Prohexadione-Ca in Apple Production: More than a Growth Regulator!

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Part B

**Modes of Action (b)**

**Benefits (b):**
- Effects on Diseases
- Effects on Insect Pests

**Tips for Efficient Usage**

**Summary / Conclusions**
Effects Caused by Prohexadione-Ca in Fruit Trees

- Reduction of shoot growth
- Reduced fruit drop
- Reduced incidence of fungal and bacterial pathogens and of insect pests
## Pathogen Incidence in Different Host-Pathogen-Pathogen Combinations after Treatment with Prohexadione-Ca

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Disease / Pathogen</th>
<th>Incidence Reduced?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Fire blight <em>Erwinia amylovora</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Pear <em>Pyrus communis</em></td>
<td>Fire blight <em>Erwinia amylovora</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Scab <em>Venturia inaequalis</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Apple powdery mildew <em>Podosphaera leucotricha</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Sooty blotch (complex of different fungi)</td>
<td>Yes</td>
</tr>
<tr>
<td>Peach <em>Prunus persica</em></td>
<td>Peach powdery mildew <em>Sphaerotheca pannosa</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Rose <em>Rosa ssp.</em></td>
<td>Black spot <em>Diplocarpon rosae</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Grape Vine <em>Vitis vinifera</em></td>
<td>Downy mildew <em>Plasmopara viticola</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Grape Vine <em>Vitis vinifera</em></td>
<td>Grey mould <em>Botrytis cinerea</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Pepper <em>Capsicum annuum</em></td>
<td>Grey mould <em>Botrytis cinerea</em></td>
<td>No</td>
</tr>
<tr>
<td>Wheat <em>Triticum aestivum</em></td>
<td>Cereal powdery mildew <em>Blumeria graminis</em></td>
<td>No</td>
</tr>
</tbody>
</table>

**Typically 40 - 80% Reduction of Incidence**
Arguments:

- Chlormequat chloride, daminozide, paclobutrazol, uniconazole
  - Alternative inhibitors of GA biosynthesis.
  - Give equivalent morphological and histological effects.
  - Many years of use in apple and pear production.
  - No effects known against fire blight or other diseases !!!

- Reduced pathogen incidence is observed only if prohexadione-Ca or the related trinexapac-ethyl are applied.

Reduced Incidence of Diseases Does not Primarily Result from Reduced Shoot Growth
Effects of Different Types of Growth Retardant on Shoot Length and Scab Incidence in Apple Seedlings

Inoculation: 14 DAT; Evaluation: 28 DAT
Mode of Action:
Modified Flavonoid Metabolism

Lowered Incidence of Diseases and Insect Pests
No Concern about Apple Fruit Coloration !!!!

Control
500 ppm ProCa
Mode of Action:
Modified Flavonoid Metabolism

Phenylalanine → Cinnamic Acid → p-Coumaroyl-CoA → Naringenin → Eriodictyol → Other Phenolics

FHT (Flavanone 3-hydroxylase)

Dihydroquercetin → Quercetin → Cyanidin → cyanidins

Quercetin → Leuco-cyanidin

Cyanidin → Proanthocyanidins → Catechin
Phenylalanine → Cinnamic Acid → p-Coumaroyl-CoA → Naringenin → Eriodictyol

Other Phenolics

Prohexadione-Ca

FHT

FNR (Flavanone reductase)

Dihydroquercetin → Quercetin

Cyanidin → Leuco cyanidin

Proanthocyanidins

Catechin

Luteoforol

Luteoliflavan

It takes a while!

10 days after treatment: ~ 7 mg/g DW
Formation of 3-Deoxyflavonoids

- **Eriodictyol**
- **Catechin**
- **Leucocyanidin**
- **Luteoforol**
- **Luteoliflavan**
Mode of Action: Modified Flavonoid Metabolism

Formation of 3-Deoxyflavonoids with Structural Similarities to Phytoalexins

Phytoalexins from *Sorghum bicolor*
# Mode of Action: Inhibition of Microbial Growth by Luteoforol

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Luteoforol</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$10^{-3}$ M</td>
<td>$10^{-4}$ M</td>
<td>$10^{-5}$ M</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Erwinia amylovora</em></td>
<td>Ea 1/79</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Erwinia amylovora</em></td>
<td>Ea 286</td>
<td>+++</td>
<td>0</td>
<td>nd</td>
</tr>
<tr>
<td><em>Erwinia amylovora</em></td>
<td>Ea 1540</td>
<td>+++</td>
<td>0</td>
<td>nd</td>
</tr>
<tr>
<td><em>Erwinia amylovora</em></td>
<td>Ea 8865</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Erwinia amylovora</em></td>
<td>Ea DCA289/01</td>
<td>++</td>
<td>0</td>
<td>nd</td>
</tr>
<tr>
<td><em>Microbacterium sp.</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><em>Streptococcus sobrinus</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alternaria brassicicola</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td><em>Alternaria solani</em></td>
<td></td>
<td>+++</td>
<td>++</td>
<td>nd</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td><em>Colletotrichum lagenarium</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td><em>Fusarium culmorum</em></td>
<td></td>
<td>+++</td>
<td>+</td>
<td>nd</td>
</tr>
<tr>
<td><em>Phytophthora infestans</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td><em>Pyricularia oryzae</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td><em>Venturia inaequalis</em></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

+++ = complete inhibition of growth
0 = no effect
nd = not determined

Fire Blight

(Gala on M.9, 4th leaf; van Buren County, MI, USA, 2000)
(Picture courtesy of Mark Longstroth, MSU)
Controlling fire blight with prohexadione-Ca is of special interest:

- General concerns about using antibiotics (e.g. streptomycin, oxytetracycline, gentamycin, kasugamycin) in plant production.

- Out of the antibiotics mentioned, gentamycin is considered to be of particular value in human medicine. Plant products contaminated with gentamycin do not qualify for export into the USA.

- Increasing problems with strains of *Erwinia amylovora* resistant against antibiotics.

*But:*

- Prohexadione-Ca cannot completely substitute antibiotics.
Problems to control blossom infections with prohexadione-Ca:

- Application required ~ 10 days prior to flowering (infection)
  - little plant surface for uptake
  - often unfavorable weather conditions
- Insufficient efficiency; mixture with other compound needed.
- Prophylactic treatment: Acceptance by growers?
Chronology:

April 25 – peak blossom

May 2 – first treatment
   (125 g/ha ai)

May 26 – severe hailstorm

July 2 – second treatment
   (125 g/ha ai)

Evaluation on:

T1 = June 13

T2 = October 1
(overwintering cankers !)

orchard trial with natural infection

K.S. Yoder et al., Virginia TU at Winchester, VA, USA (2001)
Example: Effect of Prohexadione-Ca on Scab Incidence

Prohexadione-Ca applied as Regalis® (125 g/ha a.i.); cv. Golden Delicious, 12-year old trees on M.9.
(Treatment at 5 cm of new shoot growth = end of flowering; evaluation at harvest; no fungicides applied after flowering - BASF Limburgerhof 2001)
Example: Effect of Prohexadione-Ca on the Incidence of Scab and Mildew

K.S. Yoder et al., F&N Tests 55: 46
# Reduced Incidence of Insect Pests in Pre-Treated Fruit Trees

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Insect Pest</th>
<th>Incidence reduced?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Green apple aphid <em>Aphis pomi</em> + Green citrus aphid <em>Aphis spiraecola</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Woolly apple aphid <em>Eriosoma lanigerum</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Green citrus aphid <em>Aphis spiraecola</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Potato leafhopper* <em>Empoasca fabae</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Apple <em>Malus domestica</em></td>
<td>Apple psylla <em>Psylla mali</em></td>
<td>Yes</td>
</tr>
<tr>
<td>Pear <em>Pyrus communis</em></td>
<td>Pear psylla <em>Psylla pyri</em></td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Damage by potato leafhoppers facilitates fire blight infection!
Mode of Action: Modified Flavonoid Metabolism

Formation of 3-Deoxyflavonoids with Insecticidal Activity

Luteoliflavan

Viscutin-3
The β-D-Xylose conjugate of luteoliflavan is known to be an inhibitor of insect growth
[Kubo et al., Tet. L. 28: 921-924 (1987)]

More work required for final answer!
Summary: Mode(s) of Action and Benefits for Fruit Production

Inhibition of gibberellin biosynthesis
=> less shoot growth

• reduced need for pruning
• better light penetration into canopy
• improved spray coverage
• leaves dry faster after rainfall
• earlier bud set
• improved fruit quality and storage behavior

Reduction of ethylene formation
=> increased fruit set

Makes sense ...
• when alternating trees are in the “off” year
• after severe flower losses as a result of frost damage
• to obtain moderate yield increases
• to avoid over-sized fruits

Modification of flavonoid metabolism
=> improved stress resistance

• lowered incidence of shoot fire blight
• lowered incidence of scab and mildew
• lowered incidence of insect pests
• (improved frost hardiness of flowers)
Recommendations for Practical Use
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Apogee® (27.5 % WG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targets</td>
<td>Reduction of vegetative growth; control of secondary fire blight</td>
</tr>
<tr>
<td>Maximum seasonal use rate</td>
<td>1.4 kg/ha (385 g/ha of active ingredient)</td>
</tr>
<tr>
<td>Typical rates per treatment</td>
<td>0.2 to 0.7 kg/ha (55 to 192 g/ha of active ingredient)</td>
</tr>
<tr>
<td>Number of treatments</td>
<td>1 to 3 per season</td>
</tr>
<tr>
<td>First treatment</td>
<td>At 2 to 5 cm of new shoot growth (~ 2 to 5 new leaves per shoot / ~ beginning of petal fall)</td>
</tr>
<tr>
<td>Time interval between treatments</td>
<td>2 to 4 weeks or at the beginning of new shoot growth</td>
</tr>
<tr>
<td>Recommended volume of liquid</td>
<td>1000 L/ha in a standard orchard (~ 400 L/ha per meter of crown height)</td>
</tr>
<tr>
<td>Rainfastness</td>
<td>After 12 hours</td>
</tr>
<tr>
<td>Pre-harvest interval</td>
<td>45 days</td>
</tr>
</tbody>
</table>
Application of Apogee® is of particular value in vigorous orchards.

Like other bioregulators used in fruit production, the use of Apogee® requires skill and diligence. Local experience should be used to optimize usage.

Apogee® application will result in multiple effects (e.g. less shoot growth and control of secondary fire blight). Trying to separate these effects makes no sense under practical conditions. - Priority should be given to adequate control of shoot growth.

Typically, young trees (< 3 to 4 years of age) are no candidates for treatment, except for controlling fire blight. Rates should be kept low, then.
Preparing the Spray Solution

- Do not exceed 250 ppm of active ingredient.

- Add 1-2 kg of ammonium sulfate/1000 L. (Of particular importance when hard water is used.)

- Avoid alkaline water (pH > 7). Add acidifier if needed. Optimal pH of spray solution is in the range of 4.0 to 5.5. (Rainfastness achieved after 2 to 3 hours !)

- Never co-apply Apogee® with Ca-containing products (e.g. CaCl₂ against bitter pit). Separate treatments by 2 to 3 days.

- Avoid co-application with GA₄/₇ and thinning agents. Separate treatments by 2 to 3 days.
### Trees with low vigor

- dwarfing rootstock (e.g. M.9)
- low-vigor cultivar (e.g. Cox Orange)
- age of tree: relatively young or relatively old
- low to moderate availability of nutrients and water
- regular to high expected fruit load

<table>
<thead>
<tr>
<th>Timing</th>
<th>Dosage of Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st Treatment</strong> at 2 - 5 cm of new shoot length (~ 2 - 5 new leaves per shoot / ~ beginning of petal fall)</td>
<td>20 - 40 g / 100 L</td>
</tr>
<tr>
<td><strong>2nd Treatment</strong> 2 to 4 weeks later</td>
<td>20 - 40 g / 100 L</td>
</tr>
</tbody>
</table>
Trees with **high** vigor

- less dwarfing rootstock (e.g. M 25, MM 111)
- vigorous cultivar (e.g. Jonagold)
- tree age ~ 4 to 12 years
- high availability of nutrients and water
- low fruit load (alternating trees; flower losses resulting from late frost)

<table>
<thead>
<tr>
<th>Timing</th>
<th>Dosage of Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) Treatment at 2 - 5 cm of new shoot length (~ 2 - 5 new leaves per shoot / ~ beginning of petal fall)</td>
<td>40 - 70 g / 100 L</td>
</tr>
<tr>
<td>2(^{nd}) Treatment 2 to 4 weeks later or at the beginning of new shoot growth</td>
<td>40 - 70 g / 100 L</td>
</tr>
<tr>
<td>In extreme cases: 3(^{rd}) Treatment 3 to 5 weeks after 2(^{nd}) application or at the beginning of new shoot growth</td>
<td>30 - 50 g / 100 L</td>
</tr>
</tbody>
</table>
Apogee®: => Timing of First Treatment: 
Perfect !
Apogee®: ⇒ Timing of First Treatment:
Don’t wait any longer!
Apogee®: ⇒ Timing of First Treatment:

Treatment has been too late!
Apogee®: Detailed Use Recommendations

=> Spray Application

- All factors that support a long-lasting liquid film on the leaf surface increase the uptake of prohexadione.

- A water volume of 400 l/ha per meter of crown height is recommended (next to run-off).

- Best weather conditions for application:
  - overcast sky
  - approximately 16 to 22°C
  - high relative humidity
  - period of good growing conditions expected

Apply early in the morning, late in the afternoon or early in the evening. Make use of dew formation overnight.
<table>
<thead>
<tr>
<th>Special Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Aim the second (or third) application at the top third of the crown. Here is where the most vigorous growth occurs.</td>
</tr>
<tr>
<td>- In “hopeless” situations, combine Apogee® with moderate root pruning.</td>
</tr>
<tr>
<td>- Following a severe hail damage, trees often respond with extreme vegetative growth. A single treatment with Apogee® is very effective in minimizing re-growth and maintaining a balanced tree. Return bloom and fruit production in the next season will significantly be improved. Furthermore, the treatment will protect the trees from fire blight.</td>
</tr>
</tbody>
</table>
• Very hard and/or alkaline water used without adequate adjuvants.
  => Loss of activity; re-growth later in the season.

• First application too late (e.g. at 20 cm of shoot length).
  => Poor and inhomogenous control of shoot growth; even increased shoot growth may occur!

• Application rate too low.
  => Poor activity; re-growth later in the season.

• Application rate too high.
  => In general, no negative effects in apples unless in extreme cases.

  Overdosing over several years must be avoided!
SUMMARY

Apogee® (27.5% prohexadione-Ca)

• is a modern and highly innovative product

• without any toxicological or ecotoxicological problems

• that enables the apple grower to produce fruits
  • of improved quality
  • at a higher yield level
  • at less costs.

• Fruit growers in many countries like Apogee® and the related Regalis® and have no difficulties in successful usage.
Cooperators

- **Gibberellin biosynthesis**
  - Yuji Kamiya (RIKEN, Tokyo, Japan)
  - Peter Hedden (Rothamsted Research Station, UK)

- **Ethylene formation**
  - Philip John (University of Reading, UK)
  - Klaus Grossmann (BASF)

- **Flavonoid metabolism** *(supported by the European Commission)*
  - Karl Stich (Vienna Technical University, Austria)
  - Dieter Treutter (Munich Technical University, Germany)
  - Gerd Forkmann (Munich Technical University, Germany)
  - Guglielmo Costa (University of Bologna, Italy)
  - Carlo Bazzi (University of Bologna, Italy)
  - John Speakman & Norbert Zimmermann (BASF)