RNAi-mediated control of spotted wing drosophila (*Drosophila suzukii*): efficacy challenges and biosafety considerations

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Introduction

- *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) is a rapidly emerging invasive pest in America and Europe
- first described in Germany in 2011 in the viticultural region of Palatinate in Southwest Germany
- in 2014 high yield losses were already recorded in cherries, small fruits and grapevine
- pesticide and mechanical control measures have been applied
- they are neither environmentally nor economically sustainable

*D. suzukii* is well established in Germany

- long-term solutions have to be developed
- RNAi-based pest control strategies would have some potential
RNA interference (RNAi) in insects

- Double stranded RNA (dsRNA) taken up by the insect is processed into small interfering RNAs (siRNAs) by the insect’s RNAi machinery which specifically down-regulate mRNA translation eventually leading to insect death.
- RNAi is not systemic in Drosophila.
- But: RNAi is functional in Drosophila suzukii (Taning et al. 2016)

From: Kanakala and Ghanim, 2016. Viruses, 8(12), 329
Challenges in RNAi-mediated control of *D. suzukii*

- *D. suzukii* attacks intact maturing and ripening fruits
- damage is produced by the developing larvae inside the fruits
- *D. suzukii* can rapidly produce high population densities
- fruit damages caused by *D. suzukii* can be the entrance for further pests as well as microbes
- this of major concern in grapevine

- RNAi-mediated control has to attack adult flies before egg deposition in fruits
- RNAi-mediated control has to be efficient against high population densities
- RNAi-mediated shut down of *D. suzukii* genes has to occur rapidly
- RNAi-mediated control has to be cost-effective
Objectives

- RNAi-mediated control of adult flies
- Delivery of ds RNA in attractive traps
- Optimal timing of RNAi-mediated control
Strategy

• Establishment of a gene silencing assay with published essential genes:
  – tubulin (γTub23C)
  – vacuolar ATPase (Vha26)
  – ribosomal protein S13 (RPS13)
  – ribosomal protein L19 (Rpl19)

• Delivery of ds RNA by oral feeding
  – adult flies
  – larvae
Selection of target sequences in *D. suzukii* line KEF8

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sequence homology to <em>D. suzukii</em> genome database (spottedwingflybase.org)</th>
<th>Size of ds RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>tubulin (<em>yTub23C</em>)</td>
<td>97%</td>
<td>369 bp</td>
</tr>
<tr>
<td>vacuolar ATPase (<em>Vha26</em>)</td>
<td>&gt;99%</td>
<td>457 bp</td>
</tr>
<tr>
<td>ribosomal protein S13 (<em>RPS13</em>)</td>
<td>99%</td>
<td>403 bp</td>
</tr>
<tr>
<td>ribosomal protein L19 (<em>Rpl19</em>)</td>
<td>&gt;99%</td>
<td>422 bp</td>
</tr>
</tbody>
</table>

Maximum likelihood analysis of a 1286 bp fragment of *yTub23C*

- Amplification and cloning of *D. suzukii*-specific gene fragments
Oral feeding assay with adult flies

- production of ds RNA through *in vitro* transcription
- production of ds RNA liposomes with lipofectamine according to Taning et al. (2016)
- delivery as overlay on artificial diet
- assay with starved 1 week old flies (3 males, 3 females)
  - controls on diet without ds RNA
  - controls on water agar (no food supply)
- monitoring of activity (index 0-3) and mortality
- monitoring of egg deposition
- monitoring of larval development
- monitoring of progeny flies
Results

• all flies on artificial diet survived well
• controls on water agar died within 5 days
• no impact on activity
• flies readily laid eggs into the ds RNA-incubated medium and larvae developed
• progeny flies developed in treated and untreated conditions

Number of progeny flies from 3 females per treatment (n=6); Kruskal-Wallis chi-squared = 0.98756, df = 4, p-value = 0.9117
Challenges of RNAi-mediated control of D. suzukii

- Efficient delivery of high doses of ds RNA
- Finding the best target gene
- Optimal timing of RNAi-mediated control
Timing of RNAi-mediated control

- *D. suzukii* shows a strong seasonal migration behaviour
- Summer morphs develop in fruits to high population densities and cause the damage
- In autumn, winter morphs are formed which migrate and overwinter on shelter plants
- Winter morphs survive until new fruits for reproduction are present in spring/early summer

summer morphs

winter morphs
Seasonal population dynamics of *D. suzukii*

at an overwintering site at Neustadt/W.

Captures of *D. suzukii* 2016

(total per week)

- trap 1
- trap 2

calendary weeks
Biosafety considerations

• More than 50 Drosophila species are known in Europe
• Many Drosophila species share the same habitats as *D. suzukii*
• So far, no *D. suzukii*-specific attractant is known and, therefore, many different species are caught in actual monitoring traps
• Some of these species are not well studied and molecular data are missing
• In order to be sure that no off-target effects of *D. suzukii* RNAi control occur, these Drosophilids need to be considered as well
## Biosafety considerations

### Frequent off-target drosophilids in monitoring traps at Neustadt/W. Germany

Identification by S. Alexander, DLR Rheinpfalz, Neustadt

<table>
<thead>
<tr>
<th></th>
<th>winter</th>
<th>spring</th>
<th>summer</th>
<th>autumn</th>
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</thead>
<tbody>
<tr>
<td>D. subobscura</td>
<td>D. subobscura</td>
<td>D. melanogaster</td>
<td>D. melanogaster</td>
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<tr>
<td>D. obscura</td>
<td>D. simulans</td>
<td>D. subobscura</td>
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<tr>
<td>D. testacea</td>
<td>D. hydei</td>
<td>D. buskii</td>
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<tr>
<td>D. immigrans</td>
<td>D. funebris</td>
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<td></td>
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<tr>
<td>D. phalerata</td>
<td>D. kuntzei</td>
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*D. suzukii*-specific sequences can be selected (ex. Vha26)
Conclusions

- control of *D. suzukii* by RNAi is ambitious
- an optimal target gene has yet to be identified
- improvement of RNAi delivery depends on the application method (e.g. in traps, sprays, transgenic microbes or plants)
- information of local population dynamics and migration behaviour of *D. suzukii* is needed for optimal timing of RNAi-control
- biosafety issues can be solved
  - *D. suzukii*-specific traps
  - *D. suzukii*-specific targets
Thank you