AGRONOMIA

SUSCETIBILIDADE DE *Plutella* xylostella A COMBINAÇÕES DE CEPAS DE *Bacillus thuringiensis*

MARIANA SANGUINETE SANTOS

Morrinhos, GO
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Trabalho de conclusão de curso apresentado ao Instituto Federal Goiano – Câmpus Morrinhos, como requisito parcial para a obtenção do Grau de Bacharel em Agronomia.

Orientadora: Profª. Drª. Lilian Lucia Costa
Co-Orientador: Profº. Drº. Ricardo Antonio Polanczyk

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_____	_____
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Co-Orientador	Membro
UNESP/FCAV	IF Goiano – Campus Morrinhos

_____

Profª. Drª. Lilian Lúcia Costa
Presidente - Orientador
IF Goiano – Campus Morrinhos

Morrinhos – GO
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“Dizem que quem sonha vai mais longe, mesmo que seja na imaginação”
-Maria Claudia Sanguinete Santos

Dedico,

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Sumário

RESUMO

SANTOS, Mariana Sanguinete. **Suscetibilidade de *Plutella* xylostella a combinações de cepas de *bacillus thuringiensis.*** 2017. 39p. Trabalho de conclusão de curso (Curso de Bacharelado em Agronomia). Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Morrinhos, Morrinhos, GO, 2017.

Plutella xylostella (Lepidoptera: Plutellidae), conhecida como traça-das-crucíferas, é praga chave das crucíferas. O uso de inseticidas químicos no seu controle resultou na redução das populações de inimigos naturais e organismos não alvo, além de acelerar a evolução da resistência em populações desse inseto. Bioinseticidas formulados como a bactéria entomopatogênica *Bacillus thuringiensis* (Bt) são utilizados como alternativa ao controle quimico de *P. xylostella*, porém relatos de resistência de populações desse inseto foram relatados em campo. Combinações de cepas e/ou toxinas de Bt podem ser utilizadas como estratégias de manejo da resistência. Desta forma, este trabalho avaliou a suscetibilidade de *P. xylostella* a diferentes combinações de cepas de Bt, utilizando três modelos experimentais: A1 = sete tratamentos com cepas de Bt cultivados em placas separadas (HD2, HD3, HD4, HD7, HD11, HD 12 e testemunha), A2 = 16 tratamentos com cepas de Bt cultivadas na mesma placa (HD2x3, HD2x4, HD2x7, HD2x11, HD2x12, HD3x4, HD3x7, HD3x11, HD3x12, HD4x7, HD4x11, HD4x12, HD7x11, HD7x12, HD11x12 e testemunha), e A3=16 tratamentos com cepas de Bt misturadas no momento da aplicação (HD2x3, HD2x4, HD2x7, HD2x11, HD2x12, HD3x4, HD3x7, HD3x11, HD3x12, HD4x7, HD4x11, HD4x12, HD7x11, HD7x12, HD11x12 e testemunha). Nos três modelos experimentais foram utilizadas três repetições por tratamento que consistiu de um disco de couve imerso em suspensão contendo 10^6 esporos/mL e posteriormente transferido para placas de Petri com papel filtro umedecido no seu interior. Em cada tratamento foram utilizadas 60 lagartas de 2º ínstar distribuídas em três repetições. As placas foram acondicionado em sala climatizada com ambiente e fotoperíodo controlados. Os dados foram submetidos a ANOVA, e posteriormente ao teste de Scott Knott (α= 0,05), para análise dos dados e esta foi feita com base em cálculos de mortalidade corrigida e grau de interação. A relação entre o aumento do número de toxinas com o aumento da mortalidade não foi observada em todos os tratamentos independente se as cepas foram multiplicadas juntas em placas ou

misturadas no momento de aplicação. Houve uma melhor interação entre as toxinas encontradas na cepa *Bt thunringiensis* com as cepas de *Bt finitimus*, *Bt dendrolimus*, e *Bt aizawai* que demonstraram sinergismo no tratamento de modelo A2, e efeito aditivo no modelo A3. A obtenção desta interação sinérgica está relacionada com a combinação das toxinas Cry presentes nas cepas e com a capacidade dessas toxinas se ligarem a receptores presentes nas microvilosidades apicais das células colunares do intestino médio do inseto. Combinações de cepas de *Bt* tem potencial para ser utilizadas como estratégia de manejo da resistência de *P. xylostella* a Bt bioinseticida.

Palavras-chave: Traça-das-crucíferas, Bactéria entomopatogênica, Controle Biológico.

ABSTRACT

SANTOS, Mariana Sanguinete. **Susceptibility of Diamondback moth to combinations of strains of *bacillus thuringiensis***. 2017. 39p. Completion of course work (Course of Bachelor in Agronomy). Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Morrinhos, Morrinhos, GO, 2017.

Diamondback moth (Lepidoptera: Plutellidae), known as the crucifer's moth, is a key plague of cruciferous plants. The use of chemical insecticides in their control resulted in the reduction of the populations of natural enemies and non-target organisms, besides accelerating a development of resistance in populations of this insect. The forms Bioinseticidas such as the entomopathogenic bacterium *Bacillus thuringiensis* (Bt) are used as an alternative to the chemical control of *Diamondback moth*, however, reports of resistance of populations of this type. Combinations of Bt strains and/or toxins can be used as resistance management strategies. In this work, we evaluated the susceptibility of *Diamondback moth* to different combinations of Bt strains using three experimental models: A1 = seven treatments with Bt strains grown on separate plaques (HD2, HD3, HD4, HD7, HD11, HD12 and control), A2 = 16 treatments with Bt strains cultured on the same plate (HD2x3, HD2x7, HD2x11, HD2x12, HD3x7, HD3x7, HD3x7, HD3x11, HD4x7, HD4x11, HD4x11, HD7x11, HD7x12, HD11x12 and control). A = 16 treatments with Bt strains mixed at the time of application (HD2x3, HD2x4, HD2x7, HD2x11, HD2x12, HD3x7, HD3x7, HD3x11, HD3x12, HD4x7, HD4x11, HD4x12 , HD7x11, HD7x12, HD11x12 and control). Our three experimental models required three replicates per treatment with consisted of a suspension division nightclub containing 10^6 spores/mL and later transferred to Petri dishes with filter paper moistened therein. In each treatment with 60 2nd instar larvae distributed in three replicates. The plates were conditioned in an air-conditioned room with controlled environment and photoperiod. The data were submitted to ANOVA, and to the Scott Knott test ($\alpha = 0.05$), for data analysis and this was done based on corrected mortality and interaction degree calculations. The relationship between increasing numbers of toxins with increased mortality was not observed in all independent treatments as strains were plated together or mixed at the time of application. There was a better interaction between toxins found in the *Bt thunringiensis* strain with the strains of *Bt finitimus, Bt dendrolimus*, and *Bt aizawai* that

demonstrate synergism without A2 model treatment, and additive effect without A3 model. Obtaining synergistic interaction is related to the compatibility of toxins. Strain requirements and with a solution are toxins binding to receptors present on the apical microvilli of the midgut columnar cells of the insect. Combinations of Bt strains have potential to be used as a resistance management strategy of Diamondback moth to Bt bioinsecticide.

Keywords: Diamondback moth, Entomopathogenic bacteria, Biological control.

1. Introdução

Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), conhecida como traça-das-crucíferas, é originária da região mediterrânea e destaca-se por ser a principal praga das crucíferas, no Brasil e no mundo e sua agressividade está relacionada principalmente ao seu ciclo curto (De Bortoli et al., 2013). O ataque pode ocorrer não somente em folhas novas, mas também em folhas velhas, caules e brotos, dependendo da região e época do ano, acarretando na redução do crescimento da planta e área foliar, com até 60% no rendimento final da produção (Czerpak et al., 2005).

O dano é produzido somente pelas larvas, que após a eclosão se alimentam das folhas, raspando o tecido, deixando apenas a epiderme superior transparente em formato de um pequeno orifício. Posteriormente, surgem furos no tecido da folha e, quando o ataque é intenso as folhas ficam rendilhadas (Cardoso et al., 2010; De Bortoli et al., 2014).

O controle de *P. xylostella* é feito basicamente com inseticidas químicos, devido a sua eficácia e facilidade de aplicação (De Bortoli et al., 2013). Contudo, a utilização indiscriminada resulta na seleção de populações resistentes do inseto-praga aos inseticidas, além de afetar organismos não-alvo (Boiça Júnior et al., 2005; Naika et al., 2006).

Como alternativa ao método químico de controle, destaca-se a utilização de agentes de controle biológico tais como bactérias entomopatogênicas (Fiuza et al., 2017). A bactéria *Bacillus thuringiensis* (Bt) é o microrganismo mais no controle biológico de insetos-praga devido a sua alta especificidade e seletividade (De Bortoli et al., 2017; Polanczyk et al., 2017; Van Frankenhyuzen, 2017).

O modo de ação específico e a segurança dos produtos à base de Bt estão relacionados com a produção de uma inclusão cristalina parasporal (cristal) na célula bacteriana durante a esporulação ou na fase estacionária (Vilas-Bôas et al. 2007). A toxicidade de Bt está relacionada principalmente à síntese das proteínas Cry as quais são codificadas por diferentes genes, sendo possível encontrar até cinco tipos de proteínas numa mesma célula bacteriana (Bravo et al., 2017).

Um inseto suscetível deve ingerir o bioinseticida que contém os esporos e cristais para o início da infecção. Posteriormente, os cristais são solubilizados em pH

alcalino, originando as protoxinas que em presença de enzimas digestivas (proteases) são convertidas em quatro ou mais polipeptídeos tóxicos (δ-endotoxinas). As toxinas hidrolizadas cruzam a membrana peritrófica e ligam-se a receptores específicos localizados na membrana apical das células colunares do intestino médio, interferindo no gradiente iônico e balanço osmótico da membrana apical, formando poros que aumentam a permeabilidade da membrana. O aumento na absorção de água causa lise celular e eventual ruptura e desintegração das células do intestino médio. Os esporos ingeridos junto com o cristal germinam devido a redução do pH o que causa infecção generalizada (septicemia) (Copping & Menn, 2000; Jurat-Funtes & Crickmore, 2017).

Embora o uso de Bt bioinseticidas seja promissor no manejo de *P. xylostella*, relatos de resistência de populações (Tabashinik et al., 1998; Crickmore, 2016 dificultam o manejo desse inseto-praga. Combinações de toxinas Cry de Bt são uma alternativa eficiente para manejar a resistência de insetos praga a plantas transgênicas (Huang et al., 2014; Carrière et al., 2015; Carrière et al., 2016; Wei et al., 2015), mas até o momento pouco foi explorado quanto a misturas de cepas de Bt com o mesmo objetivo (Jihen et al., 2014). Neste contexto, objetivou-se avaliar a suscetibilidade de *P. xylostella* a combinações de cepas de Bt em condições de laboratório.

2. Material e métodos

A criação dos insetos e os bioensaios foram realizados no Laboratório de Biologia e Criação de Insetos (LBCI) e no Laboratório de Controle Microbiano de Pragas (LCMP) (Departamento de Fitossanidade – FCAV/UNESP, Campus Jaboticabal).

O delineamento experimental utilizado foi o inteiramente casualizado adotando-se três formas de avaliação das combinações das cepas da bactéria conforme descrito abaixo:

Modelo A1 = constou de seis cepas de Bt cultivadas separadamente (HD2, HD3, HD4, HD7, HD11 e HD 12) mais uma testemunha e três repetições por tratamento;

Modelo A2 = constou de seis cepas de Bt, cada uma delas combinadas e cultivadas juntas na mesma placa (HD2x3, HD2x4, HD2x7, HD2x11, HD2x12, HD3x4, HD3x7, HD3x11, HD3x12, HD4x7, HD4x11, HD4x12, HD7x11, HD7x12, HD11x12) mais uma testemunha e três repetições por tratamento;

Modelo A3 = constou de seis cepas de *Bt*, cada uma delas combinadas (HD2x3, HD2x4, HD2x7, HD2x11, HD2x12, HD3x4, HD3x7, HD3x11, HD3x12, HD4x7, HD4x11, HD4x12, HD7x11, HD7x12, HD11x12) e misturadas apenas no momento da aplicação mais uma testemunha e três repetições por tratamento.

2.1 Criação de *Plutella xylostella*

A população de *Plutella xylostella* utilizada para os bioensaios foi criada no LBCI em sala climatiza com temperatura controlada de 25 ± 1°C, fotoperíodo de 14 horas e umidade relativa do ar de (70 ± 10)%, seguindo a metodologia de Barros (1998) e adaptada às condições do laboratório por De Bortoli et al. (2012).

O substrato utilizado para alimentação e oviposição da *P. xylostella* foram folhas de couve (*Brassica oleracea* var. *acephala* cv. Manteiga), cultivada em área experimental em casas de vegetação da FCAV/UNESP, Campus Jaboticabal.

A população do inseto foi coletada no dia 5 de julho de 2010 em área sem histórico de aplicação de inseticidas e foi mantida isolada, sem a introdução de novos espécimes. Essa população recebeu a denominação de SBT e no momento da realização do experimento estava na 91ª geração em laboratório.

Adultos recém-emergidos foram transferidos para gaiolas circulares transparentes, medindo 13 x 15 cm, com uma abertura lateral de 10 x 5 cm, coberta por tecido tipo "voile" para aeração e outra 10 x 2 cm para troca dos discos foliares e fechada com filme plástico de PVC. Os adultos foram alimentados com solução de mel a 10%, embebida em esponja presa na parte superior da gaiola.

No interior das gaiolas foram colocados discos de folha de couve de oito centímetros de diâmetro, como substrato para oviposição. Os discos de couve e o disco de papel foram trocados diariamente por três dias consecutivos e acondicionados em placas de Petri (9 cm de diâmetro) até a eclosão das lagartas. Posteriormente, os discos com as lagartas foram transferidos para recipientes plásticos (27 x 17 x 8 cm) e folhas de couve foram oferecidas para alimentação das lagartas. A troca das folhas dentro do recipiente foi feita diariamente até os insetos atingirem a fase pupal.

As pupas foram coletadas com o auxílio de pincel ou pinça e acondicionadas em tubos de vidro de fundo chato (8,5 x 2,4 cm) vedados com filme plástico de PVC com pequenos furos para aeração, sendo colocadas de 20 a 30 pupas por tubo. Os adultos

recém emergidos foram transferidos para as gaiolas circulares, anteriormente mencionadas.

2.2. Multiplicação das cepas de Bt

Para realizar os bioensaios foram escolhidas seis cepas de Bt, cedidas pela Embrapa Milho e Sorgo, Sete Lagoas – MG, sendo elas denominadas como: *Bt thuringiensis* HD-2, *Bt finitimus* HD-3, *Bt alesti* HD-4, *Bt dendrolimus* HD-7, *Bt aizawai* HD-11 e *Bt morrisoni* HD-12 (Valicente & Fonseca, 2004) (Tabela 1).

Tabela 1. Cepas de *Bacillus thuringiensis* utilizadas nos bioensaios de suscetibilidade de *Plutella xylostella*.

Subespécie	Cepa	Composição toxica
Bt thuringiensis	HD2	Cry1Ba
Bt finitimus	HD3	Cry1Ab/Cry1Ac, Cry2Aa, Cry2Ab
Bt alesti	HD4	Cry1Ab/Cry1Ac, Cry1Ac, Cry2Aa, Cry2Ab
Bt dendrolimus	HD7	Cry1Ab/Cry1Ac, Cry1Ac, Cry2Ab
Bt aizawai	HD11	Cry1Ab/Cry1Ac, Cry1Ac, Cry1C, Cry2Aa, Cry2Ab
Bt morrisoni	HD12	Cry1Ab, Cry1Da, Cry1Ib, Cry1Id

Para realizar a multiplicação das cepas, as mesmas foram levadas até uma câmara de fluxo laminar e, com o auxílio de uma alça de Drigalski foi transferido o material do microtubo tipo eppendorf para um tubo Falcon contendo 1500 µL de meio nutriente líquido, dos quais 500 µL foram vertidos em placa com meio nutriente sólido, vedado e colocada em estufa a 30ºC por 48horas.

Posteriormente, as placas foram raspadas, diluída em 10mL de água destilada e autoclavada em 121ºC (1 atm) e então centrifugados 1800rpm. O pelett foi separado do sobrenadante e, posteriormente, resuspendido por três vezes, para obtenção da "solução-mãe" de cada cepa.

Cada solução mãe foi diluída em uma concentração de 10^2 para a contagem dos esporos com o auxílio de uma câmara de Neubauer espelhada (Alves & Moraes, 1998). A concentração de 10^6 esporos/µL foi utilizada nos bioensaios de acordo com os modelos experimentais citados anteriormente (A1, A2 e A3).

2.3. Bioensaios de suscetibilidade

Para a condução dos bioensaios, discos foliares de couve de 8 cm de diâmetro foram mergulhados em 50 mL das suspensões das toxinas em água deionizada,

autoclavada, com 50 µg/mL Triton-X100®, por 30 segundos. A testemunha foi tratada apenas com solução de água deionizada, autoclavada e 50 µg/mL Triton-X100®.

Após a secagem (30 minutos em condição ambiente), os discos foram colocados individualmente em placas de Petri (9,5 cm de diâmetro × 2,0 cm de altura) sobre papel filtro umedecido com água deionizada. Sobre cada disco foliar foram colocadas 20 lagartas de *P. xylostella* de segundo instar. Cada disco foi considerado uma repetição e observadas três repetições por tratamento.

As placas foram envolvidas com filme plástico de PVC e mantidas em sala climatizada com temperatura de 25 ± 1°C, umidade relativa de 70 ± 10% e fotoperíodo de 12L:12E h. Após três dias foram realizadas as avaliações e registrado o número de lagartas mortas em cada tratamento. Uma lagarta foi considerada morta quando não apresentava movimentos quando tocada com um pincel de cerdas finas.

As médias obtidas de mortalidade (%) foram corrigidas segundo Abbot (1925).

Mortalidade corrigida = (Mt - Mc/100 - Mc) x 100. Sendo, Mt = Mortalidade da testemunha e Mc a mortalidade calculada de cada tratamento.

Os dados foram submetidos ao teste F da análise de variância (ANOVA) e as médias comparadas pelo teste de Scott Knott a 5% de probabilidade. As análises foram realizadas no *software* Assistat.

Em seguida, foi utilizada a classificação de Benz (1971) para determinar os níveis de interação.

1. Sinergismo subaditivo: quando dois componentes atuando em conjunto produzem um efeito maior do que o sinergismo independente, porém menor que a soma algébrica dos dois efeitos individuais.
2. Efeito aditivo: quando dois componentes atuando em conjunto produzem um leve incremento no seu efeito, em relação a atuação dos componentes individuais, porém insuficiente para ser considerado sinergismo.
3. Antagonismo: quando a interação dos componentes produz um efeito menor do que suas atuações individuais. Neste caso a interação é considerada negativa.

3. Resultados e discussão

No modelo A1 (cepas cultivadas individualmente), a cepa *Bt alesti* HD4 (Cry1Ab/Cry1Ac, Cry1Ac, Cry2Aa, Cry2Ab) causou mortalidade acima de 80 % em lagartas de segundo ínstar de *P. xylostella* (Tabela 2).

Tabela 2. Suscetibilidade de lagartas de segundo ínstar de *Plutella xylostella* a cepas de *Bacillus thuringiensis* (Modelo A1). Jaboticabal, SP, 2017.

Tratamentos	Mortalidade corrigida (%)
HD 2	35,71 ± 10,14 b
HD3	58,93 ± 6,67 a
HD4	89,31 ± 5,0 a
HD 7	69,64 ± 4,41 a
HD 11	42,85 ± 9,28 b
HD 12	67,85 ± 8,82 a

[1]Médias ± erro padrão seguidas de mesma letra na coluna não diferem pelo teste de Scott Knott a 5% de probabilidade. n = 60.

Entretanto, a mortalidade de *P. xylostella* proporcionada pelas cepas *Bt finitimus* HD3 (Cry1Ab/Cry1Ac, Cry2Aa, Cry2Ab), *Bt dendrolimus* HD7 (Cry1Ab/Cry1Ac, Cry1Ac, Cry2Ab) e *Bt morrisoni* HD12 (Cry1Ab, Cry1Da, Cry1Ib, Cry1Id) não se diferencia significativamente da cepa *Bt alesti* HD4 (Tabela 1), embora a mortalidade nessas cepas tenha sido inferior a 80%.

As cepas *Bt thuringiensis* HD2 (Cry1Ba) e *Bt aizawai* HD11 (Cry1Ab/Cry1Ac, Cry1Ac, Cry1C, Cry2Aa, Cry2Ab) proporcionaram menor mortalidade quando comparadas as demais cepas e não diferenciaram estatisticamente entre si (Tabela 2).

Van Frankenhuyzen (2009) elencou as toxinas Cry1A (b,c,d,h); Cry1B(a,d,e,f); Cry1Ca; Cry1Fa; Cry1G(b,c); Cry1I(a,b,,d,e,f); Cry1J(a,b,c,); Cry2Aa; Cry7Ba; Cry8Da; Cry9Aa; Cry9Ca; Cry9Ec; Cry22Ab e Cry32Aa como toxicas para *P. xylostella* enquanto que as toxinas Cry1Ea; Cry2A(b,c,e); Cry8Ea; Cry8Fa; Cry8Ga; Cry9Bb; Cry31Aa; Cry33Aa e Cry48/49Aa foram consideradas inócuas para essa espécie. Entretanto, é importante considerar que os resultados podem variar de acordo com a população do inseto utilizada nos experimentos (Monnerat et al., 2004; Gonzáles-Cabrera et al., 2001; Monnerat et al., 2006).

A composição toxica das cepas de Bt avaliadas no presente estudo e bastante semelhante, com exceção da cepa *Bt morrisoni* (Tabela 1). A quantidade da mesma

toxina pode variar em diferentes cepas, o que pode explicar as diferenças de suscetibilidade apresentadas na Tabela 1. A presença de alguma toxina desconhecida nas cepas além daquelas identificadas pode contribuir para a mortalidade (Monnerat et al., 2004). Cerca de 760 toxinas de Bt foram identificadas (http://www.btnomenclature.info/), mas apenas uma fração delas tem atividade inseticida conhecida (Van Frankenhuyzen, 2017).

Medeiros et al (2006), constatou que a toxina Cry1Ab foi mais toxica que a toxina Cry1C para *P. xylostella*. Entretanto, a cepa HD11 possui as duas proteínas supracitadas e não foi a cepa que proporcionou maior mortalidade (Tabelas 1 e 2). Assim pode-se afirmar que o maior número de toxinas encontradas nas cepas nem sempre se correlacionam com maior eficiência da mortalidade.

No modelo A2 (cepas cultivadas em placas), a interação HD2 x HD7; HD4 x HD11 e HD7 x HD12 proporcionaram mortalidade de *P. xylostella* acima de 80% independente do tipo de interação observada (Tabela 4).

Tabela 3. Interação de cepas de *Bacillus thuringiensis* cultivadas em placa no controle de lagartas de segundo ínstar de *Plutella xylostella* (Modelo A2). Jaboticabal, SP, 2017.

Tratamentos	Mortalidade corrigida (%)	Tipo de Interação
HD 2 x HD 3	75,00 ± 3,33 a	Sinergismo Subdativo
HD 2 x HD 4	62,49 ± 3,33 a	Antagonismo
HD 2 x HD 7	85,71 ± 1,67 a	Sinergismo Subdativo
HD 2 x HD 11	73,21 ± 7,64 a	Sinergismo Subdativo
HD 2 x HD 12	75,04 ± 13,02 a	Efeito aditivo
HD 3 x HD 4	55,35 ± 13,23 a	Antagonismo
HD 3 x HD 7	67,85 ± 4,41 a	Antagonismo
HD 3 x HD 11	51,78 ± 4,41 b	Antagonismo
HD 3 x HD 12	26,78 ± 6,01 c	Antagonismo
HD 4 x HD 7	60,71 ± 7,64 a	Antagonismo
HD 4 x HD 11	85,71 ± 10,41 a	Antagonismo
HD 4 x HD 12	60,71 ± 7,26 a	Antagonismo
HD 7 x HD 11	64,28 ± 13,02 a	Antagonismo
HD 7 x HD 12	80,36 ± 6,01 a	Efeito aditivo
HD 11 x HD 12	42,85 ± 6,01 b	Antagonismo

[1]Médias ± erro padrão seguidas de mesma letra na coluna não diferem pelo teste de Scott Knott a 5% de probabilidade.

As interações que proporcionaram menor mortalidade de *P. xylostella* foram HD11 x HD12 com 42,85% e HD3 x HD12 com 26,78% de mortalidade (Tabela 3).

De acordo com o tipo de interação, verificou-se que a maioria dos tratamentos foram antagônicos (Tabela 3). Ou seja, a interação entre as cepas foi menos toxica do que o efeito individual das cepas, sendo considerada negativa.

Destaca-se que nos tratamentos em que a cepa HD2 foi utilizada com as cepas HD3, HD7 e HD11 houve efeito sinérgico subaditivo, portanto produzem um efeito maior do que o sinergismo independente (Tabela 3). As cepas HD3, HD7 e HD11 possuem a proteína Cry1Ab (Tabela 1), classificada como a mais tóxica por Medeiros et al (2006). Ressalta-se ainda que estas cepas também possuem em comum a proteína Cry2Ab (Tabela 1) não citada pelos autores, mas que pode ter contribuído para maior mortalidade das larvas de segundo ínstar de *P. xylostella*. O efeito aditivo, leve incremento da eficiência das cepas individuais, foi observado na interação das cepas HD2 x HD12 e HD7 x HD12 (Tabela 4).

No modelo A3 (cepas misturadas no momento da aplicação), as interações HD3 x HD4 e HD4 x HD12 proporcionaram mortalidade de *P. xylostella* acima de 80% independente do tipo de interação observada (Tabela 4).

Ressalta-se que a maioria das interações no modelo A3, da mesma forma que no modelo A2, proporcionaram efeito antagônico entre as cepas (Tabela 4).

Tabela 4. Interação de cepas de *Bacillus thuringiensis* misturadas no momento da aplicação para o controle de lagartas de segundo ínstar de *Plutella xylostella* (Modelo A3). Jaboticabal, SP, 2017.

Tratamentos	Mortalidade corrigida (%)	Tipo de Interação
HD 2 x HD 3 (mist.)	62,49 ± 7,64 a	Efeito aditivo
HD 2 x HD 4 (mist.)	64,28 ± 1,67 a	Efeito aditivo
HD 2 x HD 7 (mist.)	44,63 ± 1,67 b	Antagonismo
HD 2 x HD 11 (mist.)	46,42 ± 4,41 b	Efeito aditivo
HD 2 x HD 12 (mist.)	55,35 ± 4,41 a	Antagonismo
HD3 x HD 4 (mist.)	80,36 ± 8,66 a	Antagonismo
HD 3 x HD 7 (mist.)	69,64 ± 6,01 a	Efeito aditivo
HD 3 x HD 11 (mist.)	64,28 ± 1,67 a	Efeito aditivo
HD 3 x HD 12 (mist.)	67,85 ± 2,89 a	Efeito aditivo
HD 4 x HD 7 (mist.)	35,71 ± 2,89 b	Antagonismo
HD 4 x HD 11 (mist.)	71,42 ± 6,01 a	Antagonismo
HD 4 x HD 12 (mist.)	87,49 ± 7,64 a	Antagonismo
HD 7 x HD 11 (mist.)	35,71 ± 13,02 b	Antagonismo
HD 7 x HD 12 (mist.)	35,71 ± 6,67 b	Antagonismo
HD 11 x HD 12 (mist.)	21,42 ± 6,67 c	Antagonismo

[1]Médias ± erro padrão seguidas de mesma letra na coluna não diferem pelo teste de Scott Knott a 5% de probabilidade.

Na maioria dos tratamentos combinados com as cepas HD2 e HD3 houve efeito aditivo, uma vez que as bactérias não foram cultivadas juntas (Tabela 4). Desta forma, não houve competição pelo mesmo meio nutritivo, o que garantiu provavelmente em melhor interação entre as toxinas.

As cepas HD2 e HD11 no modelo A1 utilizadas de forma isolada proporcionaram menor mortalidade quando comparadas as demais cepas (Tabela 2). No modelo A2, quando as cepas HD2 e HD11 foram multiplicadas juntas em placa, a mortalidade aumentos em cerca de 100% em relação as cepas utilizadas de forma isolada no modelo A1 (Tabela 3). No modelo A3, as cepas misturadas no momento da aplicação levaram a um pequeno incremento da mortalidade em relação ao modelo A1 (Tabela 4).

A maior taxa de mortalidade no modelo A1 foi observado com a cepa HD4 seguido pela cepa HD7, as quais apresentaram no modelo A2 e A3 redução da taxa de mortalidade, sendo a interação entre essas considerada antagônica (Tabelas 2, 3 e 4).

O antagonismo, de acordo com Tabashnik (1992) e Pinto et al. (2009), pode ocorrer pela competição entre uma ou mais proteínas presentes nas cepas pelo mesmo receptor (resistência cruzada). Segundo Jurat-Fuentes & Crickmore (2017) pode ocorrer também por uma falha na interação da toxina com o receptor presente no intestino do inseto com a toxina presente na proteína, sendo este último fato relacionado também a expressão de resistência dos insetos a campo.

O sinergismo pode ocorrer em função do maior número de toxinas presentes em uma interação entre cepas, mas para que isso ocorra as toxinas não podem competir por um mesmo receptor (resistência cruzada) sendo necessário se ligarem a diferentes receptores de foram estável para conferir toxicidade ao inseto alvo. Para melhor elucidar os mecanismos de interação e essencial o conhecimento dos receptores presentes nas microvilosidade apicais das celulas colunares do intestino médio dos insetos considerando a variabilidade populacional e entre ínstares.

Misturas de cepas pode ser uma alternativa interessante para prolongar a vida dos Bt bioinseticidas como importante tática do manejo integrado de pragas, uma vez que as plantas Bt utilizadas no controle de pragas vem tendo a sua eficácia comprometida devido aos constantes relatos de resistência (Blanco et al., 2016;

Bernardi et al., 2017) e a descoberta de novas moléculas inseticidas custa cerca de 10 vezes mais do que o desenvolvimento de novos bioinseticida (Glare et al., 2012).

CONCLUSÃO

A interação entre as cepas de Bt varia de acordo com a sua composição toxica e método de bioensaio.

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