits

The color of the tomato fruits was measured during 24 days of storage in ambient conditions with a ColorQuest Difference Meter model 25D2 (Hunter Lab). The color indicators were measured on the fluridone treated half and the control half of each fruit separately. For each fruit, four of Hunter’s “a” and “b” values at several (4 to 6) positions of fruit surface were measured. The positive values of the “a” indicator mean the level of redness and negative values for greenness, and “b” is a yellowness indicator if the values are positive.

Fruit Firmness Measurement

Six tomato fruits treated with fluridone were used in penetration test to determine the fruit firmness. Firmness was measured in tomatoes treated with fluridone and control (untreated) on the opposite side of the treated tomato fruits. The fruits were analyzed using the penetrometer Instron Model 1140 Food Testing System (Instron Ltd, Coronation Road, High Wycombe, Bucks., England). The penetrometer was fitted with the Magness Taylor Puncture- 6 mm diameter tip which punctured the fruit during each firmness measurement. The force (expressed in Newtons) needed to puncture the tomato skin (exocarp) or fruit tissue (mesocarp) was recorded as firmness. Each fruit was measured.
in two places and average values of firmness for the sample were determined. Firmness measurements of fruit skin were performed on the external surface of the fruit starting from the exocarp through the mesocarp. In order to measure fruit tissue firmness, each fruit was divided into two halves and the test was performed from the mesocarp through the exocarp/skin. Fruit tissue firmness was analyzed at the mature red stage of untreated fruits (7 days after treatment with fluridone).

DETERMINATION OF ETHYLENE PRODUCTION

After 3–13 days from the time of fluridone application, 2–3 mm scraps of pericarp tissue, about 300 mg, were collected from each of 3 fruits for determination of ethylene production. Three samples, each about 250–450 mg, were placed in 10-ml glass vials and sealed tightly. After 2 hours 1.0-ml gas samples were withdrawn and analyzed by gas chromatograph HP 4890D, equipped with a flame ionization detector and a glass column packed with Chromosorb 102. Ethylene production was expressed in nL \cdot g^{-1} \cdot h^{-1}. The experiment was repeated three times and ethylene production was measured within 3 to 13 days after treatment.

DETERMINATION OF CHLOROPHYLL CONTENT

Chlorophyll content was measured in the pericarp of tomatoes treated with fluridone and control tissues on the opposite side of the treated tomato fruits at different stages of fruit ripening. The level of chlorophyll \(a\) and \(b\) was analyzed spectrophotometrically (Bruinsma, 1963). Its content was expressed in \(\mu g \cdot g^{-1}\) fresh weight. Three tomato fruits were used for determination of chlorophyll in each treatment. The experiment was repeated twice and the chlorophyll level was measured within 5 to 14 days after treatment.

RESULTS

The halves of mature green tomato fruits treated with fluridone at 1.0% in lanolin paste became yellow after several days of herbicide application, while the fruit halves where only the lanolin paste was used (control) were red. The yellow color appeared not only in the surface layer (exocarp) of the fruit (Fig. 3a), but also inside the tomato (mesocarp and endocarp) (Fig. 3b). The results of lycopene determination show that its biosynthesis in tomato fruits is greatly inhibited by fluridone (Fig. 4).

Changes in the color of tomato halves where fluridone was applied were compared to the halves without the herbicide during 24 days of tomato fruit
Effect of fluridone on ripening of tomato fruit

A significant effect on the tomato fruit color occurred only after 16–20 days from fluridone application. Fluridone at concentration 0.1% did not affect the redness indicator “a” of tomato fruits stored for up to 24 days in ambient conditions (Fig. 5a). A higher dose of fluridone (1.0%) significantly declined the redness after 20 and 24 days’ storage of the fruit (Fig. 5b). The lower concentration of fluridone increased the yellowness indicator “b” after 20 and 24 days of treatment (Fig. 5c). A much higher influence on “b” indicator was observed in the case of exposition of tomato fruits to 1.0% fluridone. A significant increase of yellowness was noted 16 days after treatment (Fig. 5d). The yellowness indicator after 24 days of experiment reached a value almost two-fold higher in the treated half of the fruit than in the control one. A remarkably different impact of fluridone on the redness and yellowness indicates significant changes in the composition of carotenoids present in the tomato fruit. In tomato fruits exposed to fluridone, we noted a decline of red color intensity and an enhancement of yellow color intensity.

The measurements of fruit skin firmness performed on the external part of the fruit have

**Fig. 4.** The effect of fluridone at concentration of 1.0% in lanolin paste on lycopene content (μg · g⁻¹ DW ± standard deviation) in of tomato fruit pericarp; means of 3 fruits taken for analysis on September 6 (samples 1–3) and on September 21 (samples 4–6). Results of the analyses were graphically and statistically elaborated using Graph Prism program.

**Fig. 5.** The effect of fluridone on “a” greenness/redness (a, b) and “b” yellowness (c, d) indicators during 24-day storage of tomato fruits at ambient conditions. Results of the analyses were graphically and statistically elaborated using Graph Prism program. Results were presented as means of four to six replicates and the vertical bars represent confidence interval calculated using Student’s t-test at 5% of significance.
shown that there is no significant difference between firmness of the part of the tomato fruit treated with fluridone (22.33 N) and the non-treated one (22.31 N) after 7 days’ period of storage at room temperature 20°C. In the fruit tissue test showing the firmness of tissue under the tomato skin (mesocarp), the differences between firmness of the treated (15.70 N) and non-treated /control/ tomato fruits (14.51 N) were higher but still not significant. Higher fruit skin firmness (av. 22.32 N) compared to fruit tissue firmness (av. 15.11 N) can be noted (Table 1).

TABLE 1. The effect of 7-day fluridone treatment on firmness of tomato fruits.

<table>
<thead>
<tr>
<th>Part of fruit</th>
<th>Object</th>
<th>Puncture force (Newtons) mean ± standard deviation</th>
<th>LSD α = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exocarp (skin)</td>
<td>Control</td>
<td>22.31 ± 2.28</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>22.33 ± 2.68</td>
<td></td>
</tr>
<tr>
<td>Mesocarp</td>
<td>Control</td>
<td>14.51 ± 3.25</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>15.70 ± 3.03</td>
<td></td>
</tr>
</tbody>
</table>

Ethylene production was analyzed in slices of tomato fruits consisting of exocarp and mesocarp at the pink stage in the untreated part (control) and the one treated with fluridone (yellow color). The pink stage of tomato fruits was reached after different number of days from treatment, depending whether they were treated at immature or mature green stages; tomatoes treated at the mature green stage ripened earlier. Tomato fruits treated with fluridone produced similar amounts of ethylene to the control tissues on the opposite side of the same tomato fruit (Table 2).

During tomato fruit ripening the decline of chlorophyll content is a natural process. Fluridone inhibited the decline of chlorophyll content when the control part of fruit reached the pink stage (Table 3). However, in full fruit ripeness the level of chlorophyll content was low in both control and fluridone-treated tissues.

**DISCUSSION**

Fluridone, an inhibitor of phytoene desaturase, which converts phytoene to phytofluene in carotenoid biosynthesis (Fig. 1), greatly inhibited lycopene accumulation in the pericarp of tomato fruits. The inhibition of the lycopene accumulation in all parts of the tomato fruit by fluridone applied on a green fruit suggests that the herbicide easily penetrates into the deeper layers of the fruit.

<table>
<thead>
<tr>
<th>Date of treatment</th>
<th>Object</th>
<th>Days after treatments</th>
<th>Stage of fruit maturity</th>
<th>Ethylene production (nl g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 6, 2016</td>
<td>Control</td>
<td>3</td>
<td>turning</td>
<td>101.34a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>3</td>
<td></td>
<td>116.44a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6</td>
<td>red</td>
<td>48.56a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>6</td>
<td></td>
<td>99.16b</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7</td>
<td>red</td>
<td>60.52a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>7</td>
<td></td>
<td>74.69a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
<td>turning</td>
<td>85.18a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>8</td>
<td></td>
<td>99.18a</td>
</tr>
<tr>
<td>Sept. 16, 2016</td>
<td>Control</td>
<td>5</td>
<td>red</td>
<td>74.09a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>5</td>
<td></td>
<td>60.39a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7</td>
<td>pink</td>
<td>191.41b</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>7</td>
<td></td>
<td>146.48a</td>
</tr>
<tr>
<td>Sept. 21, 2016</td>
<td>Control</td>
<td>5</td>
<td>pink</td>
<td>244.34a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>5</td>
<td></td>
<td>255.81a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
<td>pink</td>
<td>208.84a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>8</td>
<td></td>
<td>195.47a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12</td>
<td>red</td>
<td>67.60a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>12</td>
<td></td>
<td>75.72a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>13</td>
<td>turning</td>
<td>132.38a</td>
</tr>
<tr>
<td></td>
<td>Fluridone</td>
<td>13</td>
<td></td>
<td>130.78a</td>
</tr>
</tbody>
</table>

Differences among means were evaluated using the Duncan test at 5% of significance, calculated separately for each term of analysis.

Fluridone is a systemic herbicide; when applied on leaves it moves to the roots (Marquis et al., 1981; Berard et al., 1978).

In the tomato fruits treated with fluridone, a significant decline of the abscisic acid (ABA) level and ethylene production, and delayed fruit ripening and softening were documented, but in contrast, exogenously applied ABA to green tomato fruits induced ethylene synthesis, and accelerated fruit coloring and softening (Zhang et al., 2009). Authors proposed that ABA may act at upstream metabolic events of ethylene action/perception and initiate the
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The ripening process by inhibiting or activating general metabolic events.

Also, other authors concluded that fluridone significantly decreased ABA level, decreased ethylene production and inhibited fruit softening in tomato (Sheng et al., 2008), apple (Chen-Shang-Wu and Zhang-Da-Peng, 2000) and peach (Cao et al., 2013) fruits.

Our results show that fluridone greatly inhibits lycopene accumulation in tomato fruits but does not affect ethylene production and fruit softening. In experiments of Zhang et al. (2009) there were also no significant differences in fruit firmness between tomatoes treated and not treated with fluridone after 7 days of storage at ambient conditions. The significant differences in tomato firmness in their studies were observed only after 28 days’ period of storage. It has to be emphasized that tomato fruits in the experiment of Zhang et al. (2009) were treated by injection of fluridone into the center of the fruit.

It is interesting that fluridone delayed chlorophyll degradation in tomato fruits during ripening but inhibited chlorophyll biosynthesis in growing plants (Berard et al., 1978; Popova, 1998; Flechter et al., 1984). However, the effect of fluridone on chlorophyll disappearance in mature green tomato fruits needs further extensive studies.

On the basis of these results, it is concluded that inhibition of lycopene accumulation by fluridone did not disturb firmness and ethylene production but delayed chlorophyll degradation during ripening of tomato fruits.

AUTHORS’ CONTRIBUTIONS

The idea of the experiments: M.S., J.G.-K.; performing the experiments: J.G.-K., M.H., R.K.; writing the paper: M.S., M.H., WW. The authors declare that they have no conflict of interest.

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