Differential aphicidal effects of chitinase inhibitors on the polyphagous homopteran Myzus persicae (Sulzer)

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Abstract: Four chitinase inhibitors, cyclo-(Proline-Tyrosine), cyclo-(Histidine-Proline), allosamidin and psammaplin A, were selected for in vitro feeding experiments with the peach-potato aphid, Myzus persicae (Sulzer), under controlled photoperiod and temperature conditions. Artificial diets were used to provide chitinase inhibitors at 10, 50 and 100 µg mL−1 to M. persicae. Except for cyclo-(Proline-Tyrosine), which did not modify aphid demographic parameters, chitinase inhibitors induced differential aphicidal effects on M. persicae. At all doses, cyclo-(Histidine-Proline) induced significant effects affecting daily fecundity, intrinsic rate of natural increase (r∞) and doubling time of population. When compared with the control diet, allosamidin decreased nymph survival and daily fecundity, increasing the doubling time of population from 1 to 1.5 days. Psammaplin A was the most toxic inhibitor when delivered via artificial diet, as it induced the death of all aphids reared at 50 and 100 µg mL−1. The results demonstrate the potential use of chitinase inhibitors as aphid management tools.

Keywords: allosamidin; artificial diet; cyclic dipeptide; insect; psammaplin A; Myzus persicae

1 INTRODUCTION

Chitin is a β-1,4 linked homopolymer of N-acetyl-glucosamine residues that occurs in the cell wall of fungi and in various structural components of arthropods, such as the exoskeleton and peritrophic membrane of insects. Because chitin does not occur in plants and vertebrates, interference with its metabolism could be considered as a suitable target for new insecticides with low vertebrate toxicity.1 Chitin degradation has been considered as a possible strategy for pest management by either enhancement or inhibition of the hydrolytic enzymes involved. Chitinases (EC 3.2.1.14) are key enzymes in the turnover of chitinous structures and are widely distributed among plants, microorganisms, invertebrates and insects. With the aim of producing insect-resistant plants, chitinase genes from various origins (bacteria, fungi, plants and insects) have been introduced in plant genomes. Only two studies concerning lepidopteran chitinases, from Manduca sexta Joh2 and Lacanobia oleracea L.,3 have reported insecticidal effects when expressed in transgenic plants. However, the present authors recently demonstrated probiotic effects with a chrysomedil (Phaedon cockerellae F.) and a bacterial (Serratia marcescens Bizio) chitinase on the aphid Myzus persicae (Sulzer) (Homoptera: Aphididae).4 As chitinases exert undesirable effects on this insect pest, a chitinase-based strategy would fail in agroecosystems where aphids cause serious threats to crops. Thus, chitinase inhibition is a strategy that is of increasing interest.

Several chitinase inhibitors isolated from various origins have been reported in the last 20 years. Insect, fungal and some bacterial chitinases included in chitinase family 18 act through different mechanisms and have been shown to exert different types of interaction with chitinase inhibitors that have competitive or non-competitive inhibitory activities. A pseudotrisaccharide named allosamidin is known competitively to inhibit chitinases5,6 and to enhance chitin biosynthesis7–9 by mimicking chitin. Two cyclic dipeptides, cyclo-(Tyrosine-Proline) and cyclo-(Histidine-Proline), inhibit chitinases by mimicking a catalytic intermediate of allosamidin.10 Psammaplin A, a brominated tyrosine-derived compound, moderately inhibits chitinase by binding near the active site of chitinases, but not as tightly as allosamidin.11 Owing to their different chemical
structures and functions in nature, chitinase inhibitors were expected to have differential effects on insects. Although chitinase inhibitors have been reported to present larvicidal effects and disturb the moulting process in lepidopteran and dipteran species, they have never been assayed in Homoptera.

The homopteran M. persicae is one of the most polyphagous insects worldwide, as it successfully develops on hundreds of plant species. In several insect species, chitin is a major component of the peritrophic membrane, as a non-cellular barrier formed by the digestive epithelium to compartmentalize digestive enzymes and protect epithelial cells from disruption and penetration of parasites. Turnover of peritrophic membrane involves chitinases, which represent a digestive target in a chitinase inhibitors strategy. Myzus persicae is deprived of peritrophic membrane, and no chitinase activity has so far been described in aphid midgut. Thus, aphids could be considered as non-digestive target pests in the context of chitinase inhibitor use. Aphids are hemimetabolous and larviparous insects: offspring develop in the genital tracts of adults. During their embryonic development, growth of larvae is influenced by the physiology of the fundatrix, and their integument chitinases, involved in cuticle remodelling, are possible targets. All these characteristics make M. persicae an interesting model to study chitinase inhibitor effects.

With the aim of managing aphid populations, we have evaluated the aphicidal effects of four chitinase inhibitors, cyclo-(Histidine-Proline), cyclo-(Proline-Tyrosine), allosamidin and psammaplin A, on M. persicae.

2 MATERIALS AND METHODS

2.1 Artificial diets
Cyclo-(Histidine-Proline), cyclo-(Proline-Tyrosine), amino acids, vitamins and mineral compounds were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Allosamidin and psammaplin A were respectively provided by Dr Sakuda (University of Tokyo, Japan) and Dr Tabudravu (University of Aberdeen, Scotland, UK). A standard diet adapted for M. persicae was used as a carrier for chitinase inhibitor dilution and as a control. The concentrations of chitinase inhibitors (10, 50 and 100 µg mL−1) incorporated into the standard diet were chosen according to Cherqui et al. The diet was prepared as described by Febvay et al. and modified by Down et al. After preparation, the artificial diet was passed through a 0.45 µm filter (Millipore Corp., Bedford, Massachusetts, USA), and diet pouches were prepared under aseptic conditions.

2.2 Insects and feeding assays
The aphid clone was established in 1999 from a single apterous female collected on a potato field near Loos-en-Gohelle (50° 27′ 27″ N, 2° 47′ 30″ E) in Northern France. The colony of M. persicae was maintained on potato plants (Solanum tuberosum L. cv Désirée) propagated from tubers in a greenhouse under parthenogenesis-inducing conditions, i.e. 20 ± 1 °C and a 16:8 h light:dark photoperiod.

Pouches of each diet (0 = control, 10, 50 and 100 µg mL−1 inhibitor) with 10–12 nymphs younger than 24 h were maintained under parthenogenesis-inducing conditions as described above. At least five replicates were carried out for each diet, and pouches were changed every second day.

Nymphal survival, prereproductive period (i.e. the period of time from birth until onset of reproduction), adult emergence and reproduction were recorded daily according to a procedure described by Saguez et al. These parameters were used to calculate the intrinsic rate of natural increase (rN) of the M. persicae population according to Le Roux et al. The variance of rN value was estimated with the ‘Petitr’ program (Pierre JS, private communication). The time necessary to double the population was estimated as

2.3 Statistical analysis
The effects of chitinase inhibitors on nymphal survival were analysed with Pearson’s χ2 test. One-way analysis of variance (ANOVA) was carried out with Statistica 5.5 software (StatSoft, Tulsa, Oklahoma, USA) to test the effects of the diets on aphid demographic parameters. Significantly altered demographic parameters were further analysed with Fisher’s positive least significant difference test (PLSD) at P < 0.05.

3 RESULTS
Among demographic parameters, prereproductive period duration was not affected by any chitinase inhibitor (Table 1, A, B, C and D). The cyclic dipeptide cyclo-(Proline-Tyrosine) did not affect M. persicae development when compared with the control diet (Fig. 1A and Table 1, A). In contrast, growth parameters were slightly modified by cyclo-(Histidine-Proline). No difference was observed in nymphal mortality at all doses compared with the control diet (Fig. 1B). However, this inhibitor significantly reduced the daily fecundity of M. persicae by 25–40% and also reduced rN values. As a consequence, the doubling time of population was only enhanced at 10 and 50 µg mL−1 (Table 1, B).

Although allosamidin did not affect nymphal mortality at 10 µg mL−1, nymphal survival of M. persicae was reduced at 50 and 100 µg mL−1 (Fig. 1C), by 40 and 60% respectively ($\chi^2 = 17.26$; DF = 9; P < 0.04 and $\chi^2 = 39.72$; DF = 9; P < 0.00). Aphids fed on all allosamidin concentrations presented a significant decrease in their rN values that was directly linked with a reduction in offspring production. As a consequence, the duration of the doubling time of aphid population was increased from 1 to 1.5 days (Table 1, C).
Psammaplin A induced important toxic effects on *M. persicae* larvae. At 50 and 100 µg mL⁻¹, all aphids died before adulthood (Fig. 1(D)). As a consequence, death of aphids at the nymphal stage precluded the determination of adult parameters. At 10 µg mL⁻¹, *M. persicae* developed normally, and no difference was determined when compared with control diet either on the larval or the adult stage (Table 1, D).

### 4 DISCUSSION

The present results showed differential effects of chitinase inhibitors. Although cyclic dipeptides induced little or no insecticidal effects, allosamidin and psammaplin A were potent developmental inhibitors against *M. persicae*. These results could be associated with the nature of interaction between chitinases and their inhibitors; the higher the affinity between chitinases and their inhibitors, the higher the inhibitory activity. Crystallographic studies associated with *in vivo* bioassays have shown that inhibitory activity is correlated with the affinity of the inhibitor for the active site of chitinase. Thus, the cyclic dipeptide cyclo-(Histidine-Proline) is better linked to the active site of *Saccharomyces cerevisiae* Meyer ex Hansen and is more potent than cyclo-(Tyrosine-Proline).¹⁰,²⁰ However, these interactions are weaker than those of allosamidin which tightly binds chitinases. Psammaplin A binds near the active site of chitinases, but its inhibitory activity is less than that of allosamidin.¹¹

At 10 and 50 µg mL⁻¹, cyclo-(Histidine-Proline) is more toxic than at 100 µg mL⁻¹. Amino acids are important sources of nitrogen for aphids, and aminopeptidases have been found in their digestive tract.²¹ As cyclic dipeptides are formed by two linked amino acids, the possibility of their hydrolysis by aminopeptidases during digestion to provide a supply of amino acids to aphids could not be ruled out. In this case, cyclo-(Histidine-Proline) would induce chitinase inhibition at the lower concentrations, but this inhibitory effect could be confounded by the supply of amino acids at the highest concentration. In contrast, psammaplin A (a derivative of tyrosine) is not a target of peptidases because tyrosine residues are linked by a disulfur bond.

Until now, published information relevant to chitinase inhibitor treatments in insects concerned only lepidoptera or diptera. Chitinase inhibitors affect the peritrophic membrane turnover, midgut chitinolytic activities and development of insects. Homoptera, including aphids, do not possess peritrophic membrane. Moreover, no chitinase and chitinolytic activity have been described yet in aphid midgut (Rahbe Y, private communication). As a consequence, a digestive target of chitinase inhibitors is excluded. However,

### Table 1. Mean (±SD) demographic parameters of *Myzus persicae* reared on diets containing chitinase inhibitors

<table>
<thead>
<tr>
<th>Demographic parameter</th>
<th>Control</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>F⁵</th>
<th>P⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) Cyclo-(Proline-Tyrosine)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of aphids assayed</td>
<td>29</td>
<td>30</td>
<td>32</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prereproductive period (days)</td>
<td>9.9 (±1.2)</td>
<td>9.9 (±1.3)</td>
<td>9.7 (±1.1)</td>
<td>10.0 (±1.1)</td>
<td>0.3</td>
<td>0.80</td>
</tr>
<tr>
<td>Daily fecundity (nymphs female⁻¹ day⁻¹)</td>
<td>1.2 (±0.4)</td>
<td>1.1 (±0.4)</td>
<td>1.1 (±0.4)</td>
<td>1.1 (±0.4)</td>
<td>0.8</td>
<td>0.51</td>
</tr>
<tr>
<td>r₀ (nymphs female⁻¹ day⁻¹)</td>
<td>0.18 (±0.04)</td>
<td>0.18 (±0.04)</td>
<td>0.17 (±0.02)</td>
<td>0.18 (±0.04)</td>
<td>0.8</td>
<td>0.49</td>
</tr>
<tr>
<td>Doubling time (days)</td>
<td>3.9 (±0.8)</td>
<td>4.0 (±0.9)</td>
<td>4.1 (±0.5)</td>
<td>4.0 (±1.0)</td>
<td>0.3</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>(B) Cyclo-(Histidine-Proline)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of aphids assayed</td>
<td>32</td>
<td>31</td>
<td>28</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prereproductive period (days)</td>
<td>10.1 (±1.3)</td>
<td>10.1 (±1.2)</td>
<td>9.9 (±0.9)</td>
<td>9.9 (±0.9)</td>
<td>0.3</td>
<td>0.82</td>
</tr>
<tr>
<td>Daily fecundity (nymphs female⁻¹ day⁻¹)</td>
<td>1.7 (±0.4)</td>
<td>1.2 (±0.5)</td>
<td>1.0 (±0.2)</td>
<td>1.3 (±0.3)</td>
<td>17.9</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>r₀ (nymphs female⁻¹ day⁻¹)</td>
<td>0.20 (±0.03) a</td>
<td>0.18 (±0.03) b</td>
<td>0.16 (±0.02) c</td>
<td>0.18 (±0.02) bc</td>
<td>7.0</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Doubling time (days)</td>
<td>3.6 (±0.6) a</td>
<td>4.0 (±0.8) bc</td>
<td>4.3 (±0.7) c</td>
<td>3.9 (±0.5) ab</td>
<td>5.5</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td><strong>(C) Allosamidin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Number of aphids assayed</td>
<td>35</td>
<td>31</td>
<td>24</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prereproductive period (days)</td>
<td>9.3 (±0.9)</td>
<td>9.7 (±0.7)</td>
<td>9.8 (±0.8)</td>
<td>9.5 (±0.6)</td>
<td>2.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Daily fecundity (nymphs female⁻¹ day⁻¹)</td>
<td>1.6 (±0.4) a</td>
<td>0.9 (±0.2) b</td>
<td>1.2 (±0.4) c</td>
<td>1.0 (±0.4) bc</td>
<td>18.5</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>r₀ (nymphs female⁻¹ day⁻¹)</td>
<td>0.20 (±0.02) a</td>
<td>0.16 (±0.02) b</td>
<td>0.16 (±0.02) b</td>
<td>0.15 (±0.04) b</td>
<td>21.3</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Doubling time (days)</td>
<td>3.4 (±0.4) a</td>
<td>4.4 (±0.9) b</td>
<td>4.4 (±0.7) b</td>
<td>5.0 (±1.5) c</td>
<td>14.4</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td><strong>(D) Psammaplin A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of aphids assayed</td>
<td>52</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prereproductive period (days)</td>
<td>9.3 (±0.7)</td>
<td>9.5 (±0.8)</td>
<td>NA</td>
<td>NA</td>
<td>2.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Daily fecundity (nymphs female⁻¹ day⁻¹)</td>
<td>1.4 (±0.4)</td>
<td>1.4 (±0.3)</td>
<td>NA</td>
<td>NA</td>
<td>0.0</td>
<td>0.84</td>
</tr>
<tr>
<td>r₀ (nymphs female⁻¹ day⁻¹)</td>
<td>0.20 (±0.03) a</td>
<td>0.20 (±0.02)</td>
<td>NA</td>
<td>NA</td>
<td>0.2</td>
<td>0.68</td>
</tr>
<tr>
<td>Doubling time (days)</td>
<td>3.6 (±0.7)</td>
<td>3.5 (±0.5)</td>
<td>NA</td>
<td>NA</td>
<td>0.8</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Values in the same row followed by the same letter indicate that they do not differ significantly according to Fisher’s PLSD test (P = 0.05).<sup>a</sup>

NA = not available, as a result of nymphal mortality.<sup>b</sup>

F, Fisher’s test value of one-way ANOVA analysis; P, value of Fisher’s test.<sup>c</sup>
Effects of chitinase inhibitors on *M. persicae*

**Figure 1.** Nymphal survival of *Myzus persicae* reared on diets containing 0 (control), 10, 50 or 100 µg mL\(^{-1}\) of (A) Cyclo-(Proline-Tyrosine), (B) Cyclo-(Histidine-Proline), (C) allosamidin and (D) psammaplin A.

several compounds have been reported to cross mesenteron cells in insects.\(^{16,22,23}\) If chitinase inhibitors could cross the digestive epithelium, then epithelial chitinases involved in cuticle degradation and remodelling during moulting would be potential action sites for chitinase inhibitors.

Chitinase inhibitors are reported to inhibit post-embryonic development of diptera and lepidoptera. For example, consumption of allosamidin and its derivatives by larvae of *Lucilia cuprina* (Wiedemann) resulted in high (85–90%) larval mortality and a reduction in body length.\(^{24}\) Allosamidin also induces an increase in larval mortality with 50% of abnormal, delayed or interrupted moulting in *Tineola bisselliella* (Hummel).\(^{24}\) Allosamidin injection in larvae of *Bombyx mori* L. and *Leucania separata* Walker strongly inhibits moulting and induces death.\(^{5}\) Likewise, larvicidal effects have been shown on *Plutella xylostella* L. reared on leaf discs treated with 500 mg kg\(^{-1}\) of psammaplin A, whereas *M. persicae* was not affected under these conditions.\(^{11}\) For the first time, the present results have highlighted an increase in homopteran larval mortality when reared on allosamidin and exposed to diets containing added psammaplin A. The present authors also observed that cuticle shedding was disturbed or larval moulting failed when insects were reared on chitinase inhibitors (data not shown). Their results also showed for the first time larvicidal effects of allosamidin and psammaplin A on immature aphids.

Remodelling of chitinous structures not only involves chitin syntheses but also chitinases during insect development. Allosamidin is responsible for chitinase inhibition but also enhances chitin biosynthesis.\(^{7–9}\) As the present authors demonstrated a reduction in fecundity in aphids, the hypothesis that chitinase inhibitors cross the mesenteron cells becomes relevant. The decrease in fecundity observed in aphids reared on allosamidin could be due to the impact of this compound on cuticle formation during larval development in the genital tract of females.

Chitinase inhibitors have shown effects against bacteria, phytopathogenic fungi and insect pests. The present results demonstrated for the first time differential aphicidal effects of chitinase inhibitors of different nature on the polyphagous aphid *M. persicae*. Thus, chitinase inhibitors are a promising lead for the development of a new strategy to protect crops. As many of these chitinase inhibitors are not presently available commercially owing to their high production cost and their chemical instability when applied on crops, it is appropriate to find new chitinase inhibitors with biopesticidal properties amenable to the commercial context.

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