UNIVERSIDADE CATÓLICA DOM BOSCO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA

Peptídeos Bioinspirados na Proteína Cry10Aa como Potencial Estratégia no Desenvolvimento de Inseticidas de Nova-Geração

Autora: Renata do Nascimento Santos
Orientadora: Dra. Maria Fatima Grossi de Sá
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Campo Grande
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“PEPTÍDEOS BIOINSPIRADOS NA PROTEÍNA CRY10AA COMO POTENCIAL ESTRATÉGIA NO DESENVOLVIMENTO DE INSETICIDAS DE NOVA-GERAÇÃO”

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“Para trabalhar com sucesso, tenha caridade
no coração e paciência na execução.”
São João Bosco

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Dedico esta tese aos meus pais, Valmir e Maria Dalva, meus grandes exemplos de empatia, humildade e honestidade, que sempre fizeram o possível e o impossível para garantir minha formação e que estiveram ao meu lado, com apoio incondicional, em cada etapa desta trajetória. Esta conquista pertence a vocês.

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BIOGRAFIA DO AUTOR

Renata do Nascimento Santos nasceu em 1996, em Campo Grande, Mato Grosso do Sul, filha de Valmir Alves dos Santos e de Maria Dalva do Nascimento Santos. Desde a adolescência, demonstrou interesse pela pesquisa científica e nutre o sonho de seguir carreira acadêmica. Iniciou sua trajetória universitária em 2014, ao ingressar no curso de Agronomia da Universidade Católica Dom Bosco (UCDB), como bolsista do Programa Universidade para Todos (PROUNI). Durante a graduação, participou ativamente do Programa de Iniciação Científica entre 2014 e 2018, período em que também realizou estágio supervisionado na Embrapa Gado de Corte, na área de Entomologia, o que consolidou seu interesse pelo controle de pragas agrícolas. Em 2019, ingressou no Mestrado em Ciências Ambientais e Sustentabilidade Agropecuária, dando continuidade às pesquisas voltadas ao manejo e controle de pragas agrícolas. Em 2021, foi aprovada em primeiro lugar no processo seletivo do curso de Doutorado em Biotecnologia, concretizando um sonho cultivado desde a graduação e dando sequência à sua formação acadêmica e científica.

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INTRODUÇÃO

CAPÍTULO I

LISTA DE ABREVIATURAS

EMBRAPA: Empresa Brasileira de Pesquisa Agropecuária
UCDB: Universidade Católica Dom Bosco
FUNDECT: Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul
FAPDF: Fundação de Apoio à Pesquisa do Distrito Federal
CAPES: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico
FINEP: Financiadora de Estudos e Projetos
FAO: Food Agriculture Organization
Bt: *Bacillus thuringiensis*
Cry: Proteínas Cristalinas
Cyt: Proteínas Citolíticas
Vip: Proteínas Inseticidas Vegetativas
DL: Dose Letal
nm: Nanômetros
mg: Miligramas
g: Gramas
kDa: Kilodalton
m: Metros
cm: Centímetros
L: Litros
mL: Mililitros
µL: Microlitros
µg: Microgramas
h: Horas
µg mL^{-1}: Microgramas por Mililitros
USD: United States Dollar

OGMs: Organismos Geneticamente Modificados

GPCRs: Receptores Acoplados a Proteína G

CPPs: Cell-Penetrating Peptides

LGICs: Ligand-gated Ion Channels

VGICs: Voltage-gated Ion Channels

nAChRs: Nicotinic Acetylcholine Receptors

PFPs: Pore-forming Insecticidal Peptides

RGCs: Receptor Guanylate Cyclases

RTKs: Receptor Tyrosine Kinases

Ach: Acetylcholine

GABA A: γ-aminobutyric Acid Type A

GluCl: Glutamate-gated Chloride Channels

sNPF: short neuropeptide F

NPF: neuropeptide F

PBAN: Pheromone Biosynthesis-activating Neuropeptide

LC_{50}: Lethal Concentration 50%

IPM: Integrated Pest Management

RMSD: Root Mean Square Deviation

RMSF: Root Mean Square Fluctuation

ANOVA: Analysis of Variance

RESUMO

As pragas agrícolas representam uma ameaça contínua à segurança alimentar e ocasionam perdas econômicas anuais superiores a US$ 220 bilhões. O manejo dessas pragas tem sido desafiado pela resistência aos inseticidas convencionais, além dos impactos ambientais e de saúde pública associados ao uso desses inseticidas. Os bioinseticidas à base de *Bacillus thuringiensis* (Bt), que utilizam proteínas pesticidas (Cry, Cyt e Vip), são alternativas relevantes, mas o uso contínuo dessas proteínas também favoreceu o desenvolvimento de resistência. Nesse contexto, os peptídeos bioativos emergem como uma nova fronteira promissora, oferecendo vantagens como maior especificidade e menor probabilidade de resistência. Este estudo teve como objetivo desenvolver e avaliar a atividade inseticida de peptídeos derivados da proteína Cry10Aa de Bt contra *Spodoptera frugiperda* e *Anthonomus grandis*. Os peptídeos foram desenhados com base na sequência da α-hélice 3, presente no domínio 1 da proteína Cry10Aa, visando potencializar sua ação. Para avaliar a segurança da aplicação, a citotoxicidade dos peptídeos foi avaliada em fibroblastos humanos (FN1). Os resultados demonstraram que a proteína Cry10Aa intacta foi mais eficaz contra *A. grandis*, embora os peptídeos (AMPCry10Aa, AMPCry10Aa_2e, AMPCry10Aa_6) também apresentaram atividade significativa. Para *S. frugiperda*, o peptídeo AMPCry10Aa_6 destacou-se na aplicação tópica (28 μg mL^{-1}), enquanto o AMPCry10Aa_5 mostrou maior potência por via injetável (56 μg mL^{-1}). Quanto à toxicidade em fibroblastos, o peptídeo AMPCry10Aa_6 não apresentou toxicidade, mesmo em altas concentrações, ao contrário do AMPCry10Aa_5 e do próprio AMPCry10Aa, que foram tóxicos apenas em doses elevadas (112 e 224 μg mL^{-1}). Ensaios *in silico* de interação molecular para elucidar os mecanismos de ação dos peptídeos mais promissores foram realizados, e estes estudos indicam que a atividade inseticida decorre de interações diretas com a membrana plasmática das células-alvo, levando à morte do inseto. Esses achados validam a estratégia de desenvolvimento de peptídeos derivados da toxina Cry10Aa como ferramentas seguras e eficazes para o controle de pragas.

Palavras-chave: *Bacillus thuringiensis*, bioinseticidas, citotoxicidade, controle de pragas, desenho racional, resistência.

ABSTRACT

Agricultural pests represent a continuous threat to food security and cause annual economic losses exceeding US$220 billion. The management of these pests has been hindered by resistance to conventional insecticides and the associated environmental and public health concerns. *Bacillus thuringiensis* (Bt)-based bioinsecticides, which employ pesticidal proteins (Cry, Cyt, and Vip), are relevant alternatives; however, their continuous use has also led to the development of resistance. In this context, bioactive peptides emerge as a promising new frontier, offering advantages such as greater specificity and a lower probability of resistance. This study aimed to develop and evaluate the insecticidal activity of peptides derived from the Cry10Aa protein of Bt against *Spodoptera frugiperda* and *Anthonomus grandis*. The peptides were designed based on the sequence of the α-helix 3 located in domain I of the Cry10Aa protein, to enhance their activity. To assess the safety of the application, the cytotoxicity of the peptides was tested in human fibroblasts (FN1). The results demonstrated that the intact Cry10Aa protein was more effective against *A. grandis*, although the peptides (AMPCry10Aa, AMPCry10Aa_2, and AMPCry10Aa_6) also exhibited significant activity. For *S. frugiperda*, the peptide AMPCry10Aa_6 stood out in topical application (28 μg mL^{-1}), whereas AMPCry10Aa_5 was more potent via injection (56 μg mL^{-1}). When the toxic potential in fibroblasts was assessed, AMPCry10Aa_6 showed no toxicity, even at high concentrations, in contrast to AMPCry10Aa_5 and AMPCry10Aa, which were toxic only at elevated doses (112 and 224 μg mL^{-1}). *In silico* molecular interaction assays were conducted to elucidate the mechanisms of action of the most promising peptides. These studies confirmed that the insecticidal activity arises from direct interactions with the plasma membrane of target cells, ultimately leading to insect death. These findings validate the strategy of developing peptides derived from the Cry10Aa toxin as safe and effective tools for insect-pest control.

Keywords: *Bacillus thuringiensis*, bioinsecticides, cytotoxicity, pest control, rational design, resistance.

1. INTRODUÇÃO

1.1 Controle de pragas na agricultura

As pragas agrícolas representam uma ameaça persistente à segurança alimentar, causando perdas de até 40% na produção agrícola mundial, o que gera prejuízos econômicos superiores a US$ 220 bilhões, dos quais pelo menos US$ 70 bilhões decorrem de danos causados por insetos-praga. Esse cenário tende a se agravar com as mudanças climáticas, podendo haver um acréscimo de 10 a 25% nas perdas para cada grau Celsius de aumento na temperatura. Atualmente, os inseticidas convencionais constituem a principal estratégia de controle, com um consumo global anual de cerca de 760 mil toneladas em 2023 (Deutsch et al., 2018; FAOSTAT, 2023; FAO, 2024).

Apesar de eficazes, muitos pesticidas não são seletivos, afetando organismos não-alvo. A obra *Silent Spring* de Rachel Carson, lançada em 1962, destacou os riscos ambientais e à saúde causados por esses químicos (Warne & Reichelt-Brushett, 2023). A exposição crônica aos agroquímicos pode gerar espécies reativas de oxigênio (ROS), promovendo estresse oxidativo, enfraquecendo as defesas celulares e contribuindo para doenças como câncer, distúrbios neurodegenerativos, hepáticos e cardiovasculares (Kaur et al., 2019; Ayilara et al., 2023). Estimativas globais indicam que, a cada ano, até 5 milhões de pessoas sofrem intoxicações graves por pesticidas, o que resulta em aproximadamente 200 mil mortes (Islam et al., 2022).

Além da saúde humana, os pesticidas ameaçam a biodiversidade e os serviços ecossistêmicos, contaminando alimentos, água, ar e solo (Zaller et al., 2022; Cech et al., 2023). Isso prejudica a ação de polinizadores, inimigos naturais e microrganismos do solo, gerando desequilíbrios ecológicos que podem culminar no colapso de cadeias tróficas e até mesmo na extinção de espécies (Rajmohan et al., 2020; Arya et al., 2022; Dhuldhaj et al., 2023). Anualmente, cerca de 710 toneladas de pesticidas alcançam os oceanos e 68 mil toneladas contaminam aquíferos (Maggi et al., 2023). Frente a esses

impactos, cresce o interesse por alternativas sustentáveis, como os biopesticidas e os organismos geneticamente modificados (OGMs), para o manejo de pragas (Abbasi-Jorjandi et al., 2020; Zhang et al., 2023).

Os biopesticidas constituem uma categoria de defensivos agrícolas em rápida expansão, com o mercado global estimado em aproximadamente US$ 5 bilhões em 2022 e projeções de crescimento para cerca de US$ 15 bilhões até 2030 (Marrone, 2024). Nesse cenário, os bioinseticidas respondem por cerca de 30% desse mercado. Entre eles, destacam-se as proteínas inseticidas Cry, produzidas por *Bacillus thuringiensis* (Bt), amplamente utilizadas tanto no controle biológico quanto em culturas transgênicas, em razão de seu modo de ação, que consiste na formação de poros no intestino de insetos-praga, levando-os à morte (Couch et al., 2023; Pacheco et al., 2023).

1.2 Proteínas Cry como agente de biocontrole de insetos-praga

A bactéria aeróbia *Bacillus thuringiensis* (Bt) é um microrganismo Gram-positivo e formador de esporos que, durante a esporulação, produz corpos de inclusão cristalinos com atividade inseticida, como as proteínas Cry, amplamente utilizadas no controle de pragas agrícolas (Jurat-Fuentes et al., 2021). A ação da Cry envolve a inserção de α-hélices na membrana intestinal dos insetos, o que forma poros letais (Figura 1). Essas proteínas inicialmente produzidas como pró-toxinas (~130 ou ~75 kDa), são solubilizadas e ativadas no intestino alcalino dos hospedeiros, gerando a toxina funcional (~60 kDa), que se liga a receptores específicos da membrana intestinal, como aminopeptidases, fosfatases alcalinas, proteínas transportadoras de cassete de ligação de caderina e proteínas associadas ao trifosfato de adenosina, levando à lise celular e morte do inseto (Maagd et al., 2001; Pardo-López et al., 2013; Endo, 2022).

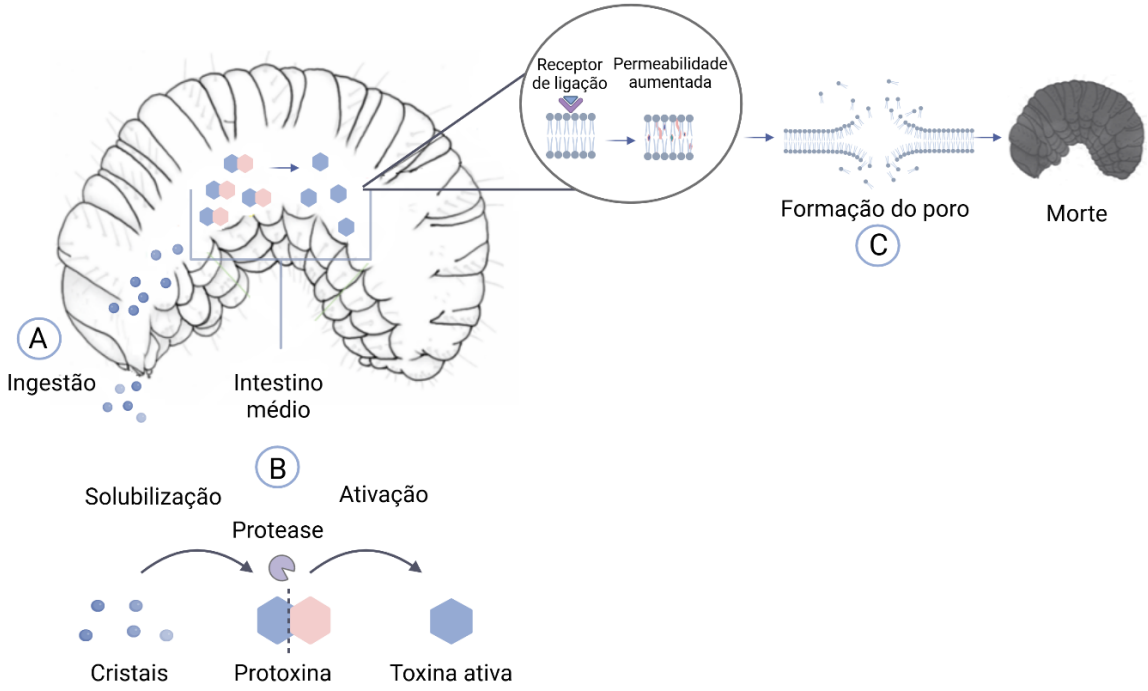

Figura 1. O mecanismo de ação das proteínas Cry em larvas de insetos ocorre em três etapas: (A) ingestão dos cristais de toxina; (B) solubilização no intestino médio alcalino, onde os cristais insolúveis são desnaturados e ativados por proteases; (C) inserção da toxina na membrana intestinal, resultando na formação de poros, paralisia do trato digestivo, lise celular e morte do inseto.

Para exercer sua atividade inseticida, as proteínas Cry apresentam uma estrutura terciária altamente conservada, composta por três domínios principais (Figura 2). O domínio I é formado por sete α-hélices antiparalelas, com uma hélice central hidrofóbica (α5) cercada por hélices anfipáticas. Este domínio, o mais conservado entre as toxinas, sofre clivagem proteolítica, o que pode estar relacionado à sua função de inserção na membrana e formação de poros. O domínio II é constituído por três folhas β antiparalelas e duas α-hélices curtas, e está associado à especificidade de ligação da toxina aos receptores e ao processo de oligomerização. Por fim, o domínio III apresenta duas folhas β antiparalelas e está envolvido na interação com os receptores e na estabilidade

estrutural durante a inserção na membrana (Adang et al., 2014; Lucena et al., 2014; Palma et al., 2014; Rios et al., 2024).

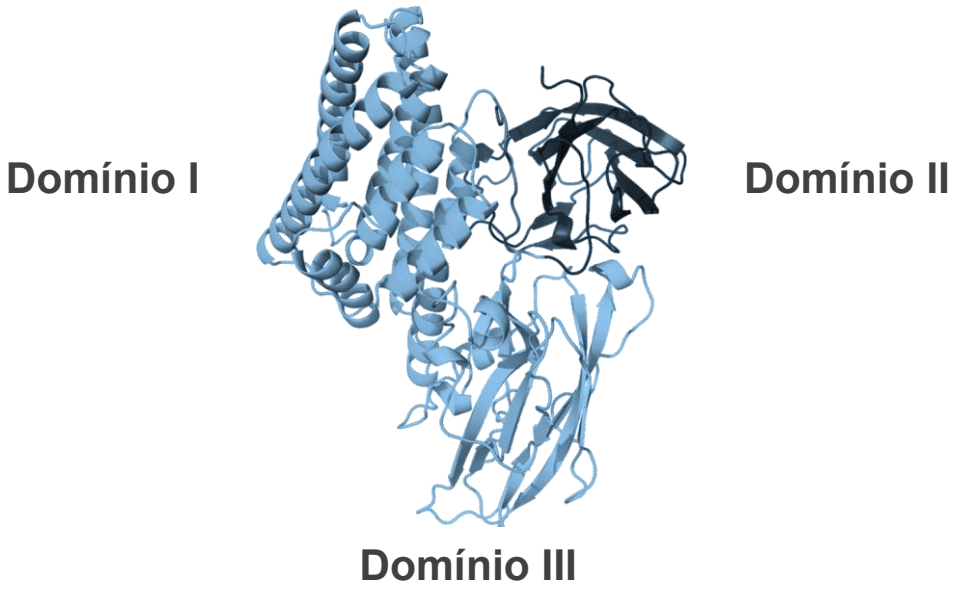

Figura 2. A estrutura geral das proteínas Cry é composta por três domínios: o domínio I, formado por sete α-hélices antiparalelas; o domínio II, organizado em três folhas β antiparalelas e duas α-hélices curtas; e o domínio III, constituído por duas folhas β antiparalelas. Gerado a partir do código PDB 6DJ4 da proteína Cry1A (Software PyMol).

Mais de 750 variantes de proteínas Cry já foram identificadas, evidenciando a ampla diversidade e a elevada especificidade dessas toxinas, o que reforça sua relevância em programas de manejo integrado de pragas (Crickmore et al., 2021; Gu et al., 2021; Panneerselvam et al., 2022). A ação dessas proteínas é direcionada a diferentes ordens de insetos, como é demonstrado na Tabela 1 (Mohanty et al., 2025). Devido a essas propriedades, são amplamente incorporadas em organismos geneticamente modificados (OGMs) que as expressam, possibilitando o controle eficiente de pragas agrícolas. Entretanto, a adoção de práticas de cultivo inadequadas tem comprometido a efetividade dessa tecnologia a longo prazo (Taylor et al., 2021; Guan et al., 2023).

Tabela 1. Famílias de proteínas Cry de *Bacillus thuringiensis* e suas respectivas ordens de insetos-alvo.

Família de proteínas	Ordem de insetos-alvo	Exemplos / observações principais	Referências
Cry1, Cry2, Cry9	Lepidoptera	Amplamente utilizadas no controle de lepidópteros; interação com receptores Caderina, APN, ALP e transportadores ABC (ABCC2); formação de oligômeros essencial para toxicidade	Vadlamudi et al. (1995); Gómez et al. (2002); Bravo et al. (2004); Endo et al. (2017); Huang et al. (2020)
Cry4A, Cry4B, Cry11A, Cry11B	Diptera	Toxinas mosquitocidas; ligação a ALP, APN, caderina e α-amilase; atuam principalmente em *Aedes* e *Anopheles*	Berry et al. (2002); Krieger et al. (1999); Fernandez et al. (2006); Chen et al. (2009a,b)
Cry3	Coleoptera	Atividade contra besouros; requer ativação proteolítica distinta; interação com Caderina e transportadores ABC (ABCB1)	Carroll et al. (1997); Park et al. (2009); Niu et al. (2020)
Cry7, Cry8, Cry10 Cry22, Cry23, Cry34, Cry55	Coleoptera	Amplo espectro contra coleópteros; diferentes famílias com modos de ação complementares	Ribeiro et al. (2019); Wang et al. (2019a); Shu et al. (2020)

1.3 Resistência aos métodos de controle atuais

Bioinseticidas e OGMs baseados em *Bt*, especialmente os que expressam as toxinas Cry e Vip, destacam-se no controle de pragas agrícolas e no desenvolvimento de cultivos transgênicos (Gangwar et al., 2021; Pinheiro & Valicente, 2021; Couch et al., 2023). O uso dessa tecnologia tem gerado múltiplos benefícios, incluindo aumento da produtividade agrícola, menor dependência de pesticidas químicos, redução das emissões de gases de efeito estufa, conservação da qualidade do solo e menores taxas de desmatamento na expansão agrícola (Kumar et al., 2020; Wu et al., 2021; Caradus, 2022). No entanto, assim como ocorreu com compostos químicos convencionais, sua eficácia tende a decrescer ao longo do tempo devido ao surgimento de populações resistentes. Os insetos-praga desenvolvem adaptações fisiológicas, como modificações nos sítios-alvo e aumento da atividade de enzimas de detoxificação, além de alterações comportamentais, como a evasão das áreas tratadas, reduzindo, assim, a efetividade dos inseticidas (Nauen et al., 2021; Bass e Nauen, 2023; Hubbard e Murillo, 2024).

O avanço da resistência tem sido agravado pela ausência de práticas agrícolas eficazes, como o manejo integrado de pragas, a rotação de culturas, a implantação insuficiente de áreas de refúgio e a falta de diversificação e rotatividade de ingredientes ativos (Taylor et al., 2021; Hubbard e Gerry, 2020; Guan et al., 2023). Desde 2010, a inovação em pesticidas tem sido limitada, concentrando-se em variações de compostos já existentes, pois o desenvolvimento de novas moléculas é dificultado por altos custos, complexidade e rigor regulatório (Sparks e Bryant, 2020). Isso torna os agricultores dependentes de poucos ingredientes ativos, tanto nos inseticidas químicos quanto nos materiais transgênicos que expressam proteínas entomopatogênicas, intensificando a pressão seletiva sobre as pragas (Phillips, 2020).

Nos últimos anos, os avanços nas áreas de biotecnologia e bioinformática têm favorecido o desenvolvimento de alternativas aos defensivos químicos sintéticos e às plantas transgênicas de primeira geração (Wang et al., 2025). Nesse cenário, os biopesticidas à base de peptídeos se destacam como uma das estratégias mais promissoras para o manejo de pragas agrícolas, devido à sua alta seletividade e ao baixo impacto ambiental. As pesquisas voltadas aos peptídeos com atividade inseticida têm avançado significativamente na proteção de culturas, culminando no lançamento de produtos inovadores, como o bioinseticida Spear®, introduzido nos Estados Unidos em 2020 (Zhang et al., 2023; Vikas e Ranjan, 2024).

1.4 Peptídeos inseticidas no manejo de pragas

Peptídeos são biomoléculas curtas, com 2 a 70 aminoácidos unidos por ligações peptídicas, podendo ser naturais ou sintéticos, e obtidos por hidrólise de proteínas, síntese química, fermentação ou engenharia genética (Zhang et al., 2023). Eles são amplamente estudados por suas funções, estrutura e aplicações, sendo empregados em áreas como a medicina, os cosméticos e a agricultura. Na proteção de plantas, atuam como antimicrobianos, reguladores de crescimento, herbicidas e inseticidas. Peptídeos inseticidas naturais derivam principalmente dos venenos de artrópodes, mas também são encontrados em outros animais, plantas e microrganismos, além de poderem ser

projetados como análogos aos compostos endógenos de insetos (Grover et al., 2021; Akbarian et al., 2022; Liu et al., 2021; Zhang et al., 2023).

Esses peptídeos apresentam eficácia contra insetos-praga por meio de diferentes modos de ação, sendo classificados em peptídeos formadores de poros, que se ligam a receptores específicos dos insetos, promovendo a formação de poros que levam à lise celular e à morte. Peptídeos inibidores de enzimas digestivas, que atuam no trato digestivo, comprometendo a absorção de nutrientes. Peptídeos neurotóxicos, que afetam canais iônicos (cálcio, sódio e potássio) e receptores neuronais. Por último, os peptídeos hormonais e neuropeptídeos, que imitam ou antagonizam hormônios essenciais a processos vitais como a ecdise e o crescimento (Figura 3) (Liu et al., 2021; Wang et al., 2022; Raisch e Raunser, 2023).

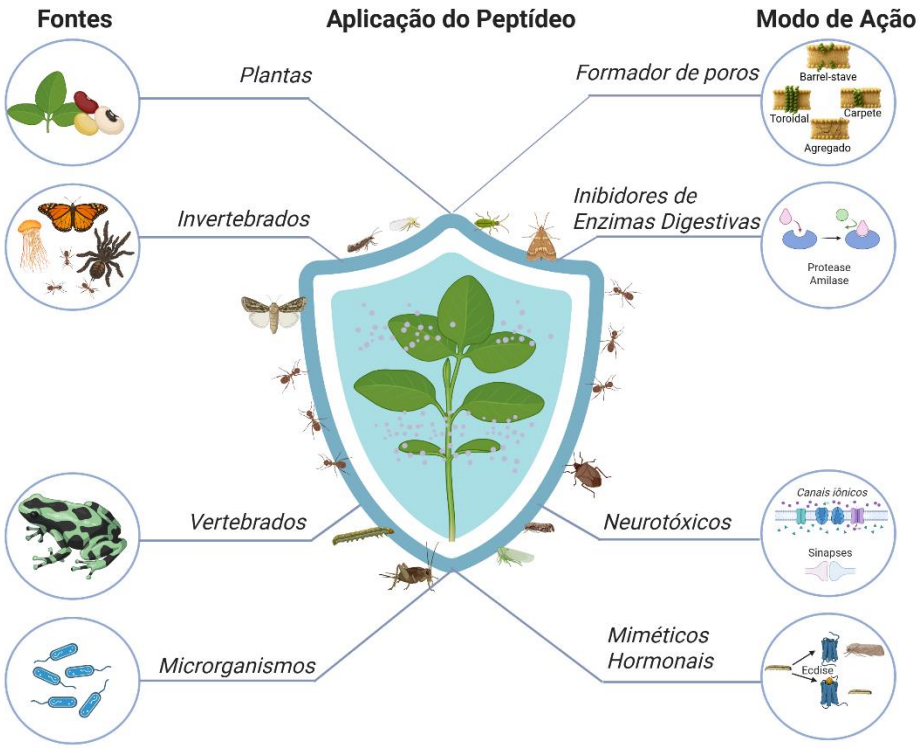

Figura 3. Representação esquemática das principais fontes, da aplicação e dos mecanismos de ação de peptídeos inseticidas. Os peptídeos, derivados de plantas, invertebrados, vertebrados e microrganismos, atuam na proteção das plantas por meio da formação de poros em membranas, inibição de enzimas digestivas, ação neurotóxica e mimetismo hormonal, comprometendo o desenvolvimento e a sobrevivência dos insetos-praga.

Peptídeos formadores de poros são moléculas, tanto naturais quanto sintéticas, capazes de se inserir em membranas celulares e formar poros transmembranais. A formação desses poros compromete a integridade da membrana, promovendo um fluxo desregulado de íons e solutos, o que pode resultar em disfunções celulares e levar à morte celular por lise, perda da homeostase ou apoptose (Jacob e Kahn, 2022; Alimohamad et al., 2023; Has e Das, 2023). A literatura destaca peptídeos formadores de poros com atividade inseticida promissora, especialmente aqueles derivados de artrópodes, como o M-Tb1a e o U9, identificados no veneno da formiga-da-guiné (*Tetramorium bicarinatum*), e a cupienina, isolada do veneno da aranha-tigre-bromélia (*Cupiennius salei*) (Araújo et al., 2022; Ascoët et al., 2023).

De modo análogo aos artrópodes, as plantas produzem peptídeos tóxicos como estratégia de defesa, destacando-se os inibidores de enzimas digestivas. Esses peptídeos bloqueiam amilases e proteases intestinais por ligação competitiva ao sítio ativo ou por induzir alterações conformacionais que reduzem a atividade enzimática, envolvendo interações hidrofóbicas, pontes salinas e ligações de hidrogênio. Como consequência, ocorrem diminuição da absorção de nutrientes, atraso no desenvolvimento, redução da fecundidade e, em alguns casos, mortalidade dos insetos (Pandey et al., 2022; Wang et al., 2022). Entre os principais exemplos estão os peptídeos do tipo Knottin (≈3 kDa), isolados de *Amaranthus hypochondriacus*, *Alternanthera sessilis* e *Chenopodium quinoa*, capazes de inibir completamente as α-amilases de *Tribolium castaneum* e *Callosobruchus chinensis* (Rane et al., 2020). Adicionalmente, inibidores de metalocarboxipeptidase do tomate e da batata potencializam a ação da toxina Cry1Ac contra *Helicoverpa armigera*, enquanto os dipeptídeos DI1 e DI2 inibem as tripsinas intestinais, promovendo mortalidade e redução de peso em *Anticarsia gemmatalis* (Chen et al., 2021; Meriño-Cabrera et al., 2022).

Peptídeos neurotóxicos também são sintetizados como estratégia de defesa ou de predação e são amplamente estudados. Essas moléculas atuam modulando os processos neurológicos dos insetos, destacando-se por sua eficácia e seletividade. Sua ação ocorre principalmente por meio de canais dependentes de ligantes (Bloomquist et al., 2023) e de canais iônicos voltagem-dependentes (Na^+, Ca^{2+} ou K^+) (Yan et al., 2025).

Entre os canais ativados por ligantes, destaca-se o peptídeo GS-ω/κ-Hxtx-Hv1a, do veneno da aranha-teia-de-funil *Hadronyche versuta*, que atua como modulador alostérico dos receptores nicotínicos de acetilcolina e é comercializado nos EUA como *Spear*®, com alta seletividade para polinizadores. Já a neurotoxina PPTX-04, isolada da aranha-lobo-de-lagoa *Pardosa pseudoannulata*, atua sobre canais de sódio voltagem-dependentes, prolongando sua ativação de modo semelhante aos piretroides (Wang et al., 2024).

Ao explorar as semelhanças com compostos endógenos dos insetos, desenvolveu-se a classe de antagonistas peptidomiméticos capazes de inibir a ação de neuropeptídeos, hormônios peptídicos e seus receptores acoplados à proteína G (GPCRs), essenciais para funções vitais nos insetos (Lyison et al., 2021; Toprak, 2020; Liu et al., 2021). Esses peptídeos participam da regulação de processos como a mobilização de lipídios (hormônio adipocinético), a síntese de hormônio juvenil (alatostatina), o crescimento (hormônio de diapausa) e o comportamento alimentar (Hou et al., 2023). A elevada especificidade dos GPCRs em insetos favorece o desenvolvimento de bioinseticidas mais seletivos e seguros (Elakkiya et al., 2019). Um exemplo promissor é o análogo de alatostanina Q6, que apresentou forte inibição do hormônio juvenil na barata *Diploptera punctata* e atividade inseticida inesperada contra a mariposa *Plutella xylostella*, indicando potencial para um amplo espectro de ação inseticida (Zhang et al., 2024).

Embora eficazes, os peptídeos inseticidas enfrentam limitações comerciais devido à degradação por enzimas dos insetos e a fatores ambientais, o que reduz sua estabilidade e biodisponibilidade, especialmente em aplicações por pulverização em larga escala (Fassolo et al., 2024; Zhang et al., 2023). Os altos custos de produção também são apontados como uma barreira, mas o bioinseticida peptídico *Spear*® mostrou viabilidade econômica comparável à de inseticidas convencionais, graças ao uso de sistemas heterólogos de produção em bactérias, fungos e plantas, que possibilitam a fabricação em larga escala (Parachin et al., 2012; Narayani et al., 2020; Zhang et al., 2023).

Ferramentas de bioinformática também têm sido essenciais para o desenvolvimento de novos peptídeos inseticidas. Algoritmos de IA e bancos, como *o Protein Data Bank*

(PDB) e o UniProt, permitem a identificação *e a* predição de propriedades estruturais (Wan et al., 2022; Zhai et al., 2025). Modelos como AlphaFold3 e ESMFold, ao integrarem dados de sequência e estrutura, também têm revolucionado o design racional de peptídeos com potencial de aplicação agrícola (Lin et al., 2023; Varadi et al., 2024; Ma et al., 2022). Além disso, estratégias como substituição de aminoácidos, ciclização, fusão com peptídeos penetrantes celulares (CPPs), conjugação com toxinas, uso de entomopatógenos transgênicos e encapsulamento com nanomateriais também têm ampliado a eficácia dos peptídeos inseticidas (Figura 4) (Civolani et al., 2025; Darif et a., 2023; Lee e Poh, 2023; Zhang et al., 2023).

Figura 4. Estratégias para melhorar a biodisponibilidade e estabilidade de peptídeos inseticidas.

Substituições de L por D-aminoácidos e inclusão de aminoácidos não naturais conferem maior resistência proteolítica aos peptídeos (Chatterjee et al., 2013; Wang et

al., 2022). Alterações nas cadeias laterais aumentam a afinidade e a seletividade, como observadas em fármacos peptídicos, como semaglutida e liraglutida (Henninot et al., 2018; Garcia e Torres et al., 2018). Ciclizações estruturais também conferem grande resistência à degradação enzimática, sendo altamente desejáveis para aplicações em campo, pois aumentam a meia-vida do peptídeo em ambientes agrícolas ou no trato digestivo dos insetos (Chen et al., 2024; Tang et al., 2025).

Para aumentar a sua toxicidade oral, estratégias como a fusão de peptídeos inseticidas com CPPs ampliam a absorção e a eficácia (Wu et al., 2024). Além disso, o uso de nanocarreadores, como metais, polímeros, lipídios e carbono, tem apresentado bons resultados. Nesse contexto, verificou-se maior toxicidade contra *Tribolium castaneum* quando peptídeos inibidores de tripsina foram encapsulados em nanopartículas de grafeno e de zinco (Mehmood et al., 2024). O uso de entomopatógenos transgênicos (*Bacillus thuringiensis*, *Metarhizium*) e de proteínas, como lectinas e Cry, também favorece a translocação intestinal de peptídeos inseticidas (Herzig et al., 2014; Sukiran et al., 2022; Ross et al., 2025). Isso já é demonstrado comercialmente em produtos da empresa Vestaron, como o Spear® LEP e o Spear®RC, peptídeos inseticidas comercializados para aplicação conjunta com bioinseticidas de *B. thuringiensis* (Vestaron, 2025).

Apesar de os principais bioinseticidas utilizados na agricultura serem de *B. thuringiensis*, existem poucos peptídeos inseticidas derivados de microrganismos ou de suas toxinas (Ortiz e Sansinenea, 2021; Zhang et al., 2023). Pesquisas *in silico* demonstraram que a toxina Cry8Aa possui uma sequência de aminoácidos que, mesmo isolada do domínio completo, mantém seu potencial de interação com membranas lipídicas (Lin et al., 2014). Partindo dessa premissa, foram desenvolvidos peptídeos antimicrobianos derivados da toxina Cry10Aa, contendo regiões catiônicas capazes de interagir com a bicamada lipídica das membranas bacterianas, o que resultou em atividade antimicrobiana positiva dessas moléculas (Rios et al., 2024).

Assim como nas bactérias, as membranas de insetos também são constituídas por uma bicamada lipídica, composta majoritariamente por fosfolipídios (Yeh et al., 1997). Assim, os peptídeos derivados da proteína Cry10Aa, desenvolvidos por Rios et al. (2024),

têm grande probabilidade de atuar na membrana dos insetos, causando efeitos inseticidas contra pragas agrícolas. Neste estudo, avaliamos a atividade inseticida de peptídeos derivados da proteína Cry10Aa contra as pragas agrícolas *Anthonomus grandis* e *Spodoptera frugiperda*. Adicionalmente, conduzimos testes de citotoxicidade em culturas de fibroblastos humanos, visando garantir a sua segurança de uso em aplicações agrícolas futuras.

Diante da complexidade dos desafios fitossanitários na agricultura, torna-se urgente o desenvolvimento de estratégias inovadoras em biotecnologia para o controle de pragas agrícolas, reduzindo a dependência de agroquímicos convencionais e ampliando a sustentabilidade do setor.

2. OBJETIVOS

Objetivo geral

Desenvolver e avaliar uma estratégia inovadora para a formulação de inseticidas de nova geração, utilizando peptídeos bioinspirados na proteína Cry10Aa, para o controle de insetos-praga de importância agrícola.

Objetivos específicos

- Avaliar o índice de sobrevivência do inseto modelo *Anthonomus grandis*, em uma dose letal de Cry10Aa, comparando os peptídeos sintéticos bioinspirados com a proteína inseticida de origem;
- Estabelecer a atividade inseticida dos peptídeos AMPCry10Aa, AMPCry10Aa_1, AMPCry10Aa_2, AMPCry10Aa_3, AMPCry10Aa_4, AMPCry10Aa_5 e AMPCry10Aa_6, por meio de metodologias de aplicação tópica e injetável na lagarta polífaga *Spodoptera frugiperda*, determinando faixas de concentração eficazes;
- Conduzir a análise da dinâmica de interação molecular por meio de abordagem *in silico*, avaliando a interação entre os peptídeos inseticidas e os fosfolipídeos presentes na membrana celular de *S. frugiperda*;
- Avaliar a citotoxicidade dos peptídeos inseticidas em células de fibroblastos humanos (FN1) *in vitro*, determinando as faixas de concentração seguras para o uso dessas novas moléculas na agricultura.

3. CAPÍTULO I

Insecticidal peptides as sustainable tools for future agriculture

Esse capítulo corresponde ao artigo de revisão da tese que foi submetido à revista: Elsevier – Biotechnology Advances (ISSN 1873-1899).

Biotechnology Advances

Insecticidal peptides as sustainable tools for future agriculture

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	The increasing global human population and the intensification of agriculture present unprecedented challenges for pest control. The escalating resistance of pests to conventional synthetic insecticides, coupled with ecological and health concerns, underscores the urgent need for innovative and sustainable management approaches. Insecticidal peptides, due to their structural diversity, molecular specificity, and biodegradability, are emerging as promising candidates for the development of next-generation bioinsecticides. This strategic roadmap synthesizes recent advances in peptide architectures, ranging from pore-forming scaffolds to designs targeting enzyme inhibition and mimicking neuroendocrine actions, with a focus on the molecular mechanisms underpinning their selectivity and efficacy. By integrating structure–function insights with translational frameworks, we identify critical knowledge gaps and propose a pathway towards biotechnological tools, including artificial intelligence (AI)-guided peptide engineering, bioinspired synthesis, and nanodelivery systems for controlled release. Our analysis positions peptide-based insecticides at the forefront of sustainable agriculture, with the potential to minimize off-target effects, reduce environmental impact, and enhance crop resilience in the face of global change.

FRANCO'S LAB

To the Editor-in-chief

Biotechnology Advances

Dear Professor Edward A. Bayer,

We are pleased to submit the manuscript entitled "**Insecticidal Peptides as Sustainable Tools for Future Agriculture**" for consideration as a Review article in **Biotechnology Advances**.

This review presents a comprehensive, up-to-date, and integrative analysis of insecticidal peptides as next-generation biotechnological tools for sustainable pest management. The manuscript addresses a pressing challenge in global agriculture: reducing dependence on synthetic chemical insecticides while maintaining crop productivity, environmental safety, and food security. By integrating recent advances in molecular biology, peptide engineering, and agrobiotechnology, the review provides a unified framework for understanding the potential of peptide-based insecticides. Adopting a systems-level perspective, insecticidal peptides are organized according to their primary modes of action, including membrane disruption, digestive enzyme inhibition, neurotoxicity, and endocrine modulation. By linking structure–function relationships to physiological targets and potential field-level applications, the review demonstrates how mechanistic diversity and biological specificity can be leveraged to mitigate resistance development and strengthen long-term integrated pest management strategies. In addition, the manuscript highlights emerging translational enablers, such as AI-guided peptide design, bioinspired engineering, and advanced delivery platforms, underscoring their role in accelerating the transition from discovery to application. In this context, the review aligns closely with the scope and interests of Biotechnology Advances.

We confirm that this manuscript is original, not under consideration elsewhere, and approved by all authors. We appreciate your time and consideration and would be honored to contribute to Biotechnology Advances.

Best regards,

Those are the referees who are suggested to evaluate this paper better.

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FRANCO'S LAB

To the Editor-in-chief

Biotechnology Advances

Competing Interests

We hereby submit the manuscript entitled “Insecticidal Peptides as Sustainable Tools for Future Agriculture” for consideration as a Review article in *Biotechnology Advances*. This manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All authors contributed collaboratively to the conceptualisation, development, critical analysis, and writing of the manuscript, have approved the final version, and agree with its submission to this journal.
The authors declare no competing financial or non-financial interests that could have influenced the conception, execution, interpretation of the results, or the writing of this manuscript.

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Insecticidal peptides as sustainable tools for future agriculture

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Abstract: The increasing global human population and the intensification of agriculture present unprecedented challenges for pest control. The escalating resistance of pests to conventional synthetic insecticides, coupled with ecological and health concerns, underscores the urgent need for innovative and sustainable management approaches. Insecticidal peptides, due to their structural diversity, molecular specificity, and biodegradability, are emerging as promising candidates for the development of next generation bioinsecticides. This strategic roadmap synthesizes recent advances in peptide architectures, ranging from pore-forming scaffolds to designs targeting enzyme inhibition and mimicking neuroendocrine actions, with a focus on the molecular mechanisms underpinning their selectivity and efficacy. By integrating structure function insights with translational frameworks, we identify critical knowledge gaps and propose a pathway towards biotechnological tools, including artificial intelligence (AI) guided peptide engineering, bioinspired synthesis, and nanodelivery systems for controlled release. Our analysis positions peptide-based insecticides at the forefront of

sustainable agriculture, with the potential to minimize off-target effects, reduce environmental impact, and enhance crop resilience in the face of global change.

Keywords: bioinsecticides; agricultural biotechnology; selective toxicity; bioinspired molecules.

Highlights

- Insecticidal peptides enable selective and sustainable pest control.
- Structure–function relationships underpin multiple insect-specific modes of action.
- Emerging molecular targets reduce resistance risks.
- AI-driven design and advanced delivery accelerate translation to the field.
- Peptide-based insecticides align with integrated pest management strategies.

1 Introduction

Insecticides have played a fundamental role in protecting crops and ensuring global food security. However, their intensive use has accelerated the evolution of resistance in pest insects (Patel et al., 2025; Singh et al., 2023; Sparks, 2025). The efficacy of these compounds is increasingly limited by metabolic, behavioral, and genetic resistance mechanisms, while their environmental impacts and health risks intensify the demand for safer and more sustainable alternatives (Liang et al., 2025; Wang et al., 2024a; Zhou et al., 2025a). These challenges compromise pest management and hinder progress toward global targets, including several Sustainable Development Goals (SDGs) established by the UN (Pu and Chung, 2024; Melo et al., 2024; Jeyaseelan et al., 2024).

Despite this, worldwide pesticide use remains high, exceeding 3.73 million tons annually, while agricultural losses from pests, pathogens, and weeds continue to

exceed 220 billion USD (FAO, 2024; FAOSTAT, 2025). Under global warming scenarios, such damage may increase by 10–25% per additional degree Celsius (Deutsch et al., 2018; Yang et al., 2024). The low selectivity of many pesticides also affects non-target organisms, and chronic exposure can induce oxidative stress and severe diseases (Kaur et al., 2019; Ayilara et al., 2023). Globally, up to 5 million people suffer severe pesticide poisoning each year, resulting in approximately 200,000 deaths, reinforcing the need for safer and more specific solutions (Islam et al., 2022).

In this context, biological control and biopesticides have advanced as promising alternatives (Galli et al., 2024; Vermelho et al., 2024), driving a market projected to reach USD 14.39 billion by 2030 (Poli and Fontefrancesco, 2024; Marrone, 2024). Among these technologies, insecticidal peptides stand out for their selectivity, low environmental persistence, and broad diversity of modes of action (Zhang et al., 2024; Schultz et al., 2024; Civolani et al., 2025). These compounds are already applied in areas such as pharmacology and cosmetology and, more recently, in agriculture, acting through pore formation, enzyme inhibition, ion channel blockade, or hormonal modulation. Moreover, such molecules could originate from arthropods, plants, microorganisms, and vertebrates (Diya et al., 2024; Wu et al., 2024; Raisch and Raunser, 2025; Zhou et al., 2025b).

Advances in synthetic biology, bioinformatics, and AI have accelerated the discovery and optimization of new peptides through structural modeling and *in silico* screening (Iram et al., 2024; Gong et al., 2024; Melo and Andrade, 2024; Arunachalam et al., 2025). Strategies such as lipopeptide modification, nanoencapsulation, and controlled-release systems enhance stability, specificity, and field performance (Patel et al., 2025; Zai et al., 2025; Pang et al., 2025). In addition to environmental benefits, insecticidal peptides may reduce costs associated with environmental degradation and biodiversity

loss, particularly benefiting smallholder farmers. Comparative life-cycle assessment (LCA) studies indicate that peptide-based bioinsecticides have a significantly smaller environmental footprint than synthetic pesticides, reducing carbon emissions and soil and water contamination (Nemecek et al., 2022; Voglhuber-Slavinsky et al., 2022).

This review integrates structural and functional analyses with practical applicability, providing a detailed examination of advances in these compounds by exploring their multiple mechanisms of action and discussing strategies for their optimization in integrated pest management. By examining structure–function relationships, it identifies critical knowledge gaps and offers strategic guidance for advancing biotechnological research in the field.

2 Classical architectures of insecticidal peptides

Insecticidal peptides (2 to 70 amino acids) (Bojarska et al., 2024; Bizzotto et al., 2024; Xie et al., 2025) exhibit great variation, reflecting the diversity of their biological functions and mechanisms of action (Zhang et al., 2023). Among the smallest insecticidal peptides described is I-3 (YLRLRFa, a short analogue of the neuropeptide F of *Acyrthosiphon pisum*), composed of only six amino acids with an approximate mass of 870 Da, with high specificity and efficacy (Zhou et al., 2025b). In contrast, one of the largest insecticidal peptides is oxytocin 1, isolated from the venom of the spider *Oxyopes lineatus*, which contains 69 amino acids (~8 kDa), has a cyclic structure, and possesses five disulfide bonds (Estrada et al., 2016). This variation highlights the importance of primary sequence and folding for specificity and mechanism of action (Agoni et al., 2025).

In conformational terms, insecticidal peptides can be grouped into five main structural classes—extended linear peptides, α-helical peptides, β-structured peptides, hybrid

α/α and α/β peptides, and cyclic peptides—each associated with distinct properties of stability, selectivity, and molecular interaction (Ji et al., 2024; Davis et al., 2025; Zhang et al., 2024). Extended linear peptides, such as the derivative of the conopeptide Bt038, lack a defined secondary structure but become functional upon interacting with cell membranes. Rich in glycine, proline, and tryptophan, they exhibit structural flexibility that facilitates penetration and disruption of target cells. In contrast, α-helical peptides, such as bicarinalin, display an amphipathic conformation that favors insertion into the lipid bilayer, leading to pore formation and cell lysis. Additionally, variations in helix folding confer different ionic selectivity and conductivity properties to these peptides (Chen et al., 2023; Ascoët et al., 2023; Rossetti et al., 2025). β-structured peptides, stabilized by disulfide bonds, interact selectively with membrane lipids, as exemplified by gomesin from spiders (Fassolo et al., 2024). Hybrid structures, such as the peptide BmP01 from the scorpion *Buthus martensii*, combine α-helices and β-sheets and act on the membrane and ion channels (Ji et al., 2024; Miyashita et al., 2024). Finally, cyclic peptides, formed by a stabilized β-sheet and three disulfide bonds in a cyclic structure with a cysteine knot motif, are represented by cyclotides and θ-defensins. They exhibit high resistance to enzymatic degradation and a prolonged half-life, making them promising for agricultural use (Hang et al., 2024; Tang et al., 2025). This structural diversity ensures multiple mechanisms of action and high specificity for insecticidal peptides.

3 Mechanisms of action and targets of insecticidal peptides

The multiple modes of action of insecticidal peptides are essential for mitigating the development of resistance in insect pests. By acting through simultaneous or complementary mechanisms, these compounds reduce the selection of resistant populations, especially when integrated into strategies involving monitoring and

product rotation (Pu and Chung, 2024). Their high selectivity for insect-specific targets also minimizes impacts on non-target organisms, including vertebrates and beneficial insects, reinforcing their low toxicity and contribution to sustainable practices (Scieuzo et al., 2024; Zhou et al., 2025a). Table 1 summarizes the main insecticidal mechanisms and their molecular targets recently described, which are discussed throughout this strategic framework. Figure 1 visually integrates these mechanisms, bringing together pore-forming peptides, enzyme inhibitors, neurotoxins, and hormonal mimetics, highlighting their targets and physiological effects to facilitate a comparative understanding of their functional diversity.

Table 1 | Insecticidal mechanisms of action of different classes of bioactive peptides reported in the literature between 2021 and 2025. Categorized by mode of action, molecular target, pore or receptor type, physiological effects, and representative examples, encompassing natural and synthetic compounds from diverse origins with bioinsecticidal potential.

Category	Molecular Target	Mode of Action	Physiological Effects	Peptide	References
Pore-formers in cell membrane					
Barrel-stave	Lipid bilayer of the cell membrane	Peptides align parallel, forming a transmembrane channel lined by peptides	Loss of membrane integrity, ionic leakage, cell lysis	Oxyopinina 1	Wang et al (2024b)
Toroidal pore	Lipid bilayer of the cell membrane	Interaction with lipids to form pores connecting leaflets of the bilayer, promoting positive curvature	Controlled permeabilization, membrane destabilization, cell death	Cupienina-1a	Kuhn-Nentwig (2021)
Carpet	Lipid bilayer of the cell membrane	Accumulation of peptides on the surface until rupture, detergent-like action	Sudden loss of membrane integrity, cell lysis	U9	Ascoët et al (2023)
Aggregate	Lipid bilayer of the cell membrane	Formation of peptide-lipid complexes creates unstable pores	Depolarization, loss of homeostasis, and cell lysis	Kalata B1	Huang et al (2024) Deegala et al (2025)
Digestive Enzyme Inhibitors					
Reversible competitive	Trypsin	Specific inhibition of intestinal trypsin by zymogen-derived sequence	Reduced growth, selective mortality in *P. interpunctella* larvae	N-terminal trypsin pro-peptide	Hemmati et al (2021)
Reversible competitive	Trypsin	Inhibition and transcriptional reprogramming of intestinal epithelium	Digestive, hormonal, and immune disturbances; reduced reproduction	Synthetic tripeptides derived from SKTI	Santos et al (2025)
Non-competitive	Trypsin and α-amylase	Binding to allosteric sites, causing enzyme conformational change	Partial enzymatic activity inhibition: systemic and multifactorial impact	Plant defensins	Mulla and Tamhane (2023)
Mimetic Insecticidal Neuropeptides and Neurotoxins					
Ligand-gated ion channels (LGICs)	nAChRs	Agonism, desensitization, or positive allosteric modulation	Synaptic blockade, paralysis, neuroexcitation	GS-ω/κ-Hxtx-Hv1a	Bloomquist et al (2023) Ross et al (2025)
Ligand-gated ion channels (LGICs)	$GABA_a$	Receptor antagonismo	Neural hyperexcitability, seizures, death	Scorpion and spider toxins	Raisch and Raunser, (2023) Chen and Hawthorne (2025)

Ligand-gated ion channels (LGICs)	GluCl	Agonism/antagonismo	Signaling inhibition, motor function collapse	Spider and nematode-derived toxins	Rosa et al (2024) Guo et al (2023)
Voltage-gated ion channels (VGICs)	Na^+V	Stabilization in the open state or blockage	Sustained depolarization, paralysis	α- and β-toxins (spiders)	He (2025)
Voltage-gated ion channels (VGICs)	K^+V	Inhibition of closing, prolonging the action potential	Prolonged depolarization, neuromotor colapse	κ-hexatoxin-type toxins	Zhou et al (2022)
Voltage-gated ion channels (VGICs)	$Ca^{2+}V$	Channel blockage	Neurotransmitter release inhibition	ω-Atypitoxins (spiders)	Yan et al (2025)
Voltage-gated ion channels (VGICs)	Ryanodine receptors (RyR)	Activation or inhibition	Distortion of Ca^{2+} release, muscle dysfunction	Scorpion toxins	AlShammari et al (2023)
Neuropeptide receptors	Neuropeptide hormone receptors	Competitive agonism or antagonismo	Hormonal and metabolic deregulation	Synthetic mimetic neuropeptides	Paschapur et al (2025)
Alternative Channel Modulators	TRP channels	Thermal, chemical or mechanical modulation	Aberrant sensory stimuli, disorientation	Experimental peptides	Zhang et al (2022)
Alternative Channel Modulators	KCa channels	Inhibition of excitability regulation	Synaptic dysfunction, collapse of electrical signaling	Emerging targets	Paschapur et al (2025)
Hormonal and Mimetic Insecticidal Peptides					
Allatostatins (type C)	AstR-C receptor in the corpora allata	Binding to GPCR inhibits JH synthesis	Developmental delay / selectivity	Agonists Q6, A15	Kahveci et al (2024)
Various peptides	Neuropeptide-dependent GPCRs	Act via GPCRs in multiple phases of mating behavior	Regulation of attraction, courtship, copulation, and post-copulation	Several neuropeptides	Ombuya et al (2025)
Allatostatins (A, B, C)	Corpora allata (juvenile hormone – JH biosynthetic enzymes)	Bind to GPCRs in corpora allata, rapidly inhibiting JH synthesis	Developmental delay, infertility, typical IGR effects, and interference with cuticle formation	Natural allatostatin; derivatives Q6 and A15	Dou and Jurenka (2023) Kahveci et al (2024) Zhou et al (2025b)
Allatotropins	Corpora allata	Stimulate JH synthesis	Accelerated development and reproductive stimulation	Allatotropin	Lee et al (2024)
PBAN / Pyrokinins	Pheromone-producing glands	Activate intracellular cascades mediated by Ca^{2+} increase	Stimulate pheromone production; inhibition reduces reproductive behavior	PBAN, pyrokinin	Dou and Jurenka (2023) Kahveci et al (2024)

sNPF (short neuropeptide F)	Appetite/metabolism-regulating neurons	Modulate feeding behavior pathways; agonists/antagonists alter intake	Behavioral starvation, growth disorders	Natural sNPF; I-3 analogue (N-terminal tyrosine-modified)	Weger e Rittschof (2024) Force and Debernard (2025) Shen et al (2025) Zhang et al (2025)
NPF (neuropeptide F)	Appetite/metabolism-regulating neurons	Regulation of appetite and growth	Changes in energy metabolism and growth	NPF	Weger and Rittschof, 2024) Force e Debernard (2025)
Tachykinin-related peptides	Neurons and peripheral tissues	Modulate muscle contractions and sensory signals	Behavioral and motor alterations	TRP	Urbański et al (2022)
Kinins	Malpighian tubules	Regulation of water balance	Alterations in excretion and osmotic homeostasis	Kinin	Feng (2025)
Diuretic hormones	Malpighian tubules	Stimulate fluid secretion	Increased diuresis	DH (Diuretic Hormone)	Yoon et al (2025)
Ecdysis/molt hormones	Epidermal cells and prothoracic glands	Regulate ecdysteroid synthesis and molting gene expression	Molt control and cuticle formation	Ecdysis-triggering hormone (ETH) and others	Weger and Rittschof (2024) Force and Debernard (2025) Shen et al (2025)

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Figure 1: Classes and mechanisms of action of insecticidal peptides in insects, represented by *Spodoptera frugiperda*. a, Overview of the main structural classes of insecticidal peptides, including cyclic peptides, β-sheet–rich peptides, mixed α–β structures, α-helices, mixed α–α structures, and linear peptides. These compounds act on different physiological systems of insects, such as the neural, endocrine, epithelial, and digestive systems, exerting their effects through interactions with specific neural and hormonal receptors, membrane pore formation, or inhibition of digestive enzymes. **b**, Schematic representation of the four primary modes of action of insecticidal peptides. **1**, Neurotoxic peptides that interfere with neurotransmission by modulating voltage-gated or ligand-gated ion channels, affecting membrane potential and synaptic communication. **2**, Hormonal and mimetic peptides that act predominantly via G protein–coupled receptors (GPCRs), receptor guanylate cyclases (RGCs), or receptor tyrosine kinases (RTKs), regulating processes such as reproductive behavior, lipid mobilization, metabolism, feeding, juvenile hormone synthesis, and neuromuscular modulation. **3**, Membrane pore-forming peptides that induce disruption of the lipid bilayer through different structural models, including toroidal pores, carpet, barrel-stave, aggregates, and disordered forms. **4**, Digestive enzyme–inhibitory peptides that bind to midgut proteases and carbohydrases, such as trypsin and α-amylase, thereby impairing digestion and nutrient absorption. Taken together, the figure illustrates the structural and functional diversity of insecticidal peptides and their relevance as selective tools for agricultural pest control.

3.1 Membrane pore-forming peptides

Understanding how peptides interact with cellular membranes is essential for their rational design, as membrane lipid composition and organization determine their selectivity and effectiveness. Among them, pore-forming insecticidal peptides (PFPs) can be compared to AMPs, whose activity involves insertion into the lipid bilayer, promoting permeabilization and cell death (Ascoët et al., 2023; Oliveira Júnior et al., 2025). Although widely studied in microorganisms, mechanisms of membrane destabilization, such as pore formation and loss of cellular integrity, also apply to eukaryotic cells, including those of insects (Kawmudhi et al., 2025). The selectivity of PFPs arises from differences in lipid composition between insects and vertebrates,

with insect membranes exhibiting a higher proportion of anionic phospholipids, such as phosphatidylserine, which favor interaction with cationic peptides and reduce side effects in non-target organisms (Heath et al., 2018).

PFPs are among the best-characterized classes of insecticidal peptides, being mostly amphipathic and capable of disrupting the transmembrane barrier, leading to osmotic imbalance, ion loss, and cell lysis (Ma et al., 2024; Zhou et al., 2024; Jing, 2025). Their modes of action are described by classical permeabilization models: “barrel-stave,” “toroidal pore,” “carpet,” and “aggregate” (Volovik et al., 2024; Olmo and Andreu, 2025). In the barrel-stave model, peptides form transmembrane channels lined by peptide units, as observed for oxiopin 1 (~5.2 kDa), an amphipathic α-helix from *Oxyopes kitabensis* (Gagandeep et al., 2024; Scieuzo et al., 2024; Wang et al., 2024b). The toroidal pore model involves peptides that induce positive membrane curvature, forming pores composed of lipids and peptides, such as cupienin-1a, an amphipathic peptide isolated from the venom of the spider *Cupiennius salei* (~3.9 kDa), whose helix–loop–helix structure favors interaction with anionic lipids and shows toxicity against *S. frugiperda* Sf21 cells, with in vivo insecticidal effects against *Drosophila melanogaster* (Kuhn-Nentwig, 2021; Araújo et al., 2022). In the carpet model, peptides accumulate on the membrane until they promote its rupture, as seen for peptide U9 (~2 kDa) from *T. bicarinatum*, which is active in *D. melanogaster* S2 cells (Ascoët et al., 2023). The aggregate model describes the formation of irregular and transient pores from peptide–lipid complexes, typical of cyclic peptides such as the cyclotide kalata B1 (~2.9 kDa), whose structural stability and affinity for insect phospholipids support its insecticidal activity, including cytotoxicity against *S. frugiperda* Sf9 cells (Huang et al., 2024; Deegala et al., 2025).

The structural diversity, distinct permeabilization modes, and high selectivity of PFPs for phospholipids characteristic of insect membranes reinforce their potential as sustainable alternatives to chemical pesticides, reducing environmental risks and impacts on vertebrates (Kawmudhi et al., 2025; Bermúdez-Puga et al., 2025).

3.2 Digestive enzyme-inhibiting peptides

Insecticidal peptides that inhibit digestive enzymes are small molecules that compromise the nutrition, development, and survival of phytophagous insects by blocking essential enzymes of the midgut, the leading site of digestion, absorption, and associated physiological functions such as osmoregulation and immunity (Almeida-Barros et al., 2021; Mulla and Tamhane, 2023). Although there is no standardized classification, these peptides can be grouped according to the target enzyme, the mode of inhibition, and the structural origin (Sahayaraj and Balasubramanian, 2016; Napoleão et al., 2019).

Proteases, especially serine proteases, account for most protein digestion in insects. Protease inhibitors block enzymatic activity through competitive binding to the active site or through conformational changes that reduce catalytic capacity, mediated by hydrophobic interactions, salt bridges, and hydrogen bonds, resulting in impairments to insect development, reproduction, and population dynamics (Hartl et al., 2010; Akbar et al., 2018). Examples include metallocarboxypeptidase inhibitors from tomato and potato, which potentiate the action of the Cry1Ac toxin against *Helicoverpa armigera*, and the dipeptides DI1 and DI2, which inhibit intestinal trypsins and cause mortality and weight reduction in *Anticarsia gemmatalis* (Chen et al., 2021; Meriño-Cabrera et al., 2022).

α-Amylase inhibitors also stand out, as starch is the primary carbohydrate source for herbivorous insects. Among them, defensins, 4–5 kDa peptides with an αβ structure stabilized by four disulfide bonds, are able to inhibit α-amylases and intestinal hydrolases, showing insecticidal activity against *Acanthoscelides obtectus*, *Zabrotes subfasciatus*, *Callosobruchus chinensis*, and other pests (Pelegrini et al., 2008; Liu et al., 2006; Bukhteeva et al., 2022). Knottin-type peptides, isolated from *Amaranthus hypochondriacus*, *Alternanthera sessilis*, and *Chenopodium quinoa* (3 kDa), also completely inhibited the α-amylases of *Tribolium castaneum* and *C. chinensis*. These compounds block the hydrolysis of α-1,4-glycosidic bonds, preventing the release of simple sugars and, consequently, compromising insect nutrition, development, and survival (Janeček et al., 2014; Rane et al., 2020).

Beyond enzymatic inhibition, these peptides can induce systemic physiological effects, such as hormonal and reproductive alterations, and exert selective pressure associated with resistance. However, mutations or compensatory overexpression of target enzymes tend to impose adaptive costs on insects, contributing to the sustainable reduction of pest populations (Izadi, 2025).

3.3 Neurotoxic peptides

Peptides can exert an immediate insecticidal effect by acting on specific targets of the nervous system, inducing paralysis and neuromotor collapse. Neurotoxic peptides stand out as an emerging class of bioactive compounds with high potential for selective and environmentally sustainable bioinsecticides, due to their high molecular specificity and strong affinity for neurophysiological targets conserved in insects, which reduces risks to vertebrates and non-target organisms (Fassolo et al., 2024; Robinson et al., 2023; Yan et al., 2025).

These peptides are synthesized by spiders, scorpions, predatory insects, and marine animals, reflecting an evolutionary trajectory that favored highly specialized structures for neuromuscular modulation. In the insect nervous system, they act as chemical messengers or synaptic modulators, affecting vital functions such as locomotion, feeding, growth, and reproduction (Fassolo et al., 2024; Raisch and Raunser, 2023; Wang et al., 2024b; Zhou et al., 2022).

Neurotoxic peptides act directly on ion channels, causing paralysis, neuromotor disorientation, and death. At the molecular level, the primary targets of neurotoxins belong to ligand-gated ion channels (LGICs) and voltage-gated ion channels (VGICs), whose interactions determine the observed physiological effects (Raisch and Raunser, 2023; Guo et al., 2023). Among LGICs, nicotinic acetylcholine receptors (nAChRs) stand out as the primary excitatory mediators of the insect central nervous system (Raisch and Raunser, 2023; Yuan et al., 2024). These receptors are ion channels activated by acetylcholine (ACh) binding, allowing cation influx and propagation of the nerve impulse. Peptides may act as persistent agonists, positive allosteric modulators, or antagonists, promoting desensitization or blockade of synaptic transmission (Lu et al., 2022; Raisch and Raunser, 2023; Thany, 2025; Yuan et al., 2024).

The peptide GS-ω/κ-Hxtx-Hv1a (Spear®), approximately 4 kDa, isolated from the spider *Hadronyche versuta*, acts as a positive allosteric modulator of insect nAChRs, promoting controlled neuroexcitation, high selectivity, and low toxicity in vertebrates (Bloomquist et al., 2023; Ross et al., 2025). In contrast, antagonist peptides bind to active or allosteric sites of receptors, blocking ionic influx and synaptic transmission. This mechanism, typical of scorpion and spider toxins, involves antagonism of γ-aminobutyric acid type A (GABA A) receptors, glutamate-gated chloride channels

(GluCl), and inhibitory Cl^- channels, leading to neural hyperexcitability, convulsions, and insect death (Raisch and Raunser, 2023; Chen and Hawthorne, 2025).

VGICs, including Na^+V, K^+V, $Ca^{2+}V$ channels and ryanodine receptors, are essential for nerve impulse conduction and muscle contraction. Peptides may block these channels or alter their kinetics, destabilizing the membrane potential and leading to neuromotor collapse (Raisch and Raunser, 2023). Examples of this mode of action include spider α- and β-peptide toxins that act on Na^+V channels, altering their kinetic properties and leading to depolarization and collapse of motor control (Silver et al., 2018; He et al., 2025). ω-Atyptoxin block $Ca^{2+}V$ channels, inhibiting neurotransmitter release at presynaptic terminals and severely impairing synaptic communication (Yan et al., 2025). κ-Hexatoxin-type toxins inhibit K^+V channels, which are essential for membrane repolarization, thereby amplifying neurophysiological imbalance (Tamadon et al., 2019; Zhou et al., 2023; AlShammari et al., 2023).

The identification of new targets, such as calcium-activated potassium channels and transient receptor potential channels, has expanded the possibilities for innovative modes of action that can overcome resistance to conventional insecticides while maintaining selectivity. Although neurotoxins act rapidly, alternative strategies also aim to interfere with fundamental insect life-cycle stages (Nesterov et al., 2015; Zhang et al., 2022; Paschapur et al., 2025).

3.4 Hormonal and mimetic insecticidal peptides

In contrast to fast-acting neurotoxins, hormonal insecticidal peptides and their mimetics act in a chronic and regulatory manner, interfering with long-term physiological processes such as development, metabolism, and reproduction, mainly through the dysregulation of endocrine pathways mediated by G protein-coupled receptors

(GPCRs). These peptides integrate the neuroendocrine and endocrine systems of insects and are predominantly peptide neurohormones that act on multiple target tissues via GPCRs, as well as through tyrosine kinase or guanylate cyclase receptors (Orchard and Lange, 2024).

The mode of action of these compounds is dependent on specific receptors, regulating essential functions such as lipid mobilization via the adipokinetic hormone receptor (Toprak, 2020; Orchard and Lange, 2024), juvenile hormone synthesis mediated by the allatostatin receptor (Wegener and Chen, 2022), development and growth through the diapause hormone receptor (Shen et al., 2018), and feeding behavior (Hou et al., 2023).

Structurally and functionally, insect hormonal peptides are organized into well-defined families, including allatostatins (A, B, and C), allatotropins, short neuropeptide F (sNPF), neuropeptide F (NPF), pyrokinins, pheromone biosynthesis-activating neuropeptide (PBAN), tachykinin-related peptides, kinins, diuretic hormones, and ecdysis hormones (Liu et al., 2021).

The exploration of these systems has driven the development of highly specific peptidomimetic antagonists that selectively interfere with insect-exclusive targets, particularly neuropeptide GPCRs (Elakkiya et al., 2019). Many of these peptide’s exhibit amidated C-termini, disulfide bridges, and affinity for GPCRs of subfamilies A or B, and may also indirectly modulate critical hormonal pathways, such as those of juvenile hormone and ecdysteroids, through the regulation of key enzymes (Su et al., 2023; Noriega et al., 2025).

Among functional examples, allatostatins rapidly inhibit juvenile hormone biosynthesis in the corpora allata, causing developmental delays and infertility, whereas PBAN and

pyrokinins regulate pheromone production through calcium-dependent intracellular cascades, affecting reproductive behavior (Dou and Jurenka, 2023; Kahveci et al., 2024). Widely studied neuropeptides such as proctolin, kinins, PBAN, and allatostatins have been targeted for the development of analogs capable of blocking or modulating their physiological actions (Johnson et al., 2003; Elakkiya et al., 2019; Nässel, 2021, 2024). Bioassays with fifteen kinin analogs indicated that peptides IV-3, IV-5, and IV-10 exhibited significant insecticidal activity against the soybean aphid *Aphis glycines* (Wu et al., 2020; Zhang et al., 2020; Nässel, 2021). In turn, the sNPF analog I-3, modified at the N-terminus by the addition of tyrosine, showed a lower LC_{50} than the native peptide against aphids. Meanwhile, allatostatin-derived peptides such as Dippu-AstR, Q6, and A15, obtained through structural modeling, induced significant mortality in species such as *Diploptera punctata* and *Plutella xylostella* (Zhang et al., 2025a).

Analogs were developed based on advances in structural modeling, molecular docking, and machine learning, optimizing affinity, stability, and permeability, such as the addition of tyrosine to the N-terminal end of the sNPF I-3 analog, which reduced the LC_{50} compared to the natural peptide against aphids, in addition to increasing lipophilicity, favoring interaction with cell membranes (Zhou et al., 2025b; Zhang et al., 2025b). Taken together, these results support the use of hormonal peptides and mimetics as a selective, environmentally safe, and technically mature alternative for the rational control of agricultural pests, supported by modern molecular design approaches and peptide–GPCR interaction mapping (Xin et al., 2024; Golinelli et al., 2024; Ge et al., 2025).

4 Peptides in the agrochemical industry

In recent years, the focus of major agrochemical companies on producing generics, at the expense of developing new molecules, has reduced the introduction of pesticides with novel modes of action, favoring pest resistance and compromising agricultural productivity (Sparks, 2025; Raisch and Raunser, 2023). This scenario is exacerbated by more stringent regulatory requirements and growing consumer concerns regarding toxic and polluting compounds. Thus, the search for natural, effective, and safe molecules has become a significant challenge for the agrochemical industry (Ormancey et al., 2023). In this context, peptides stand out for their high efficacy in plant protection, acting as immune inducers, antimicrobials, growth regulators, herbicides, and insecticides, combining broad availability, high efficiency, and environmental compatibility (Zhang et al., 2023).

The integration of these innovations into platforms that combine computational prediction, synthesis, biological evaluation, and advanced formulation has accelerated the introduction of solutions to market and driven the transition toward more resilient, low-impact agricultural systems. In addition to helping overcome pest resistance, peptides reduce dependence on conventional chemical insecticides and directly align with the Sustainable Development Goals (SDGs) 2 (Zero Hunger), 12 (Responsible Consumption and Production), 13 (Climate Action), and 15 (Life on Land), reinforcing the role of peptides in sustainable pest management and global food security (Liu et al., 2021; Ormancey et al., 2023; Varadi et al., 2024).

The main commercial products based on bioactive peptides are summarized in Table 2, including the bioinsecticide Spear®, derived from neuropeptides from spider toxins and selective for pest nicotinic receptors, with low impact on pollinators (Zhang et al., 2023), and Sero-X®, formulated with plant cyclopeptides that combine high insecticidal efficacy with safety for bees (Oguis et al., 2019). Additionally, biostimulants such as

Saori, Keylan, and Hicure use natural elicitor peptides to improve plant nutrition and tolerance to biotic and abiotic stresses, promoting crop growth and resilience (Pinheiro et al., 2018; Huang et al., 2024).

Table 2 | Insecticidal products containing peptides as active ingredients. Description of composition, mode of action, molecular targets, and target sites and mechanisms of action.

Type	Commercial Name	Active Peptide	Developer	Launch Year	Patent Registration	Main Target	Mode of Action
Mimetic neuropeptides and neurotoxins	Spear T®	GS-OMEGA/KAPPA-HXTX-HV1A	Vestaron (EUA)	2020	WO2022136442A2	Thrips, whiteflies, aphids, and mites	Acts on nicotinic acetylcholine receptors causing paralysis and death
	Spear® LEP	GS-OMEGA/KAPPA-HXTX-HV1A	Vestaron (EUA)	2023	US20080107640A1.	Lepidoptera	Acts on nicotinic acetylcholine receptors causing paralysis and death
	Spear® RC	GS-OMEGA/KAPPA-HXTX-HV1A	Vestaron (EUA)	2023	US6245531B1	Lepidoptera of major crops	Acts on nicotinic acetylcholine receptors causing paralysis and death
Other Modes of Action	Sero-X®	Cyclotides from *Clitoria ternatea*	Innovate AG	2017	US4267281A	Lepidoptera, Hemiptera, and Coleoptera	Behavior modification including egg-laying interruption and feeding inhibition
	SAORI®	Peptides derived from Harpin protein	Plant Health Care	Not publicly disclosed	US5484600A	Phytopathogens	Activates plant defense mechanisms
	Hicure®	Mixture of amino acids and hydrolyzed animal protein peptides	Syngenta	Not publicly disclosed	US8236336B2	-	Stimulates natural plant production processes, assisting in protein formation

5 Perspectives and Conclusions

Despite recent advances, the large-scale application of insecticidal peptides still faces significant limitations, notably their remarkably low field bioavailability due to degradation by UV radiation, pH fluctuations, and temperature variations, which compromises their efficacy (Wu et al., 2024). In addition, limited knowledge about interactions with soil microbiota and multitrophic effects hampers robust environmental assessments, together with the lack of integrated predictive modeling, regulatory barriers, and technological challenges for production at commercial scale (Scieuzo et al., 2024; Pu and Chung, 2024).

To overcome these constraints, strategies such as peptide cyclization inspired by cyclotides (e.g., kalata B1), incorporation of non-natural amino acids (Aib and D-amino acids), modifications at the N- and C-termini, PEGylation, conjugation to cell-penetrating peptides, and encapsulation formulations, including adhesive microgels, have been explored to enhance stability and application efficiency under field conditions (Herzig et al., 2018; Wu et al., 2024).

The advancement of these approaches has been accelerated by artificial intelligence and machine learning, which enable virtual screening, sequence optimization, and prediction of interactions with target receptors. Tools such as AlphaFold3, ESMFold, and RoseTTAFold allow highly accurate structural modeling, identification of functional regions, and rational suggestions for modifications, reducing development time and costs and enabling insecticides targeted to specific pests (Gressel et al., 2022; Abramson et al., 2024).

Bioinspiration from natural toxins found in spiders, scorpions, and plants has been central to the development of more stable and effective analogues, such as those

derived from the Hv1a peptide from *Hadronyche versuta*, which exhibit increased resistance to degradation and improved oral activity (Herzig et al., 2018). These examples highlight the potential of integrating molecular biology, peptide chemistry, and chemical ecology.

The four main classes of insecticidal peptides offer a balance between speed of action, selectivity, and applicability. Pore-forming peptides and neurotoxins display rapid action, making them suitable for acute infestations. In contrast, digestive enzyme inhibitors and hormonal mimetics show slower, chronic activity but with high selectivity and reduced environmental impact, making them ideal for preventive and long-term strategies within the framework of Integrated Pest Management (IPM) (Pu and Chung, 2024).

This functional diversity enables the development of portfolios tailored to different agricultural scenarios. It helps mitigate pest resistance, as peptides present multiple modes of action and high selectivity, with low toxicity to non-target organisms, including beneficial insects and vertebrates (Scieuzo et al., 2024; Zhou et al., 2025a).

In perspective, the success of insecticidal peptides will depend on the convergence of bioinspiration, artificial intelligence, and molecular engineering, combined with overcoming critical challenges related to field bioactivity, environmental assessment, and regulation. The adoption of integrated approaches that combine basic science, predictive modeling, and technological development is essential to consolidate these compounds as strategic tools in integrated pest management and in the sustainable agriculture of the future.

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Contributions

R.N., A.F.L.F., V.S. and O.L.F contributed to writing, editing and researching data for the article and contributing to the discussion of this manuscript. M.F.G.S. and O.L.F. contributed to discussing, reviewing and editing the manuscript before submission.

Competing interests

The authors declare no competing interests.

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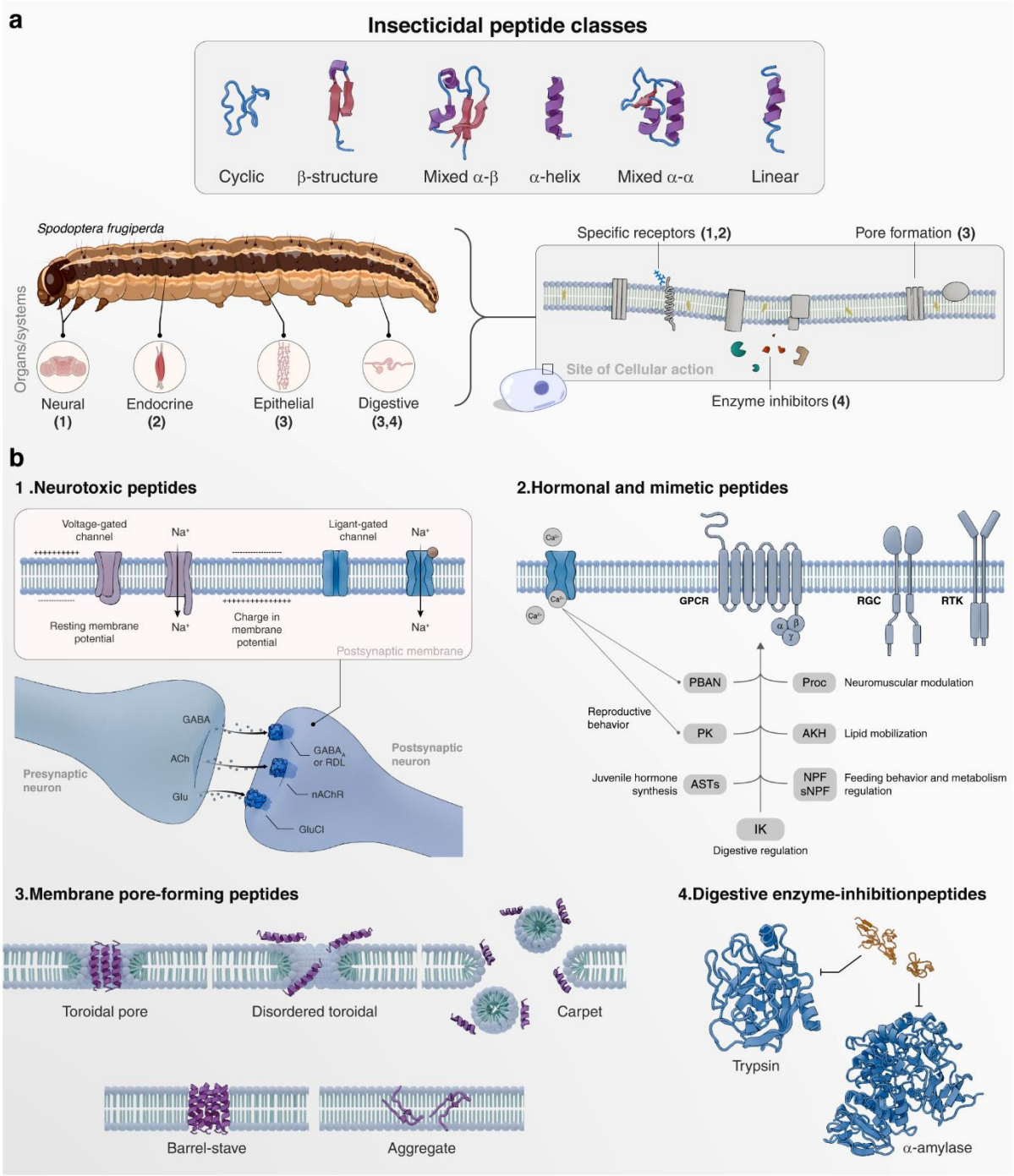
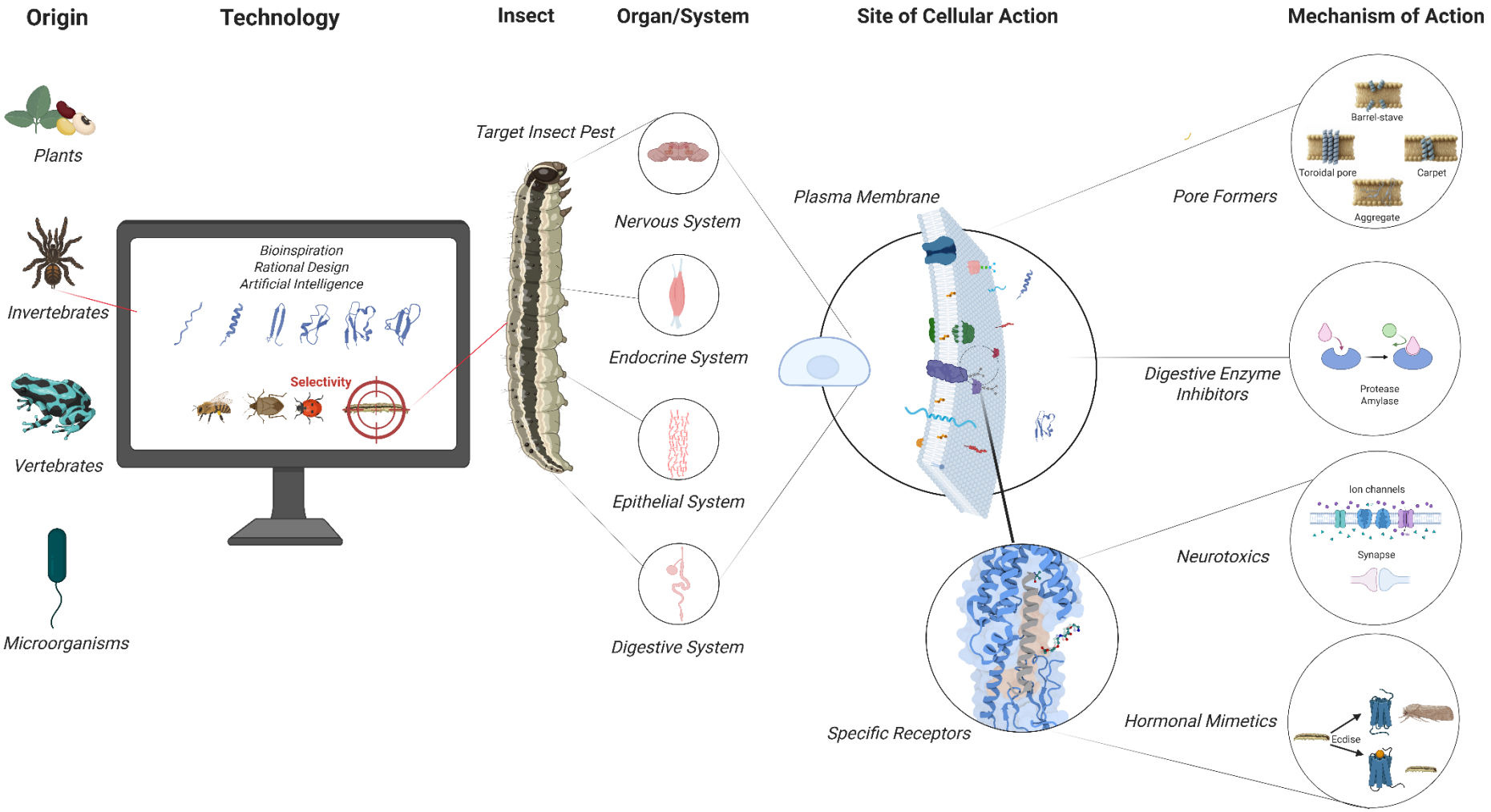

a
Insecticidal peptide classes
Cyclic
β-structure
Mixed α-β
α-helix
Mixed α-α
Linear
Spodoptera frugiperda
Organs/systems
Neural (1)
Endocrine (2)
Epithelial (3)
Digestive (3,4)
Specific receptors (1,2)
Pore formation (3)
Site of Cellular action
Enzyme inhibitors (4)
b
1 .Neurotoxic peptides
Voltage-gated channel
Ligant-gated channel
Na+
Resting membrane potential
Charge in membrane potential
Postsynaptic membrane
GABA
ACh
Glu
Presynaptic neuron
Postsynaptic neuron
GABA/Cl or RDL
nAChR
GluCl
2.Hormonal and mimetic peptides
GPCR
RGC
RTK
PBAN
Proc
Neuromuscular modulation
Reproductive behavior
PK
AKH
Lipid mobilization
Juvenile hormone synthesis
ASTs
NPF sNPF
Feeding behavior and metabolism regulation
IK
Digestive regulation
3.Membrane pore-forming peptides
Toroidal pore
Disordered toroidal
Carpet
Barrel-stave
Aggregate
4.Digestive enzyme-inhibitionpeptides
Trypsin
α-amylase

Graphical Abstract



4. CAPÍTULO II

Insecticidal activity of bioinspired peptides rationally designed from the Cry10Aa protein

Esse capítulo corresponde ao artigo de pesquisa da tese, que será submetido à revista ACS Omega (ISSN 2470-1343/ IF 4.2).

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Insecticidal activity of bioinspired peptides rationally designed from the Cry10Aa protein

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Abstract: The development of new selective and safe insecticidal molecules is essential in light of the increasing resistance to *Bacillus thuringiensis* (Bt) proteins and the limitations of traditional chemical insecticides. In this study, a parental peptide (AMPCry10Aa) was selected from the α-helix 3 of the Cry10Aa protein, and from this peptide, six variants were rationally designed. Physicochemical characterization revealed an increase in positive charge (+2 to +3) compared to the parental (+1), along

with significant variations in hydrophobicity and hydrophobic moment. In bioassays with *Anthonomus grandis*, both the Cry10Aa protein and variants such as AMPCry10Aa_2, _3, _4, and _6 reduced survival by 55–70%, confirming that functional deconstruction retains insecticidal activity. In *Spodoptera frugiperda*, the AMPCry10Aa_6 peptide showed the highest activity when applied topically (~50% mortality at 14 μg mL^{-1}), whereas AMPCry10Aa_5 was more effective by injection (~60% mortality at 56 μg mL^{-1}), associated with the establishment of multiple hydrogen-bonding interactions with phospholipids. Cytotoxicity assays demonstrated that AMPCry10Aa_6 exhibited no deleterious effects on human fibroblasts up to 224 μg mL^{-1}, indicating a wide safety margin. These results demonstrate that minor structural modifications modulate selectivity and efficacy, enabling a balance between insecticidal activity and safety. In summary, the AMPCry10Aa_6 peptide is a promising candidate for the development of selective, sustainable bioinsecticides.

Keywords: *Bacillus thuringiensis*, bioinsecticides, cytotoxicity, peptide–membrane interaction, sustainable pest control.

Introduction

Contemporary agriculture faces the dual challenge of increasing food production for a growing population while reducing the environmental impacts of agricultural management, which the indiscriminate use of chemical insecticides has historically exacerbated (Wang et al., 2024; Zhou et al., 2025). This scenario is further intensified by climate instabilities, which increase the occurrence of pests and crop losses, directly threatening global food security (Singh et al., 2023; Sparks, 2025). Thus, the

development of innovative, effective, and environmentally sustainable pest control strategies has become urgent (Civolani et al., 2025).

The traditional control model, centered on synthetic insecticides and later expanded to biotechnological technologies based on *Bacillus thuringiensis* (*Bt*), presents critical limitations. The pesticidal proteins of *Bt* revolutionized pest management by reducing dependence on conventional chemicals; however, intensive use and inadequate management practices have accelerated the evolution of resistance in several insect populations, compromising the durability of these tools (Gupta et al., 2021; Wang et al., 2024b; Jin et al., 2023). This context underscores the need for new platforms that can overcome these barriers.

Among the emerging alternatives, approaches based on computational tools are particularly notable. The rational design of molecules, combined with *in silico* modeling and simulations, enables the planning of compounds with multiple mechanisms of action, capable of delaying the evolution of resistance and more accurately predicting their efficacy (Ormancey et al., 2023; Varadi et al., 2024). In this scenario, insecticidal peptides emerge as a promising platform, with competitive advantages such as high selectivity for target species, low environmental persistence, and a diversity of mechanisms of action, including pore formation in cell membranes, inhibition of digestive enzymes, modulation of ion channels, and hormonal mimicry (Ascoët et al., 2023; Gujjarlapudi et al., 2023; Yan et al., 2025; Zhang et al., 2024).

In this context, Bt Cry proteins play a strategic role not only as established bioinsecticides but also as a source of inspiration for designing new synthetic peptides. The tertiary structure of these proteins is highly conserved and composed of three functional domains, with Domain I, rich in α-helices at the N-terminal region, directly

associated with cell membrane interaction and pore formation, a mechanism shared with several bioactive peptides (Li et al., 1991; Mohanty et al., 2025; Rios et al., 2024). *In silico* studies have shown that α-helix 3 of Cry Domain I interacts directly with cell lipid bilayers, maintaining its activity even when isolated from the protein (Lin et al., 2014).

Based on this principle of functional deconstruction, antimicrobial peptides (AMPs) inspired by the Cry10Aa protein were developed, exhibiting broad-spectrum activity against both Gram-positive and Gram-negative bacteria. This confirms the feasibility of exploring specific functional regions to generate smaller, more selective, and effective molecules (Rios et al., 2024). The Cry10Aa protein is recognized for its ability to form pores in insect intestinal cells (Ribeiro et al., 2019). The antimicrobial activity already validated in peptides derived from its pore-forming domain reinforces its relevance as a bioinspired model. Furthermore, the structural similarity between bacterial cell membranes and insect intestinal membranes, both rich in negatively charged phospholipids, strengthens the hypothesis that Cry10Aa-derived peptides may also exert insecticidal action (Yeh et al., 1997; Koehbach & Craik, 2019).

From this perspective, bioinspired approaches based on Cry proteins represent a promising strategy for developing selective insecticides. Thus, the objective of this study was to design and evaluate insecticidal peptides derived from the Cry10Aa protein, obtained through rational design, as a bioinspired strategy for the selective control of economically important agricultural pests, using *Anthonomus grandis* and *Spodoptera frugiperda* as model systems.

Results and Discussion

Cry proteins produced by *Bacillus thuringiensis* (Bt) are widely used in biological control and in transgenic crops due to their ability to form pores in the membranes of the insect midgut epithelium (Couch et al., 2023; Pacheco et al., 2023). In parallel, recent studies have highlighted the potential of antimicrobial peptides with pore-forming activity as promising alternatives for pest management (Araújo et al., 2022; Ascoët et al., 2023).

In this study, we demonstrate for the first time that peptides derived from the Cry10Aa toxin retain insecticidal activity against two economically important agricultural pests, A. grandis and S. frugiperda, even in the absence of the complete structural domains of the protein. This finding is unprecedented and promising, as these peptides act as insecticides independently of binding to specific receptors, unlike Cry proteins. Considering that mutations in these receptors constitute one of the main mechanisms of resistance to Cry proteins, the proposed approach may represent an effective strategy to overcome this limitation and expand the available alternatives for pest control (Amezian et al., 2024).

Physicochemical characterization of Cry10Aa-derived peptides revealed significant structural modifications that help explain the activity patterns observed in bioassays (Figure 7; Table 1). Overall, the variants showed an increase in positive charge relative to the parental peptide (+1), reaching +2 or +3. This charge increment promotes electrostatic interactions with insect cell membranes, enhancing insecticidal activity. Among the variants, AMPCry10Aa_2, AMPCry10Aa_5, and AMPCry10Aa_6, all with a +3 charge, stood out as promising candidates due to their higher electrostatic affinity for negatively charged surfaces.

Table 1. Sequences and physicochemical properties (charge, hydrophobicity, and hydrophobic moment) of the parental peptide (AMPCry10Aa) and variants (AMPCry10Aa_1, AMPCry10Aa_2, AMPCry10Aa_3, AMPCry10Aa_4, AMPCry10Aa_5, and AMPCry10Aa_6), developed based on the functional deconstruction of the Cry10Aa protein from *Bacillus thuringiensis*.

Peptides	Sequences	Charge	Hydrophobicity	Hydrophobic moment	Molecular Mass (Da)
AMPCry10Aa	IINVLTSIVTPIKNQLDKYQ	+1	0.540	0.059	2299.74
AMPCry10Aa_1	KDNLKTIIVTAIKNILDKYQ	+2	0.334	0.169	2330.79
AMPCry10Aa_2	IKNVLKSIVTPAKNQLDKYQ	+3	0.264	0.012	2299.74
AMPCry10Aa_3	IIKDLLKIVTPIANQLIKYQ	+2	0.640	0.036	2323.89
AMPCry10Aa_4	IINKDTLKVIPIKAQLDIYQ	+1	0.516	0.074	2325.82
AMPCry10Aa_5	IINVKTSLKTIIKNALDKIQ	+3	0.413	0.110	2252.77
AMPCry10Aa_6	IINVLKSILKPIKNQADKYI	+3	0.471	0.950	2310.85

Additionally, a trend toward reduced hydrophobicity was observed in some sequences. This parameter is critical because it is associated not only with activity but also with selectivity. Lower hydrophobicity values reduce the risk of toxicity to mammalian cells (Chen et al., 2007), supporting the safe use of these peptides in biotechnological applications. In this regard, AMPCry10Aa_2 exhibited the lowest hydrophobicity, suggesting higher selectivity, whereas AMPCry10Aa_3 was the most hydrophobic, which may translate into higher insecticidal efficacy but also a potential cytotoxic risk. Thus, the diversity of profiles obtained reinforces the strategy of generating variants with varying degrees of balance between efficacy and safety.

Another notable parameter was the hydrophobic moment, which showed significant variation among the variants. AMPCry10Aa_6 exhibited the highest value, followed by AMPCry10Aa_1 and AMPCry10Aa_5, suggesting a greater capacity to interact with cell membranes and, consequently, higher insecticidal potential. Conversely, the lowest values were recorded for AMPCry10Aa_2 and AMPCry10Aa_3, indicating

mechanisms of action possibly less dependent on amphipathicity. This variation demonstrates that no single set of properties can explain biological performance; instead, a combination of physicochemical interactions contributes in complementary ways (Omardien et al., 2018).

Overall, the integration of these results shows that the variants presented distinct and promising profiles. The combination of a higher positive charge and moderate hydrophobicity suggests an advantageous strategy that maintains insecticidal potential without excessively increasing toxicity to non-target organisms. In this context, two variants stood out: AMPCry10Aa_6, combining high charge with the highest hydrophobic moment, and AMPCry10Aa_1, which showed a similar profile. These findings reinforce the importance of fine-tuning charge, hydrophobicity, and hydrophobic moment as a foundation for the rational design of new insecticidal agents.

The relationships between these physicochemical characteristics are key parameters for biological activity, as confirmed by bioassays with *A. grandis* and *S. frugiperda*. Evaluation in *A. grandis* showed that the Cry10Aa protein and the variants AMPCry10Aa, AMPCry10Aa_2, AMPCry10Aa_3, AMPCry10Aa_4, and AMPCry10Aa_6 significantly reduced pest survival (55–70%). These data indicate that the functional deconstruction of the original protein preserved essential structural elements for membrane disruption, even in the absence of the complex three-dimensional structure. Furthermore, variants combining higher positive charge and elevated hydrophobic moment maintained consistent performance, confirming the relevance of these properties as determinants of activity. Notably, AMPCry10Aa_6 stood out, demonstrating the importance of the synergistic interaction between charge and amphipathicity.

The insecticidal assays confirmed the efficacy of the Cry10Aa protein against *A. grandis,* in agreement with previous studies that demonstrated a drastic reduction in pest survival by up to 100% after ingestion of the treatment (Aguiar et al., 2012; Ribeiro et al., 2017). Innovatively, it was observed that peptide variants derived from Cry10Aa Domain I also exhibited significant activity, with survival rates reduced to 55–70% (Figure 1).

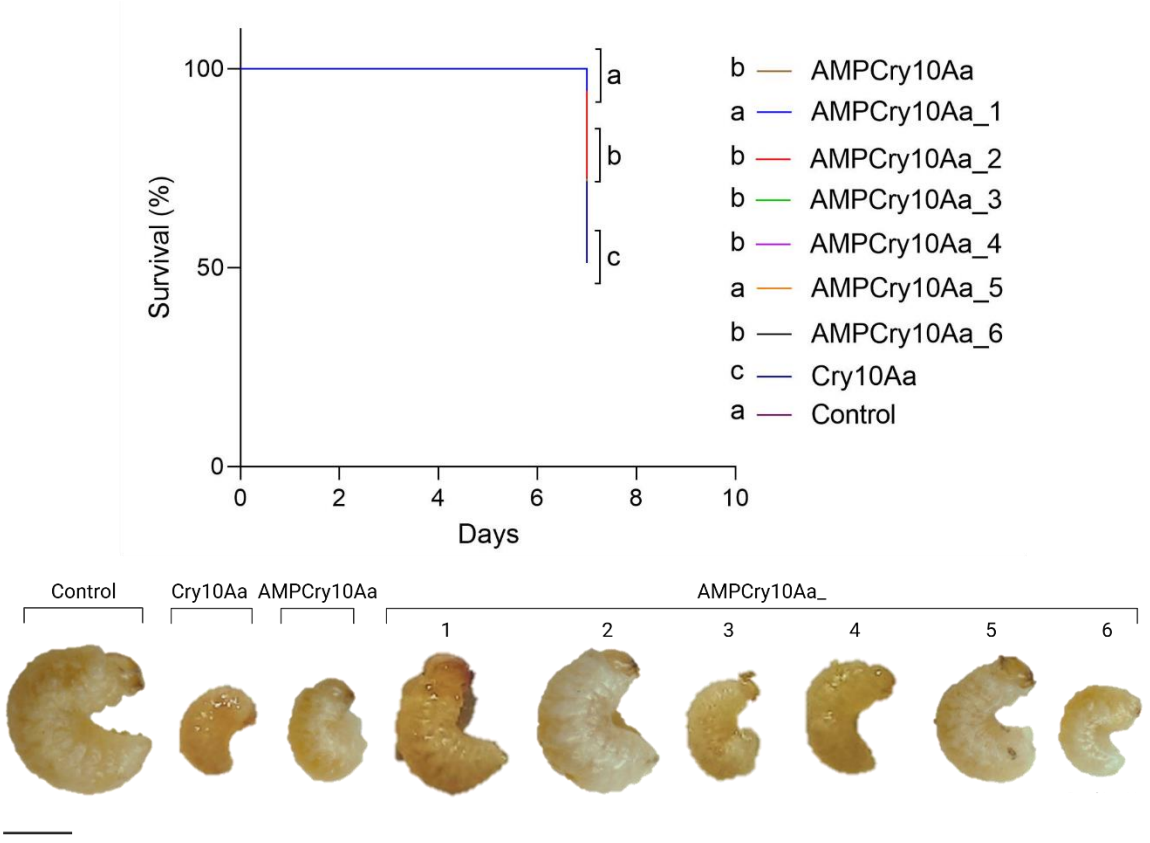

Figure 1. Survival curves of *Anthonomus grandis* and phenotypic changes associated with ingestion of a diet containing 14 µg g^{-1} of the parental peptide (AMPCry10Aa), variants (AMPCry10Aa_1, AMPCry10Aa_2, AMPCry10Aa_3, AMPCry10Aa_4, AMPCry10Aa_5, and AMPCry10Aa_6), Cry10Aa protein, and ultrapure water (Control). Kaplan-Meier analyses (GraphPad Prism 9.0) revealed significant differences between groups (log-rank test, $p < 0.05$; $n = 90$). Scale bar, 2 mm.

This result is particularly relevant, as it demonstrates that, even without the protein's complex three-dimensional structure, the derived peptides retain essential elements

that promote insecticidal activity. Analysis of the Kaplan-Meier curves revealed statistically significant differences between the treated groups and the control, confirming the robustness of the data. Variants such as AMPCry10Aa, AMPCry10Aa_2, AMPCry10Aa_3, AMPCry10Aa_4, and AMPCry10Aa_6 exhibited behavior similar to that of the parental peptide, indicating that rational sequence modification did not compromise insecticidal function while diversifying the activity profiles.

In experiments with *S. frugiperda*, the survival graphs exhibited a more heterogeneous pattern, where the route of application was a decisive factor in peptide performance. In topical application (Figure 2 and Figure S1), the AMPCry10Aa_6 peptide showed the best result, with an approximate 50% reduction in survival at 14 μg mL^{-1}. This effect is consistent with its physicochemical properties, particularly the high hydrophobic moment, which facilitates penetration through the cuticle and initial contact with external cell membranes.

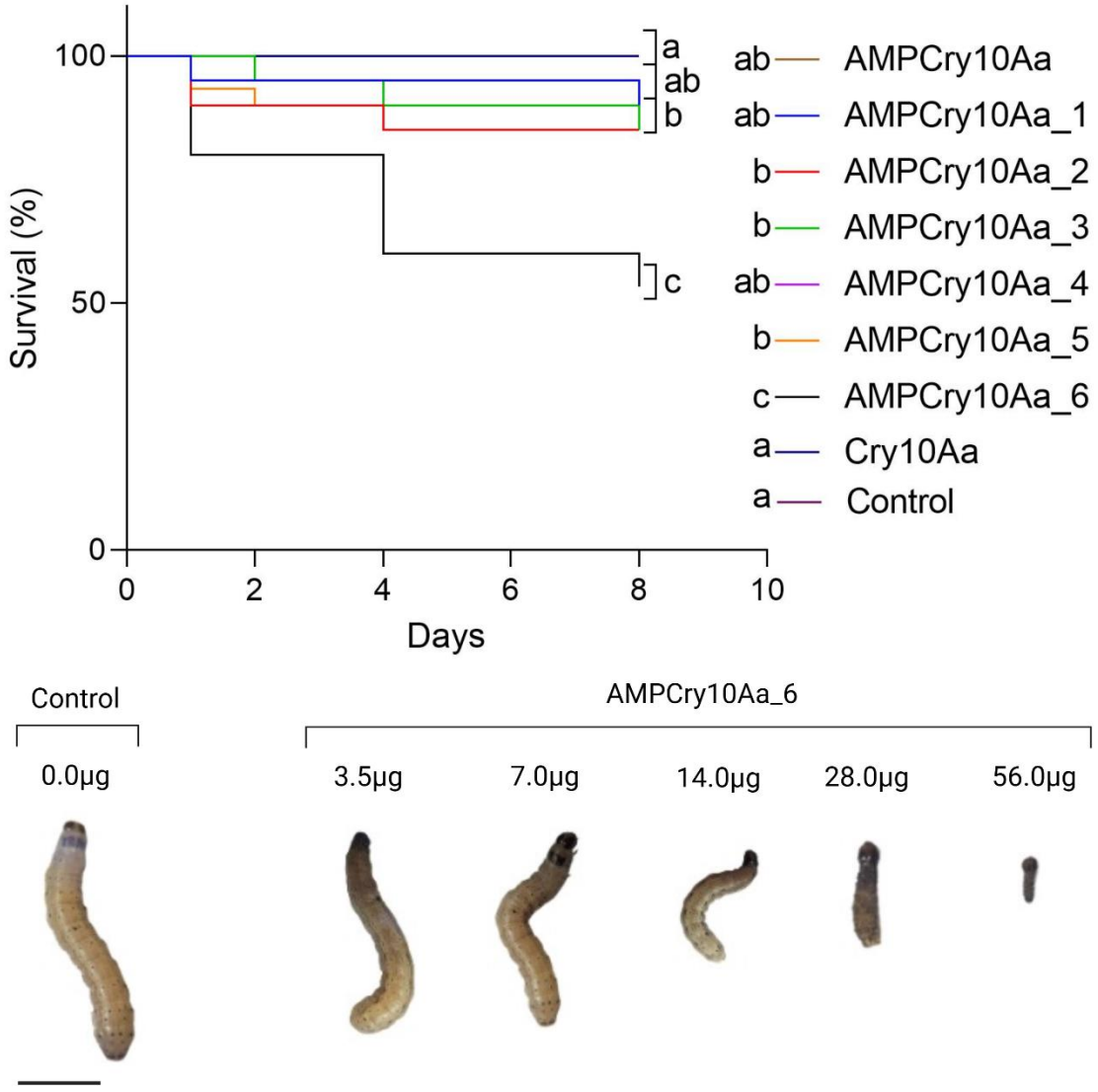

Figure 2. Survival curves of *Spodoptera frugiperda* and phenotypic changes in 3rd-instar larvae subjected to topical application of the parental peptide (AMPCry10Aa), variants (AMPCry10Aa_1 to AMPCry10Aa_6), Cry10Aa protein, and ultrapure water (Control) at a concentration of 14 µg mL^{-1}. Different letters indicate significant survival differences between groups (log-rank test, p-value <0.05; N: 30). Scale bar, 1 cm.

The profiles indicate a progressive reduction in survival as a function of AMPCry10Aa_6 concentration, however from the dose of 14 µg mL^{-1} onward, the survival rate remained stable, even with further increases in peptide concentration. In contrast, the original peptide and some variants exhibited more modest performance, suggesting that not all introduced modifications translate into greater topical efficacy.

In the injectable application (Figure 3 and Figure S2), the results showed a distinct scenario. In this case, the AMPCry10Aa_5 peptide stood out, reducing survival to approximately 40% at the highest tested concentration (56 µg mL^{-1}). Analysis of the survival curve indicates that the effect of this peptide was more consistent across concentrations, differing significantly from the control. This higher efficacy can be explained by molecular dynamics data, which indicate multiple and diverse hydrogen-bonding interactions between AMPCry10Aa_5 and different phospholipids (POPS, POPE, and POPI). This binding profile suggests a multisite affinity with the membrane, which becomes particularly advantageous when the peptide is directly injected into the hemolymph, bypassing the cuticular barrier that restricts its action in topical assays.

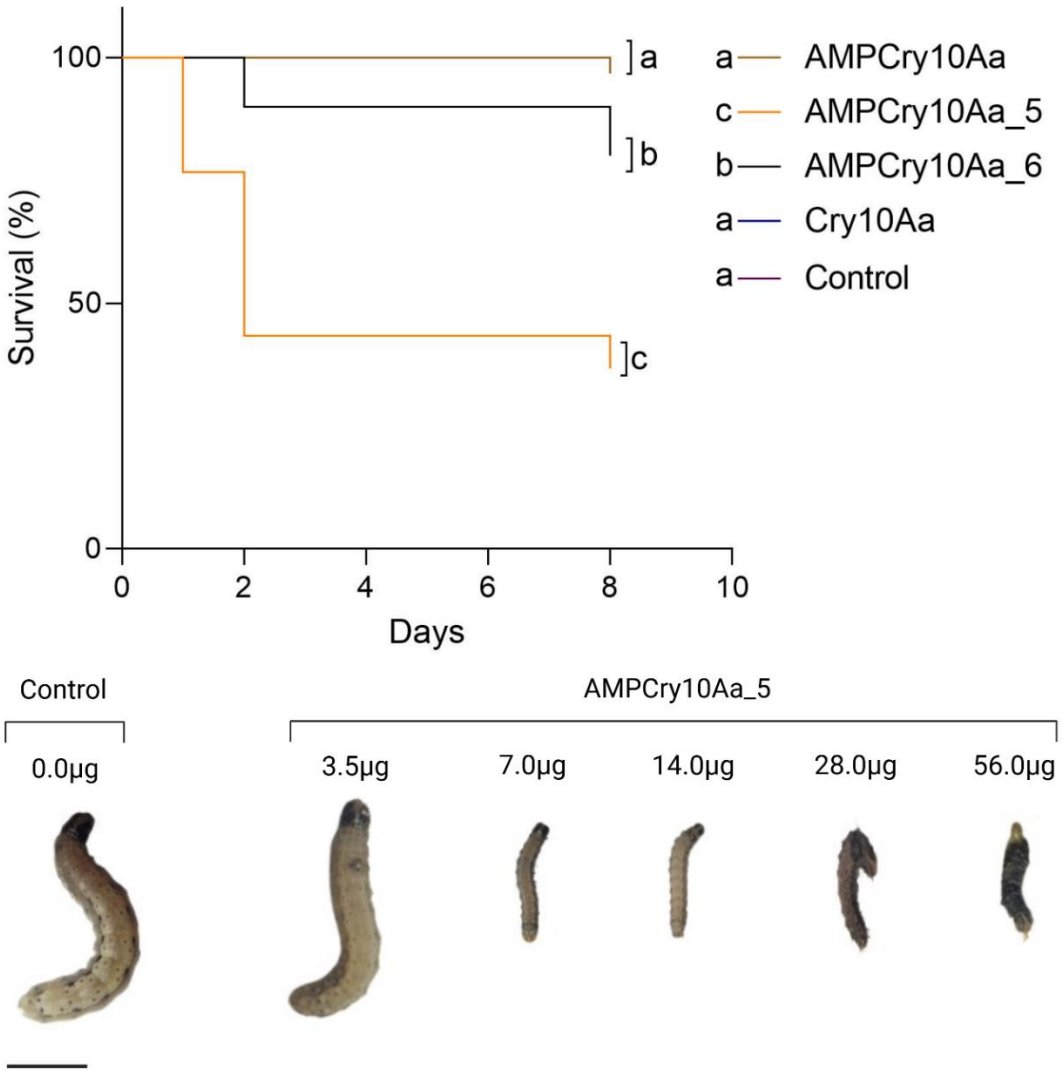

Figure 3. Survival curves of *Spodoptera frugiperda* and phenotypic changes in 3rd-instar larvae subjected to injected application of the parental peptide (AMPCry10Aa), variants (AMPCry10Aa_1 to AMPCry10Aa_6), Cry10Aa protein, and ultrapure water (Control) at a concentration of 56 µg mL^{-1}. Different letters indicate significant survival differences between groups (log-rank test, p-value <0.05; N: 30). Scale bar, 1 cm.

The results obtained are also consistent with previous observations by Rios et al. (2024), who reported vigorous antimicrobial activity of AMPCry10Aa_5 and AMPCry10Aa_6 against *Escherichia coli*. This parallel suggests that the mechanism of action of these peptides involves membrane disruption, mediated by electrostatic attraction between the peptides' positive charges and the negatively charged surfaces of bacterial and insect cells (Yeh et al., 1997; Koehbach & Craik, 2019). The convergence of antimicrobial and insecticidal activities reinforces the biotechnological potential of these peptides, which may serve as multifunctional bioinspired molecules. Molecular dynamics analyses further support this interpretation (Figure 4).

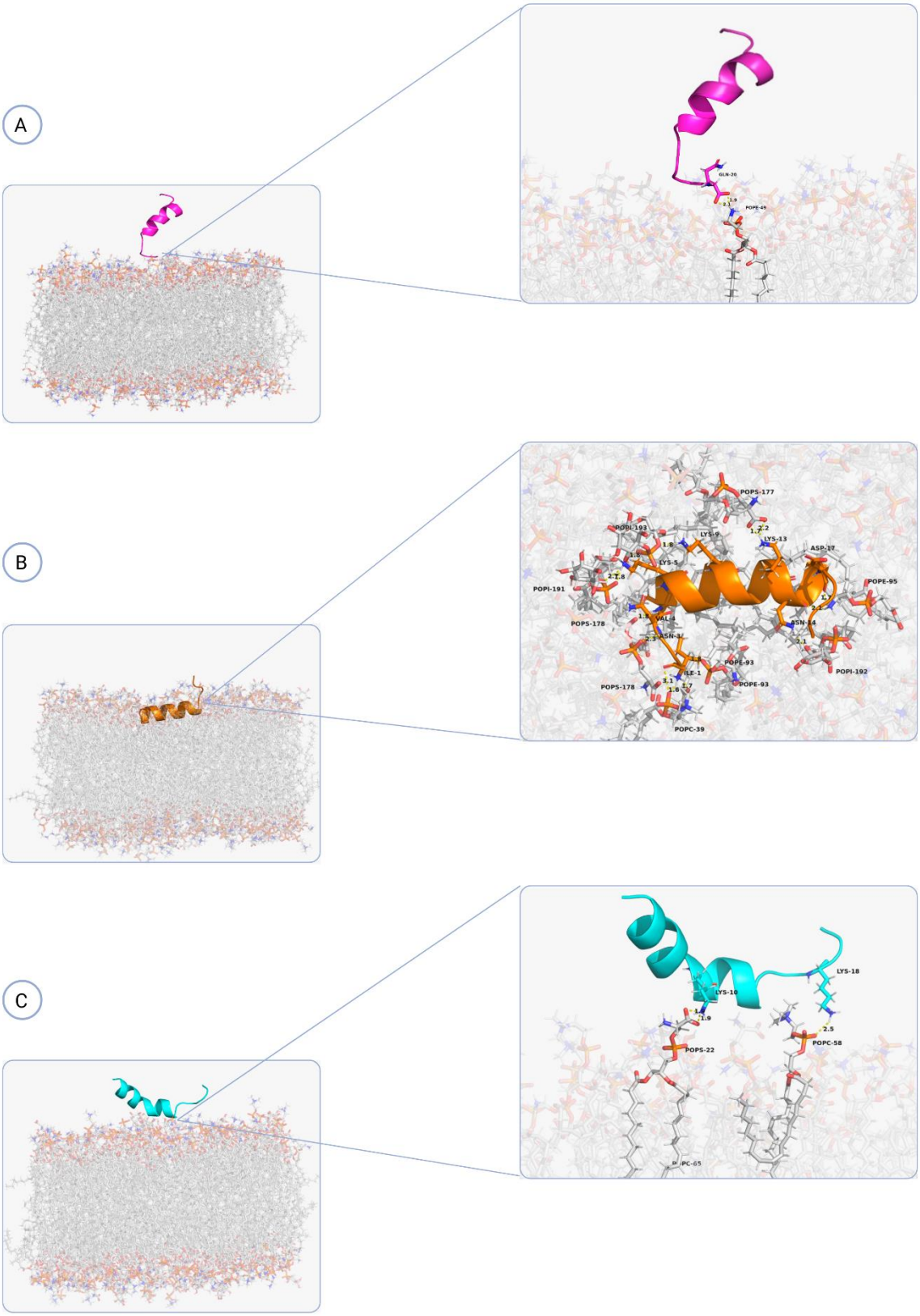

Figure 4. Interactions of (A) the parental peptide AMPCry10Aa, (B) the variant AMPCry10Aa_5, and (C) the variant AMPCry10Aa_6 with the lipid bilayer of *Spodoptera frugiperda* cell membranes, obtained through molecular docking simulations. The representations highlight anchoring modes and residues involved in membrane interactions.

The AMPCry10Aa_6 peptide primarily established interactions with phosphatidylethanolamine (PE) residues, a significant component of the insect cell membrane (42%), thereby favoring its anchoring in the lipid bilayer (Table 2). The AMPCry10Aa_5 peptide formed an increasing number of diverse hydrogen bonds, including with POPS and POPI, indicating stronger, multisite affinity for the membrane. This difference in behavior may explain the higher efficacy of AMPCry10Aa_5 in the injectable assay, as direct contact with the hemolymph bypasses the cuticular barrier.

Table 2. Interactions of the parental peptide (AMPCry10Aa) and the variants (AMPCry10Aa_5 to AMPCry10Aa_6) with the lipid bilayer of *Spodoptera frugiperda* cell membranes, obtained through molecular docking simulations.

	Residue	Peptide position	Atom	Residue	Membrane position	Atom	Occupancy (%)	Interaction
AMPCry10Aa	Ile	1	N	POPC	114	O13	14.3	HB
	Ile	1	N	POPI	177	O13	14.7	HB
AMPCry10Aa_5	Val	4	N	POPS	178	O22	35.9	HB
	Lys	5	NZ	POPI	191	O14	22.6	HB
	Lys	5	NZ	POPI	193	O13	13.0	HB
	Ile	2	N	POPE	93	O13	13.0	HB
	Ile	1	N	POPC	23	O13	17.5	HB
	Lys	9	NZ	POPI	193	O13	31.4	HB
	Lys	13	NZ	POPS	177	O13	18.7	HB
	Asn	14	ND2	POPC	21	O32	22.5	HB
	Asn	14	O	POPE	95	N	30.3	HB
	Asp	17	O	POPE	95	N	25.2	HB

	Lys	18	NZ	POPE	101	NZ	29.8	HB
	Lys	10	NZ	POPE	129	O14	15.6	HB
	Lys	6	NZ	POPE	118	O14	18.9	HB
AMPCry10Aa_6	Ile	1	N	POPE	120	O14	15.0	HB
	Ile	2	N	POPE	120	O14	13.0	HB
	Ile	1	N	POPE	124	O14	11.2	HB

[A] 42% de phosphatidylethanolamine (POPE), 36% de phosphatidylcholine (POPC), 15% de phosphatidylinositol (POPI), e 7% de phosphatidylserine (POPS).

Isoleucine stood out among the amino acids that interacted with phospholipids, showing contacts with POPE (AMPCry10Aa_6 and AMPCry10Aa_5), POPC (AMPCry10Aa_6 and AMPCry10Aa_5), and POPI (AMPCry10Aa), the three most abundant phospholipids in the cell membrane of *S. frugiperda*. Being hydrophobic, like valine, it tends to insert into the fatty acid region of the bilayer, thereby stabilizing the peptide–membrane association, which explains the interactions observed in the docking (Sato and Felix, 2006). Additionally, lysine interacted with POPI and POPS, attributed to electrostatic attraction between its positive charge and the negative charges of these phospholipids (Marukovich et al., 2015).

To evaluate conformational stability, molecular dynamics simulations were performed in an aqueous medium, analyzing the root mean square deviation (RMSD) and the root mean square fluctuation per residue (RMSF), parameters that indicate structural stability and flexibility under conditions close to physiological ones. The three peptides (AMPCry10Aa, AMPCry10Aa_5 and AMPCry10Aa_6) remained structurally stable throughout the simulations. However, AMPCry10Aa_6 presented the lowest mean RMSD and RMSF values and a lower tendency to increase over time, indicating

greater global stability and lower flexibility. The parental peptide AMPCry10Aa exhibited the highest values, being the least stable, while the AMPCry10Aa_5 variant showed intermediate behavior. These results indicate that the variants generated by Joker were more stable than the parental peptide (Figure 5).

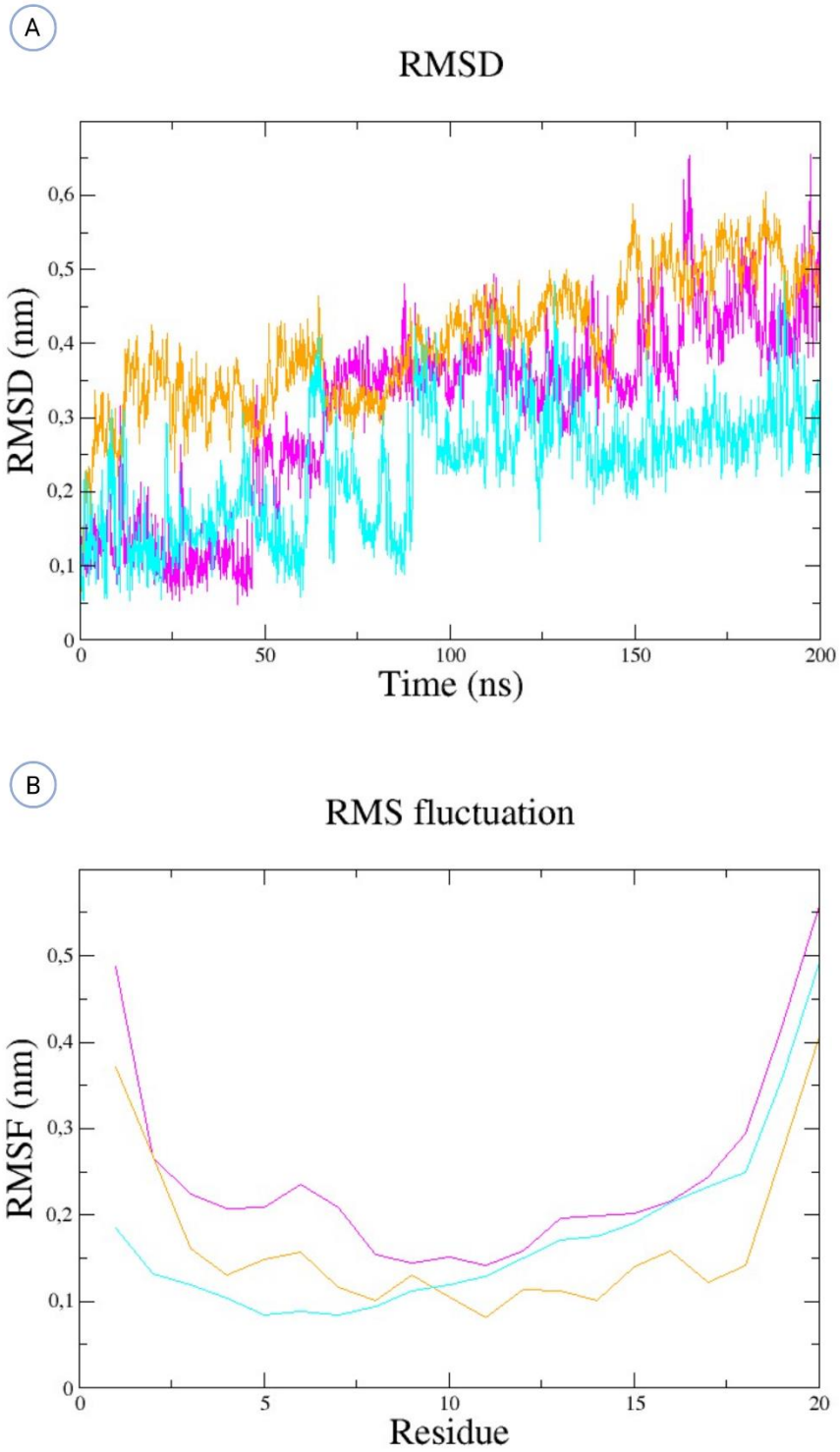

Figure 5. Representation of the root mean square deviation (A) and the root mean square fluctuation (B) of parameters obtained from molecular dynamics simulations in water, with 150 mM NaCl to simulate physiological conditions (GROMACS software v5.0.4). (Pink) the parental peptide AMPCry10Aa, (orange) the variant AMPCry10Aa_5, and (blue) the variant AMPCry10Aa_6.

Another relevant finding is the peptide selectivity. While Cry10Aa, AMPCry10Aa, and AMPCry10Aa_5 exhibited cytotoxicity only at concentrations much higher than those used in the bioassays (≥112 µg mL^{-1}), AMPCry10Aa_6 showed no significant toxicity against human fibroblasts at any tested concentration (Figure 6). This profile is highly desirable for the development of bioinsecticides, as it indicates a broad safety margin, combining insecticidal efficacy with mammalian cell safety. Considering that the effective concentrations against *S. frugiperda* ranged from 28 to 56 µg mL^{-1}, a particularly relevant safety margin is observed, especially for the AMPCry10Aa_6 peptide.

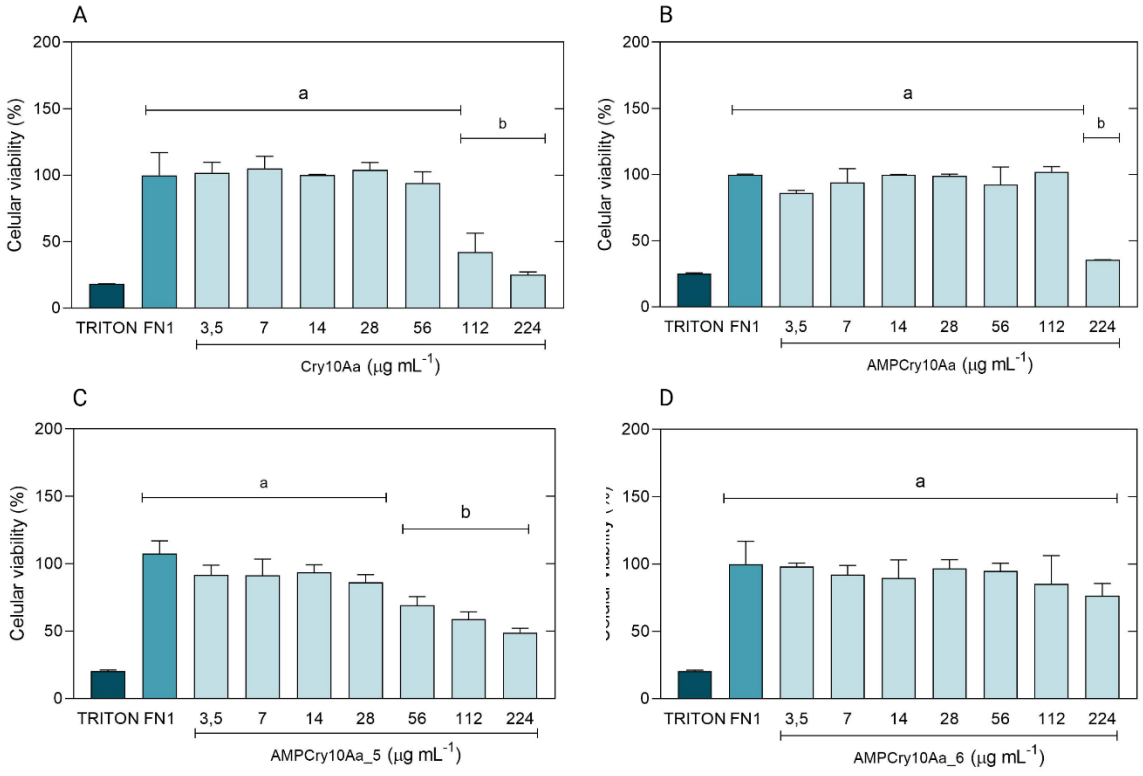

Figure 6. Cytotoxic activity of (A) Cry10Aa protein, (B) AMPCry10Aa peptide, (C) AMPCry10Aa_5 peptide, and (D) AMPCry10Aa_6 peptide against human fibroblast (FN1) cell cultures. Positive control (T) Triton; negative control (FN1). Different letters indicate significant differences according to $p < 0.05$ (one-way ANOVA followed by Tukey's test). Data represent mean ± SD.

In summary, the results presented here expand the understanding of the functional diversity of Cry proteins and their derived fragments. The demonstration that small peptides can maintain and even enhance insecticidal activity across different species and application routes opens new perspectives for the development of selective bioinsecticides.

Conclusions and Prospects

Insecticidal peptides exhibit broad spectrum and multiple mechanisms of action, with potential to prevent the development of resistance in agricultural pests. Our study revealed that the Cry10Aa protein can serve as a bioinspiration model for the synthesis of insecticidal peptides. Six variants were designed, analyzed, and tested for activity against major agricultural pests (*A. grandis* and S. *frugiperda*). The results demonstrated that the AMPCry10Aa_6 peptide stood out in topical assays, whereas AMPCry10Aa_5 performed best in injection assays, characterizing them as promising molecules for the development of new bioinsecticides. *In silico* analyses revealed interactions of these peptides with *S. frugiperda* membrane phospholipids, highlighting the role of isoleucine in its association with phosphatidylethanolamine, supporting a possible pore-forming mechanism. These findings confirm that the rational design of peptides, combining the dermaseptin B pattern with the Cry10Aa sequence, was successful in generating potential bioinsecticidal solutions for the safe and sustainable control of agricultural pests.

Experimental Section

Design of Cry10Aa-Derived Peptides

The design of the peptides in this study was based on the Cry10A protein (Figure 7A), which was selected for its central role of α-helix 3 in the pore-forming mechanism in cellular membranes, a characteristic associated with the bioactive function of these proteins (Rios et al., 2024). The original sequence of this region (IINVLTSIVTPIKNQLDKYQEFFDKWEPA) was analyzed. From it, a parental peptide, AMPCry10Aa, was defined, with a reduced sequence of IINVLTSIVTPIKNQLDKYQ (Figure 1B), which served as the basis for rational modification.

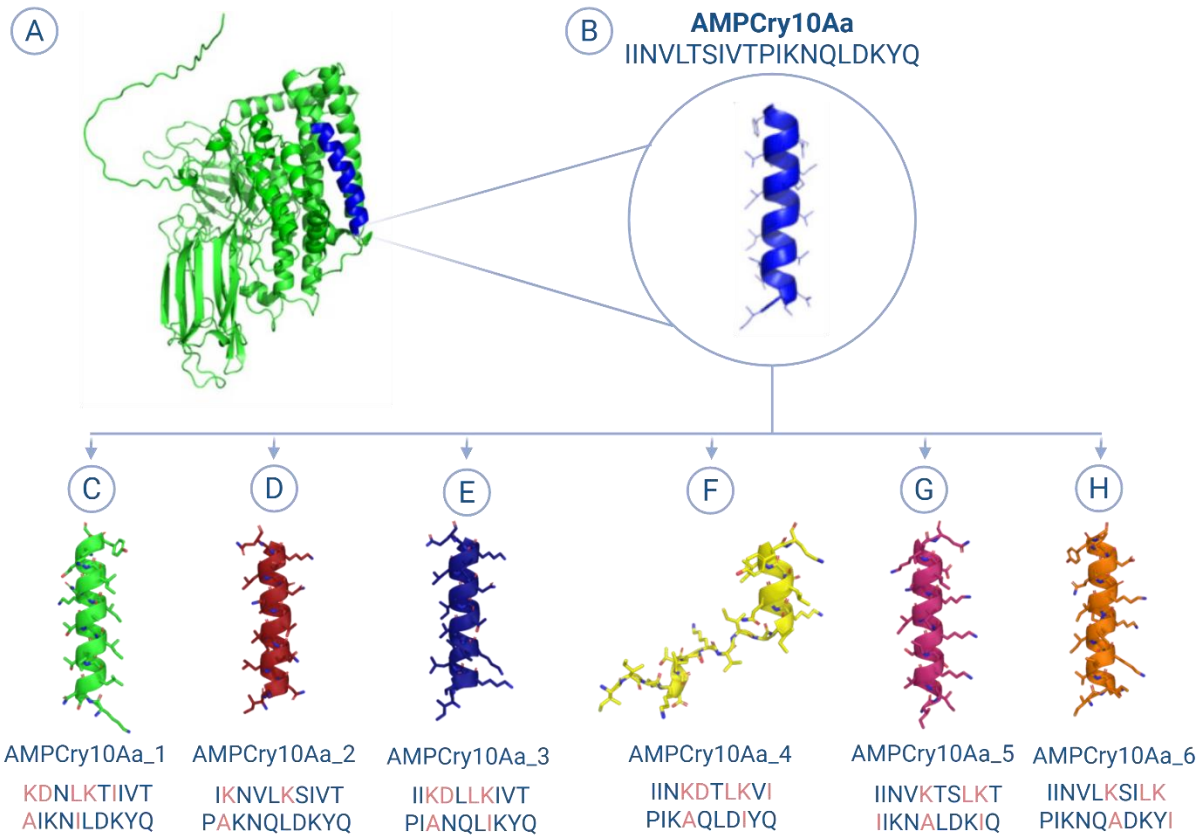

Figure 7. Molecular model of the Cry10Aa protein represented as ribbons (A) and a highlight of the 20-amino-acid sequence of α-helix 3 in light blue (B), used as the parental sequence for the development of the variants. Derived peptides: (C) AMPCry10Aa_1, (D) AMPCry10Aa_2, (E) AMPCry10Aa_3, (F)

AMPCry10Aa_4, (G) AMPCry10Aa_5, and (H) AMPCry10Aa_6, which comprised the experimental panel evaluated in this study. All structures were generated using PyMOL v. 1.8.

In the next step, this sequence was optimized using the Joker algorithm, a tool that introduces controlled variations based on known structural patterns. For this, the characteristic pattern of the dermaseptin family was used as a model: K-[ADEGNQST]-x-[AGL]-K-x-[AILV]-x(3)-A-x(3)-[AGILV]. In this arrangement, the residues K and A represent fixed positions, maintained across all sequences; the amino acids in brackets represent variable positions; the symbol “x” acts as a wildcard, accepting any amino acid; and the number in parentheses indicates consecutive repetitions of the same element. Based on this logic, the algorithm generated a set of variant peptides derived from the parental sequence. As a result, the sequences AMPCry10Aa_1 (Figure 1C), AMPCry10Aa_2 (Figure 1D), AMPCry10Aa_3 (Figure 1E), AMPCry10Aa_4 (Figure 1F), AMPCry10Aa_5 (Figure 1G), and AMPCry10Aa_6 (Figure 1H) were obtained, which constituted the experimental panel evaluated in this study.

Physicochemical Characterization of the Peptides

The designed variants, along with the parental sequence, were analyzed using the HeliQuest software, which allowed evaluation of physicochemical properties such as hydrophobic moment, net charge, and average hydrophobicity, based on the projection of an α-helix over a 20-residue window (Eisenberg et al., 1984). Peptide synthesis was carried out by Peptide 2.0 Inc. (USA) using FMOC (N-9-fluorenylmethoxycarbonyl) chemistry, ensuring a minimum purity of 95%. Molecular masses were confirmed by MALDI-ToF mass spectrometry, using the Ultraflex MALDI-TOF III instrument (Bruker Daltonics).

Insecticidal Assays on Target Pest Species

Insecticidal assays were conducted using the species *Anthonomus grandis* and *Spodoptera frugiperda*, two agriculturally relevant pest models. *A. grandis* (Coleoptera) was selected due to previous evidence of Cry10Aa protein insecticidal activity against this pest (Ribeiro et al., 2019), and *S. frugiperda* (Lepidoptera) represents a significant economic pest due to its polyphagous behavior, being widely used in bioassays for evaluating new bioactive compounds. The combined use of these two species enabled assessment of peptide efficacy across different insect orders.

Insecticidal Assay in *Anthonomus grandis* (Coleoptera)

Larvae of *A. grandis* used in the tests were obtained from the insect rearing facility at Embrapa Genetic Resources and Biotechnology. Insects were maintained in plastic Petri dishes containing artificial diet, under controlled incubation conditions: 28 °C, 50–60% relative humidity, and a 12-hour photoperiod (Vasquez et al., 2023). The six modified variants, along with the parental peptide AMPCry10Aa and the Cry10Aa protein, were incorporated into the diet at a concentration of 14 µg of peptide per gram of diet, corresponding to the previously established LD_{50} for Cry10Aa against this species.

The treated diet was distributed in 60 mm × 15 mm Petri dishes, into which eggs were placed at the developmental stage where the head capsule was already formed. Bioassays were conducted in three independent replicates, each with 30 larvae per treatment, for a total of 90 biological units per experimental group. Positive controls included the Cry10Aa protein and the AMPCry10Aa peptide, while the negative control

consisted of the artificial diet without any compounds. Larval survival rate was recorded 7 days after treatment, based on the ratio of initial eggs, hatched individuals, and mortality over the evaluation period.

Insecticidal Assay in *Spodoptera frugiperda* (Lepidoptera)

Colonies of *S. frugiperda* used in the experiments were provided by Embrapa Genetic Resources and Biotechnology and maintained under laboratory conditions at 25°C, 60% relative humidity, and a 14-h photoperiod. Insects were reared in glass tubes and fed on an artificial diet to ensure population uniformity. For the bioassays, two larval stages were selected according to the methodology used. Third-instar larvae were used for topical application and injection assays. In the topical application assay, larvae received two µL of peptide solutions at different concentrations (3.5, 7, or 14 µg mL^{-1}), applied to the prothoracic region. All six modified variants, the parental peptide AMPCry10Aa, and the Cry10Aa protein were evaluated as positive controls. Ultrapure water was used as a negative control. Each treatment included 30 individuals, and survival rates were recorded at 1, 2, 4, 6, and 8 days post-application, for peptides that showed reduced survival rates, higher concentrations (28 and 56 µg mL^{-1}) were tested to assess dose-dependent responses. In the injection assay, peptides that showed the best performance in the topical test, along with the positive (Cry10Aa and AMPCry10Aa) and negative (ultrapure water) controls, were selected. Each fourth-instar larva received 2 µL of peptide solution, at concentrations ranging from 3.5 to 56 µg mL^{-1}, injected dorsally using Hamilton® 10 µL syringes. Survival was monitored at the same time intervals, providing additional insights into insecticidal efficacy and potential mechanisms of action.

Peptide–Insect Cell Membrane Interaction (Molecular Docking)

The three-dimensional structures of peptides AMPCry10Aa (PDB ID: 8T3H) and AMPCry10Aa_5 (PDB ID: 8T3N) are deposited in the RCSB Protein Data Bank (Burley et al., 2024; Rios et al., 2024), while the structure of AMPCry10Aa_6 was predicted from its primary sequence using the PEP-FOLD 4 server (Rey et al., 2023). All simulations were performed using the GROMACS v2023.4 molecular dynamics package (Páll et al., 2020). To simulate a biological environment, plasma membrane models were built based on a composition of approximately 42% phosphatidylethanolamine (POPE), 36% phosphatidylcholine (POPC), 15% phosphatidylinositol (POPI), and 7% phosphatidylserine (POPS) (Yeh et al., 1997). Lipid bilayers representative of the *S. frugiperda* cuticle were generated using the CHARMM-GUI membrane builder (Jo et al., 2008), comprising 200 lipids (100 per leaflet) in a rectangular box parameterized with the CHARMM36m force field (Huang et al., 2016). Peptides were placed 30 Å above the upper monolayer, and the system was solvated with explicit TIP3P water, yielding a box of approximately 69 × 69 × 120 Å³. Ions were added for neutralization, along with 150 mM NaCl to mimic physiological conditions. Initial membrane equilibration followed the six-step CHARMM-GUI protocol, comprising three temperature-coupling phases (1.25 ns each) and three simultaneous temperature- and pressure-coupling phases (5 ns each). Subsequently, 200-ns production runs were conducted in GROMACS v2023.4 under the NPT ensemble (300 K, 1 bar). Resulting trajectories were analyzed to calculate root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and hydrogen bond donor–acceptor angle frequencies. Hydrogen-bond occupancy was evaluated in VMD using cutoff values of ≤ 3.2 Å for distance and ≤ 60° for angle. Finally, a comparison between the parental peptide AMPCry10Aa and its Joker-generated variants provided

mechanistic evidence for how structural modifications affect membrane insertion potential, helping to explain the differences observed in insecticidal activity.

Risk Assessment (Cytotoxicity in Human Fibroblasts)

To investigate the safety profile of the developed peptides, cytotoxicity assays were conducted on human fibroblasts (FN1), cultured in RPMI medium with 10% fetal bovine serum. The cytotoxicity of the Cry10Aa protein, the parental peptide AMPCry10Aa, and its variants, AMPCry10Aa_5 and AMPCry10Aa_6, were assessed using the MTT colorimetric assay (Mosmann, 1983; Sieuwerts et al., 1995). A suspension of approximately 1×10^5 cells mL^{-1} was plated in 96-well plates with varying peptide concentrations (3.5 to 224 µg mL^{-1}). Plates were incubated at 37 °C for 24 hours in a 5% CO_2 incubator. After incubation, the medium was aspirated and discarded. 10 µL of MTT solution diluted in PBS was added to the cell pellet, and plates were incubated for 4 hours at 37 °C in the dark. After incubation, 60 µL of solubilizing solution (isopropyl alcohol + hydrochloric acid) was added to dissolve formazan crystals. Plates were read using a Multiskan GO microplate reader (Thermo Fisher) after 10 minutes of constant agitation. Absorbance was measured at 540 nm, and cell viability percentages were calculated relative to untreated controls.

Statistical Analysis

For the *A. grandis* and *S. frugiperda* assays, survival data were analyzed using the Kaplan-Meier method, appropriate for time-to-event studies, describing mortality dynamics over the experimental period. Differences between treatments were assessed using the log-rank test, allowing direct comparison of survival curves. In

cytotoxicity assays, cell viability percentages were calculated, and group means were subjected to one-way ANOVA. When significant differences were detected, Tukey's post-hoc test was applied for multiple comparisons, adopting a significance level of $p<0.05$. All statistical analyses were performed using GraphPad Prism version 9.0, ensuring methodological standardization and reliability in result interpretation.

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5. APÊNDICE

Figuras suplementares: Capítulo II

Figuras Suplementares do Capítulo II

Insecticidal activity of bioinspired peptides rationally designed from the Cry10Aa protein

Este arquivo inclui:

Figura. S1 e S2

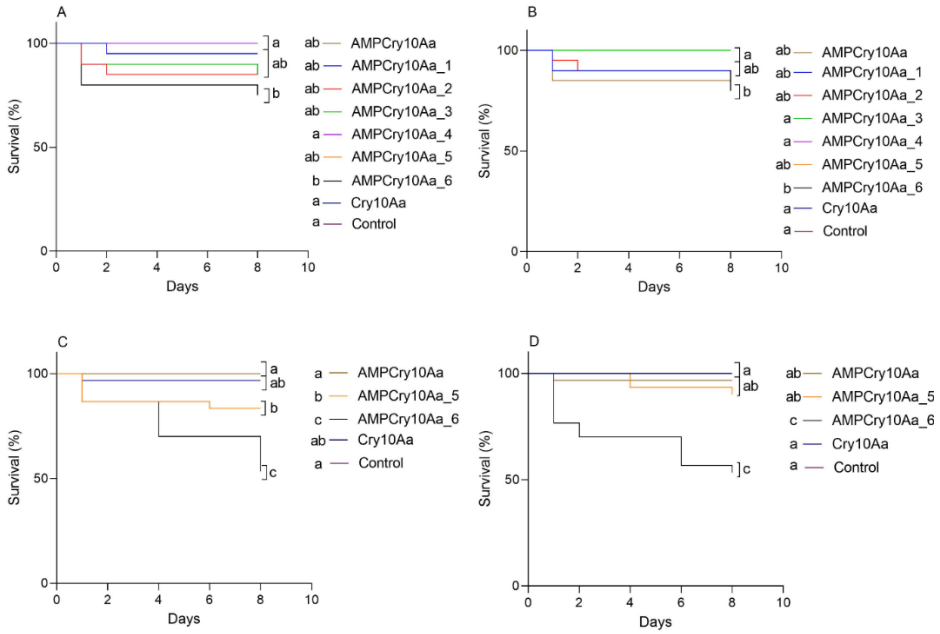

Figure S1. Survival curves of *Spodoptera frugiperda* in 3rd-instar larvae subjected to topical application of different treatment concentrations: (A) 3.5 µg mL⁻¹; (B) 7 µg mL⁻¹; (C) 28 µg mL⁻¹; (D) 56 µg mL⁻¹. The parental peptide (AMPCry10Aa), variants (AMPCry10Aa_1 to AMPCry10Aa_6), Cry10Aa protein, and ultrapure water (Control) were evaluated. Different letters indicate significant survival differences between groups (log-rank test, p-value <0.05; N: 30).

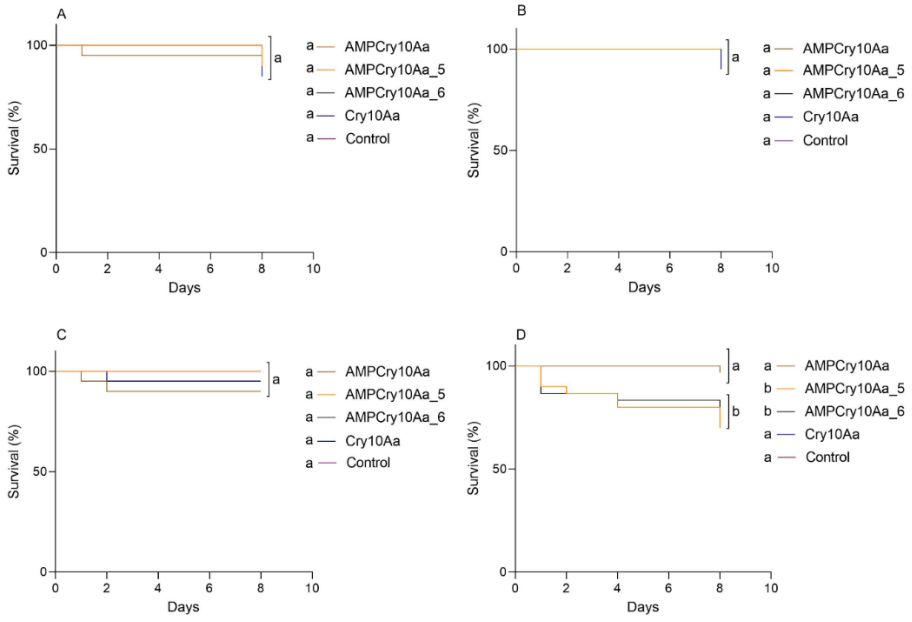

Figure S2. Survival curves of *Spodoptera frugiperda* and phenotypic changes in 3rd-instar larvae subjected to injected application of different treatment concentrations: (A) 3.5 µg mL⁻¹; (B) 7 µg mL⁻¹; (C) 14 µg mL⁻¹; (D) 28 µg mL⁻¹. The parental peptide (AMPCry10Aa), variants (AMPCry10Aa_1 to AMPCry10Aa_6), Cry10Aa protein, and ultrapure water (Control) were evaluated. Different letters indicate significant survival differences between groups (log-rank test, p-value <0.05; N: 30).

6. DISCUSSÃO GERAL

Esta tese abordou o potencial dos peptídeos inseticidas como alternativas biotecnológicas e sustentáveis para o manejo de pragas, visando superar os desafios associados aos inseticidas convencionais, como os impactos ambientais, os riscos à saúde e a evolução da resistência. O Capítulo I apresenta uma revisão do estado da arte sobre pesquisas e o desenvolvimento de produtos comerciais à base de peptídeos inseticidas, abordando suas fontes, mecanismos de ação e tecnologias inovadoras voltadas à criação de novas moléculas, bem como estratégias que asseguram sua aplicabilidade no contexto agrícola. Dando continuidade ao tema discutido na revisão, o Capítulo II descreve a caracterização da atividade inseticida de peptídeos derivados da proteína entomotóxica Cry10Aa, com ênfase em sua ação *in vivo* contra *A. grandis* e *S. frugiperda*. Em conjunto, os capítulos desta tese contribuem para o avanço da biotecnologia agrícola, evidenciando o potencial dos peptídeos como soluções bioinseticidas para o controle seguro, eficaz e sustentável de pragas agrícolas.

Os peptídeos são moléculas estudadas por suas funções, estrutura e aplicações, sendo amplamente empregados na área da medicina, como antimicrobianos (AMPs), no tratamento de diabetes e obesidade (Ozempic® e Mounjaro®), além de suas aplicações em cosméticos e, mais recentemente, na agricultura. No contexto agrícola, destacam-se por seu papel na proteção de plantas, atuando contra fitopatógenos, além de atuar como reguladores de crescimento, herbicidas e inseticidas. Os peptídeos inseticidas de origem natural são encontrados, sobretudo, nos venenos de artrópodes, mas também podem ser derivados de outros animais, plantas e microrganismos, ou ainda desenvolvidos por bioengenharia como análogos de compostos endógenos de insetos (Grover et al., 2021; Akbarian et al., 2022; Liu et al., 2021; Zhang et al., 2023).

Os avanços em biologia sintética e bioinformática, impulsionados pelo uso de algoritmos de inteligência artificial, têm permitido não apenas a predição estrutural,

mas também a identificação de interações moleculares-chave para o desenvolvimento de novos inseticidas peptídicos (Gressel, 2022; Zhang et al., 2023; Zai et al., 2025). Essa abordagem foi aplicada no desenvolvimento de peptídeos derivados da proteína Cry10Aa, que apresentaram expressiva atividade inseticida contra *A. grandis* e *S. frugiperda*, conforme evidenciado no Capítulo II desta tese, o que evidencia o potencial dessas tecnologias para a criação de novas moléculas com ação inseticida. Além disso, o Capítulo II destaca uma inovação de caráter disruptivo, pois o uso da proteína Cry10Aa para gerar variantes representa não apenas o primeiro relato de peptídeos derivados de proteínas Cry, mas também um dos raros exemplos de peptídeos inseticidas originados de microrganismos, como a bactéria *B. thuringiensis*.

Além dos modelos de predição, os avanços em bioinformática têm permitido esclarecer os mecanismos de ação dos peptídeos inseticidas, orientando o desenvolvimento de moléculas com diferentes mecanismos de ação. Ferramentas de *docking* molecular, ao revelar suas interações com receptores e alvos específicos, têm ampliado o conhecimento e acelerado a aplicação dessa estratégia na proteção de plantas (Meriño-Cabrera et al., 2022; Zhow et al., 2025). No Capítulo II, essa ferramenta foi aplicada para prever a interação dos peptídeos com a membrana fosfolipídica de *S. frugiperda*, indicando que o provável mecanismo de ação das variantes desenvolvidas e testadas no estudo envolve a formação de poros.

O processo de formação de poros em membranas está amplamente descrito para peptídeos antimicrobianos (AMPs), mas ainda é pouco explorado no contexto de peptídeos inseticidas (Ascoët, S. et al., 2023; Oliveira-Junior et al., 2025). Estudos pioneiros com AMPs evidenciaram o papel fundamental do aminoácido hidrofóbico isoleucina na interação com membranas bacterianas (Juvvadi et al., 1996). De forma semelhante, essa interação também foi observada nas variantes de Cry10Aa avaliadas no Capítulo II por meio do *docking* molecular, nas quais a isoleucina apresentou o maior número de interações por meio de pontes de hidrogênio com o fosfolipídio fosfatidiletanolamina. Esse resultado pode ser atribuído à semelhança estrutural entre as membranas bacterianas e as celulares de insetos, ambas caracterizadas por uma carga negativa decorrente de sua composição fosfolipídica (Yeh et al., 1997; Koehbach & Craik, 2019).

Além da formação de poros em membranas, os peptídeos inseticidas relatados na literatura também podem atuar de outras formas. Alguns funcionam como inibidores de enzimas digestivas no trato intestinal dos insetos, reduzindo a absorção de nutrientes. Outros apresentam efeito neurotóxico, interferindo nos canais iônicos de cálcio, sódio e potássio, bem como em receptores neuronais. Há ainda os peptídeos hormonais e neuropeptídeos, capazes de mimetizar ou antagonizar hormônios fundamentais para processos vitais, como a ecdise e o crescimento (Liu et al., 2021; Raisch & Raunser, 2025). Esses são os principais tópicos discutidos na revisão do Capítulo I, que apresenta diferentes soluções inseticidas baseadas em peptídeos. A diversidade funcional desses compostos constitui um arsenal biotecnológico versátil, permitindo a escolha do peptídeo mais apropriado para cada tipo de praga, cultura ou estratégia de controle.

Os peptídeos que inibem enzimas digestivas apresentam ação mais lenta e cumulativa, mas se destacam pela maior seletividade, o que reduz o impacto ambiental e aumenta a segurança para organismos não alvo. Seu mecanismo envolve a inibição de α-amilases e de proteases intestinais, comprometendo a absorção de nutrientes e, consequentemente, o crescimento, a reprodução e, em alguns casos, a sobrevivência dos insetos (Rane et al., 2020; Pandey et al., 2022). Apesar de serem a estratégia menos explorada na literatura recente, o que pode ser observado no Capítulo I, esses peptídeos apresentam grande potencial como ferramentas de controle preventivo e de longo prazo.

Os peptídeos neurotóxicos, por sua vez, têm ganhado destaque com o lançamento,® nos EUA, em 2020, do bioinseticida Spear®. Derivado do veneno da aranha *Hadronyche versuta*, esse peptídeo atua como modulador alostérico positivo dos receptores nicotínicos de acetilcolina (AChRs) em insetos, aumentando a sensibilidade desses canais à acetilcolina (ACh) sem causar hiperestimulação tóxica prolongada (Bloomquist et al., 2023). Esse mecanismo resulta em uma neuroexcitação controlada, assegurando alta seletividade em relação a insetos e baixa toxicidade em vertebrados (Ross et al., 2025). Essas neurotoxinas se destacam pela ação rápida, comparável à dos inseticidas convencionais, mas sem os efeitos adversos típicos desses compostos químicos, o que as torna ideais para o controle de infestações agudas.

Em contraste com as neurotoxinas de ação rápida, os peptídeos inseticidas hormonais e miméticos atuam de forma crônica ao regular processos essenciais, como o desenvolvimento, o metabolismo e a reprodução. Ainda assim, essa classe vem ganhando destaque devido à sua alta seletividade, abrindo espaço para o desenvolvimento racional de compostos por meio de ferramentas modernas, como modelagem estrutural tridimensional, docking molecular e algoritmos de aprendizado de máquina, que permitem a otimização da potência, seletividade e perfil ambiental de agentes inseticidas (Xin et al., 2024; Ge et al., 2025). Isso demonstra que as ferramentas de bioinformática podem ser aplicadas para o desenvolvimento de diferentes classes de peptídeos inseticidas, seja um potencial formador de poros como os descritos no Capítulo II, ou peptídeos miméticos que possuem alta seletividade.

A seletividade é um fator essencial no desenvolvimento de inseticidas de nova geração. Embora eficazes, muitos pesticidas convencionais carecem desse atributo, afetando organismos não alvo. A exposição crônica a agroquímicos pode induzir a formação de espécies reativas de oxigênio (EROs), desencadeando estresse oxidativo, comprometendo as defesas celulares e favorecendo o surgimento de doenças como câncer, distúrbios neurodegenerativos, hepáticos e cardiovasculares (Kaur et al., 2019; Ayilara et al., 2023). Em contrapartida, os peptídeos destacam-se pela alta seletividade, baixa persistência ambiental e múltiplos modos de ação (Zhang et al., 2024; Schultz et al., 2024; Civolani et al., 2025). A realização de testes de segurança, como o ensaio de citotoxicidade descrito no Capítulo II, são fundamentais para assegurar que essa nova tecnologia seja efetivamente segura para a saúde humana. Além de impactar a saúde humana, os pesticidas representam uma séria ameaça à biodiversidade e aos serviços ecossistêmicos, contaminando alimentos, água, ar e solo (Zaller et al., 2022; Cech et al., 2023).

Os peptídeos inseticidas entram nesse cenário como uma alternativa disruptiva, que atende às demandas de controle de pragas, sem os efeitos colaterais dos inseticidas químicos convencionais. Entretanto, a eficácia demonstrada em condições laboratoriais não garante automaticamente a mesma performance em cenários de campo. Questões como degradação ambiental, estabilidade em formulações, interação com microbiota do solo e efeitos sobre organismos não alvo ainda precisam ser exploradas em maior profundidade. Além disso, a avaliação de segurança limitou-se a fibroblastos FN1, o que restringe as conclusões sobre toxicidade. Testes

adicionais em diferentes linhagens celulares e em organismos benéficos são necessários para confirmar a seletividade dessas moléculas.

Para superar alguns desses obstáculos, têm sido exploradas estratégias como modificações estruturais, fusão com CPPs, conjugação com toxinas, uso de entomopatógenos transgênicos e encapsulamento em nanomateriais (Fassolo et al., 2024; Zhang et al., 2023; Lee & Poh, 2023; Civolani et al., 2025). Os peptídeos inseticidas avaliados no Capítulo II foram testados em estudos prévios quanto a sua estabilidade. Após 12 horas de exposição, observou-se degradação quase completa, evidenciando a janela de ação desses compostos e, dependendo de sua aplicabilidade, a necessidade de tecnologias que prolonguem sua estabilidade ou acelerem sua atividade (Rios et al., 2024).

Outro ponto crítico refere-se à variação da resposta entre espécies. Enquanto a proteína Cry10Aa intacta mostrou maior eficácia contra *A. grandis*, alguns peptídeos derivados, como o AMPCry10Aa_6, tiveram melhor desempenho contra *S. frugiperda*. Essa diferença sugere que a composição lipídica das membranas e mecanismos de absorção distintos entre insetos podem influenciar o resultado, exigindo estudos comparativos mais detalhados.

Do ponto de vista prático, o peptídeo AMPCry10Aa_6 merece destaque por apresentar baixa citotoxicidade, configurando-se como um candidato promissor para estudos futuros. Por outro lado, algumas variantes demonstraram toxicidade em doses elevadas, o que reforça a importância de uma triagem rigorosa antes da realização de ensaios em escala agrícola. Outro ponto central refere-se à viabilidade econômica: embora o bioinseticida Spear® já evidencie competitividade em relação a inseticidas convencionais, questões como custos de produção, estabilidade em condições de campo e exigências regulatórias ainda constituem barreiras relevantes. Esses fatores explicam, em parte, a maior exploração dos peptídeos nas áreas farmacêutica e cosmética, onde o valor agregado dos produtos justifica investimentos mais altos. Ainda assim, o exemplo do Spear® demonstra que a produção em larga escala pode ser viabilizada por meio de sistemas heterólogos baseados em bactérias, fungos e culturas vegetais, o que abre caminho para sua aplicação também na agricultura (Parachin et al., 2012; Narayani et al., 2020; Zhang et al., 2023).

Quanto à resistência, ainda que os peptídeos apresentem mecanismos de ação distintos dos inseticidas sintéticos e das proteínas Cry completas, não se pode descartar a possibilidade de seleção de populações resistentes. Portanto, recomenda-se que sua utilização futura seja pensada dentro de programas de manejo integrado de pragas, com rotação de moléculas e associação a OGMs e bioinseticidas já consolidados.

Em síntese, esta tese demonstra que os peptídeos derivados da Cry10Aa constituem uma estratégia inovadora e segura para o manejo de pragas agrícolas, trazendo evidências experimentais de sua eficácia e seletividade. No entanto, para consolidar seu uso, ainda são necessários estudos adicionais em organismos não alvo, análises de estabilidade em condições ambientais reais, avaliação de custo-benefício em escala industrial e integração em programas de manejo integrado de pragas.

A principal contribuição deste trabalho foi estabelecer uma prova de conceito robusta de que fragmentos da proteína Cry10Aa podem originar peptídeos bioinspirados com atividade inseticida, abrindo caminho para novas pesquisas e aplicações na agricultura sustentável.

7. CONCLUSÕES E PERSPECTIVAS

Os resultados apresentados nesta tese reforçam o papel estratégico dos peptídeos inseticidas como alternativas biotecnológicas inovadoras e sustentáveis para o manejo de pragas agrícolas. Ao reunir uma revisão abrangente e dados experimentais inéditos, este trabalho demonstrou que os peptídeos possuem características, como alta seletividade, múltiplos mecanismos de ação e baixa persistência ambiental, o que os torna candidatos promissores frente às problemáticas causadas pelos inseticidas convencionais.

O desenvolvimento inédito de variantes derivadas da proteína entomotóxica Cry10Aa representa um avanço significativo, ao demonstrar sua eficácia no controle de pragas de elevada importância agrícola, como *A. grandis* e *S. frugiperda*. Esses achados confirmam o potencial das proteínas de *B. thuringiensis* como modelos de bioinspiração para o design racional de inseticidas peptídicos, ampliando o leque de moléculas microbianas aplicáveis ao manejo sustentável de pragas.

Outro aspecto central desta tese foi a integração entre bioinformática, modelagem molecular e ferramentas de inteligência artificial, que possibilitou a predição estrutural e a elucidação de interações moleculares chave. A identificação da formação de poros como provável mecanismo de ação das variantes de Cry10Aa demonstra como tais abordagens podem orientar a descoberta de novas moléculas e otimizar seu desempenho em sistemas biológicos complexos. A diversidade funcional descrita na revisão, abrangendo desde inibidores de enzimas digestivas até neurotoxinas e miméticos hormonais, ressalta ainda a versatilidade dos peptídeos e abre espaço para a seleção de estratégias mais específicas de acordo com a cultura, a praga-alvo ou a urgência da infestação.

Além de evidenciar a eficácia inseticida, esta tese também destacou a segurança potencial dos peptídeos derivados da proteína Cry10Aa, uma vez que os ensaios de citotoxicidade indicaram baixa toxicidade em células humanas. Esse atributo, aliado à seletividade e à baixa persistência ambiental, contribui para consolidar os peptídeos

como alternativas mais alinhadas à preservação da biodiversidade e à redução dos impactos causados pelos pesticidas químicos sobre ecossistemas e saúde pública

Apesar desse potencial, a aplicação em escala comercial ainda enfrenta desafios relacionados à estabilidade e biodisponibilidade. Estratégias como modificações estruturais, conjugação com CPPs e o encapsulamento em nanomateriais surgem como soluções viáveis para ampliar a estabilidade e a eficácia desses bioinseticidas. Além disso, perspectivas futuras apontam para a necessidade de expandir os estudos em condições de campo, de modo a validar a durabilidade e a eficiência dos peptídeos em ambientes agrícolas reais. Avaliações ecotoxicológicas mais abrangentes também se fazem necessárias para assegurar a ausência de efeitos indesejados em organismos não alvo e em serviços ecossistêmicos essenciais, como polinização e ciclagem de nutrientes. A padronização desses protocolos de segurança e eficácia será fundamental para acelerar a regulamentação e a comercialização dessa nova tecnologia em controle de pragas.

Por fim, a integração de bioinseticidas peptídicos em programas de manejo integrado de pragas representa um caminho estratégico para reduzir a dependência de pesticidas químicos e diversificar as ferramentas de controle disponíveis. O uso combinado de peptídeos com outras abordagens biotecnológicas, como entomopatógenos e plantas transgênicas, poderá potencializar sua eficácia e contribuir para a construção de sistemas agrícolas mais resilientes e sustentáveis. Ao integrar seletividade, eficácia e segurança ambiental, aliados ao potencial de inovação tecnológica, os peptídeos inseticidas emergem como pilares para o desenvolvimento de uma nova geração de defensivos agrícolas, capaz de atender às demandas globais por produtividade, sustentabilidade e segurança alimentar.

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