

**Combining *Steinernema carpocapsae*
and *Bacillus thuringiensis* strains for
control of Diamondback Moth
(*Plutella xylostella*)**

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1. Introduction

1.1 *Plutella xylostella*

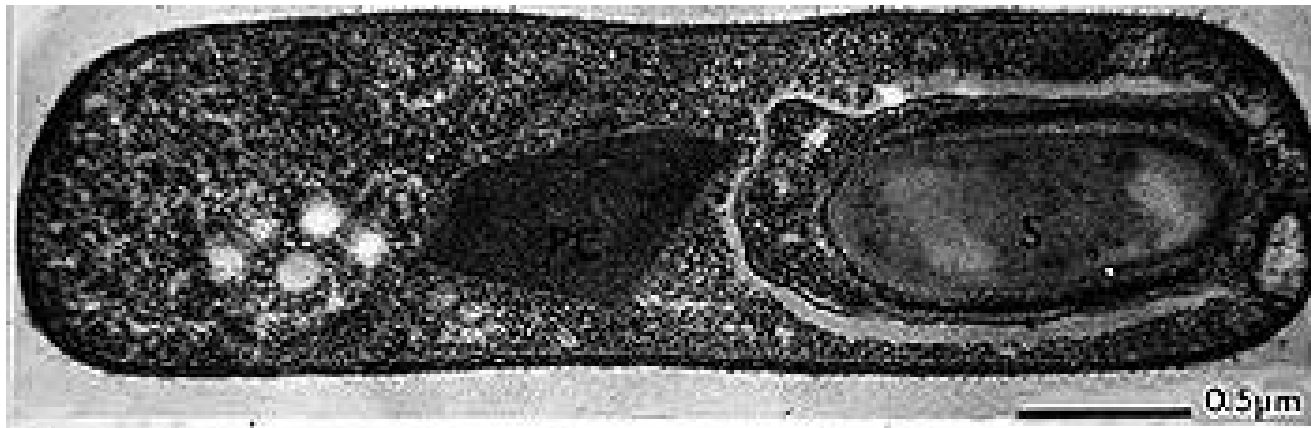


Diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae)

- **major pest of crucifers worldwide**
- **has developed insecticide resistance**

1. Introduction

1.2 *Bacillus thuringiensis* (Bt)

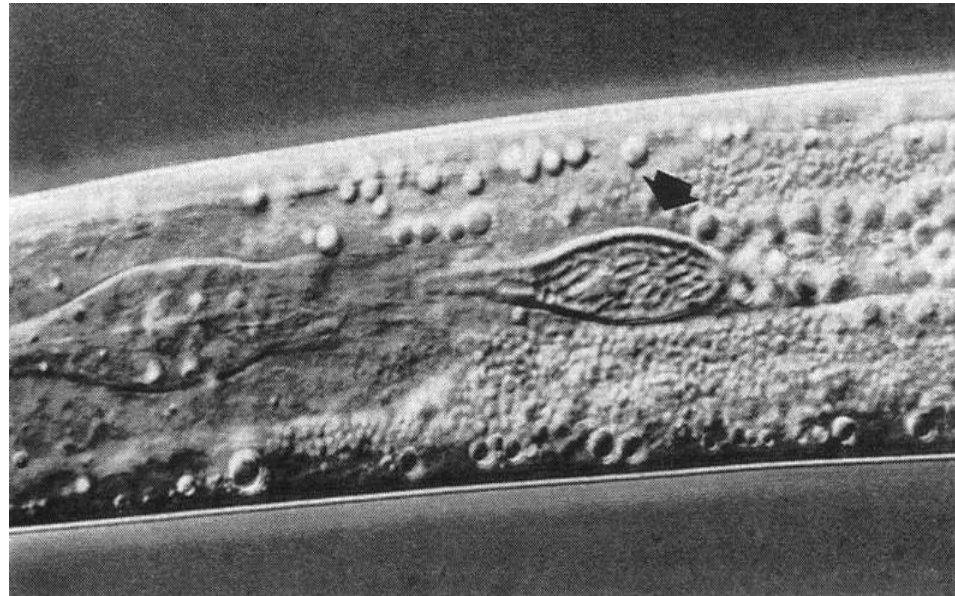


- lepidopteran specific biological insecticide
- not harmful for natural enemies
- resistance of DBM recorded from East Asia
- resistance transient

1. Introduction

1.3 Entomopathogenic Nematodes (*Steinernema carpocapsae*)

- novel agent against DBM
- *Steinernema carpocapsae*
→ most efficient species
- surfactant-polymer-
formulation (SPF) → 0,3%
Rimulgan and 0,3% Xanthan.



1. Introduction

1.4 Objectives

- **LC₅₀ of *S. carpocapsae* for *P. xylostella* 3rd instar: 9 IJs/larva (Schroer *et al.* 2004, Nematology 7, in press)**
- **LC₅₀ of Dipel (Btk) for *P. xylostella* 3rd instar: 34 ng/cm²
LC₅₀ of XenTari (Bta) for *P. xylostella* 3rd instar: 27 ng/cm²**
- **Can we make use of synergistic effects if combining Bt and EPN?**
- **Does the Cyt1A protein of Bti also support toxicity of lepidopteran specific proteins?**
- **combination of dosis:
5 or 3 IJs/larva (LC₂₅ or LC₁₅ *S. c.*)
+ 20 ng/cm² (LC₃₀ Dipel or LC₄₀ XenTari)
→ interaction between Bt and EPN or Btk, Bta and Bti**

2. Material and Methods

2.1 Material

- ***Plutella xylostella*: early 3rd instar larvae, reared in the laboratory on savoy cabbage (*Brassica oleracea* convar. *capitata* var. *sabauda*) at room temperatur**
- ***Bacillus thuringiensis*: 7 Bt-products from 3 serotypes: Bt var. *kurstaki* (Btk), Bt var. *aizawai* (Bta) und Bt var. *israelensis* (Bti).**

Table 1: Origin and formulation of Bt products

product		origin	formulation
Bt var. <i>kutstaki</i>	Dipel ES	Staehler Agrochemie GmbH & Co. KG, Germany	emulsible oil suspension
Bt var. <i>aizawai</i>	XenTari	Valent BioSciences Corporation, USA	waterdispersible granulate
Bt var. <i>israelesis</i>	Biomük	Biofa, Germany	waterdispersible granulate

2. Material and Methods

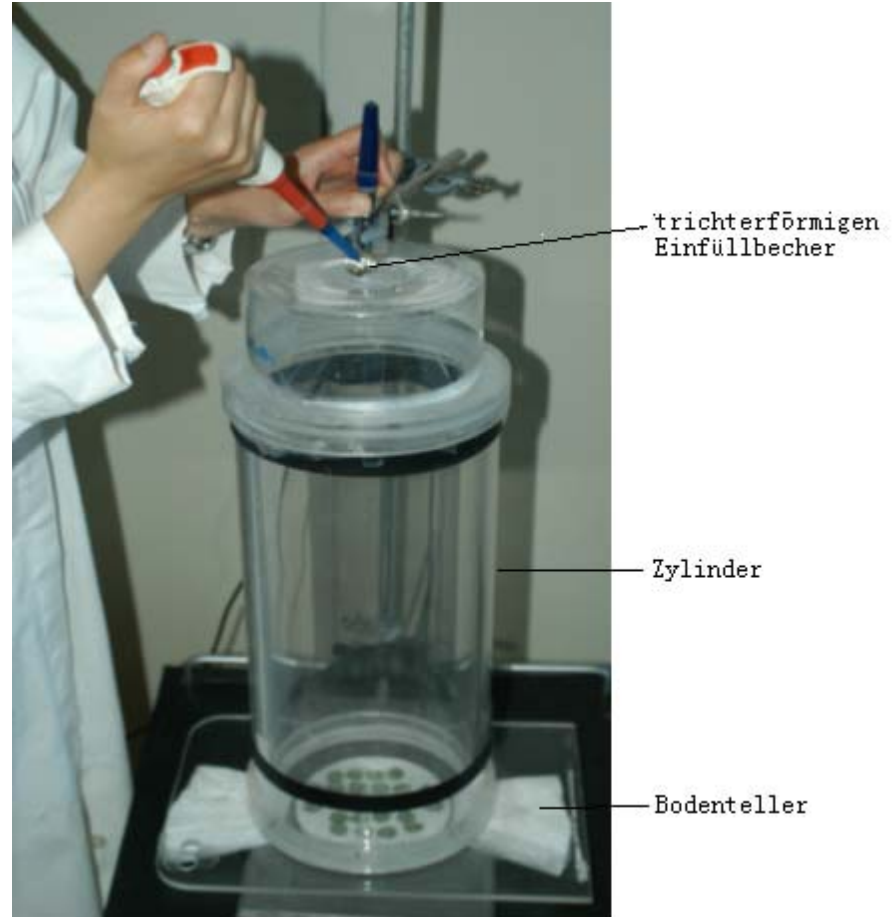
2.1 Material

- ***Steinernema carpocapsae* (EN03):**
 - from e-nema GmbH in Ralsdorf, Germany
 - propagate *in vivo* in *Galleria mellonella* larvae
 - stored at 15°C
 - used during 6-20 days after harvesting
- **cabbage for the experiment:**
 - *Brassica oleracea* var. *capitata*
- **surfactant-polymer-formulation (SPF):**
 - 0,3% Rimulgan and 0,3% Xanthan.

2. Material and Methods

2.2 Methods

- **Bt suspension application :**
 - 1 ml Bt suspension
 - 2cm² cabbage leaf disks
 - with 2000 hPa pressure
 - using an air-brush nozzle
 - 47.5 cm high cylinder
 - Ø16.5cm



2. Material and Methods

2.2 Methods

- Nematode application:
 - 40ml suspension
 - 2cm² cabbage leaf disks
 - using a flat-fan nozzle Teejet® (TP8003E) in 55 cm distance
 - volume-flow of 0,96 l/min at 2000 hPa pressure



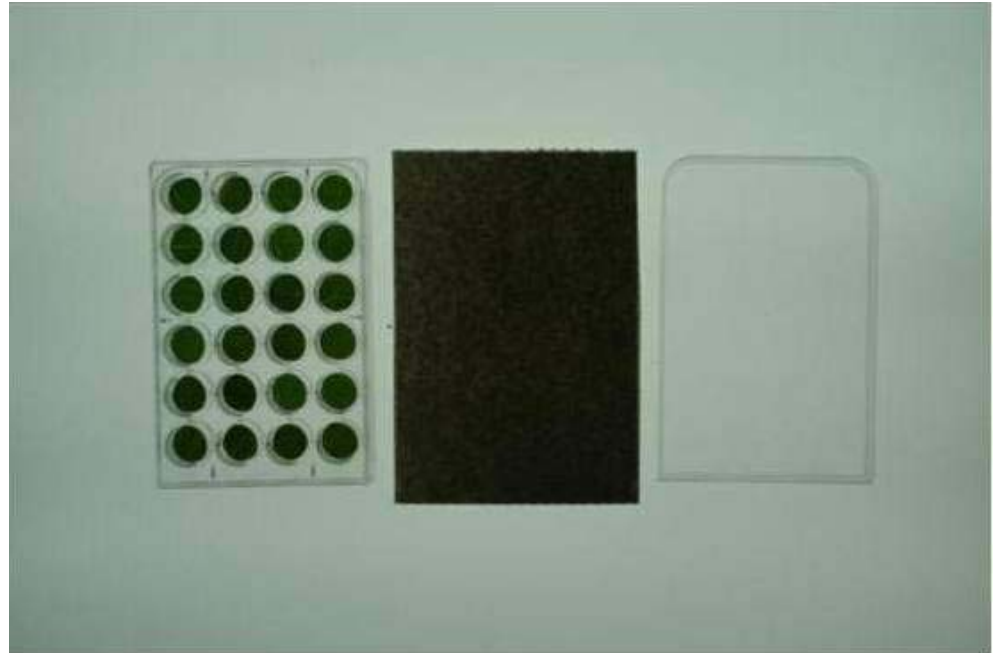
Table 2 application agent, dosis and spraying apparatus

	application agent	dosis	spraying apparatus
1	Dipel + XenTari + Bti Biomük	20+20+20ng/cm²	air-brush
2	Dipel	20ng/cm²	air-brush
3	XenTari	20ng/cm²	air-brush
4	Bti Biomük	20ng/cm²	air-brush
5	Dipel + Xentari	20+20ng/cm²	air-brush
6	Dipel + Bti Biomük	20+20ng/cm²	air-brush
7	Xentari + Bti Biomük	2+20ng/cm²	air-brush
8	Dipel + <i>S.c.</i> EN03	20ng/cm²+5IJs/L.	air-brush + flat-fan nozzle
9	Dipel + H₂O	20ng/cm²+40ml	air-brush + flat-fan nozzle
10	<i>S.c.</i> EN03	5IJs/L.	flat-fan nozzle
11	XenTari + <i>S.c.</i> EN03	20ng/cm²+5IJs/L.	air-brush + flat-fan nozzle
12	XenTari + H₂O	20ng/cm²+40ml	air-brush + flat-fan nozzle
13	SPF + XenTari + <i>S.c.</i> EN03	0,3%+0,3%+10⁻⁵+3IJs/L.	flat-fan nozzle
14	SPF	0,3%+0,3%	flat-fan nozzle
15	XenTari	10⁻⁵	flat-fan nozzle
16	<i>S.c.</i> EN03	3IJs/L.	flat-fan nozzle
17	SPF + XenTari	0,3%+0,3%+10⁻⁵	flat-fan nozzle
18	SPF + <i>S.c.</i> EN03	0,3%+0,3%+3IJs/L.	flat-fan nozzle
19	XenTari + <i>S.c.</i> EN03	10⁻⁵+3IJs/L	flat-fan nozzle

2. Material and Methods

2.2 Methods

- **2 cm² cabbage leaf disks**
→ into 24er-well plates
→ one 3rd instar DBM larva into each well
- **one plate with 24 replications**
- **incubate at 80% RH and 25°C**
- **evaluate mortality after 48h**



2. Material and Methods

2.3 Statistic analysis

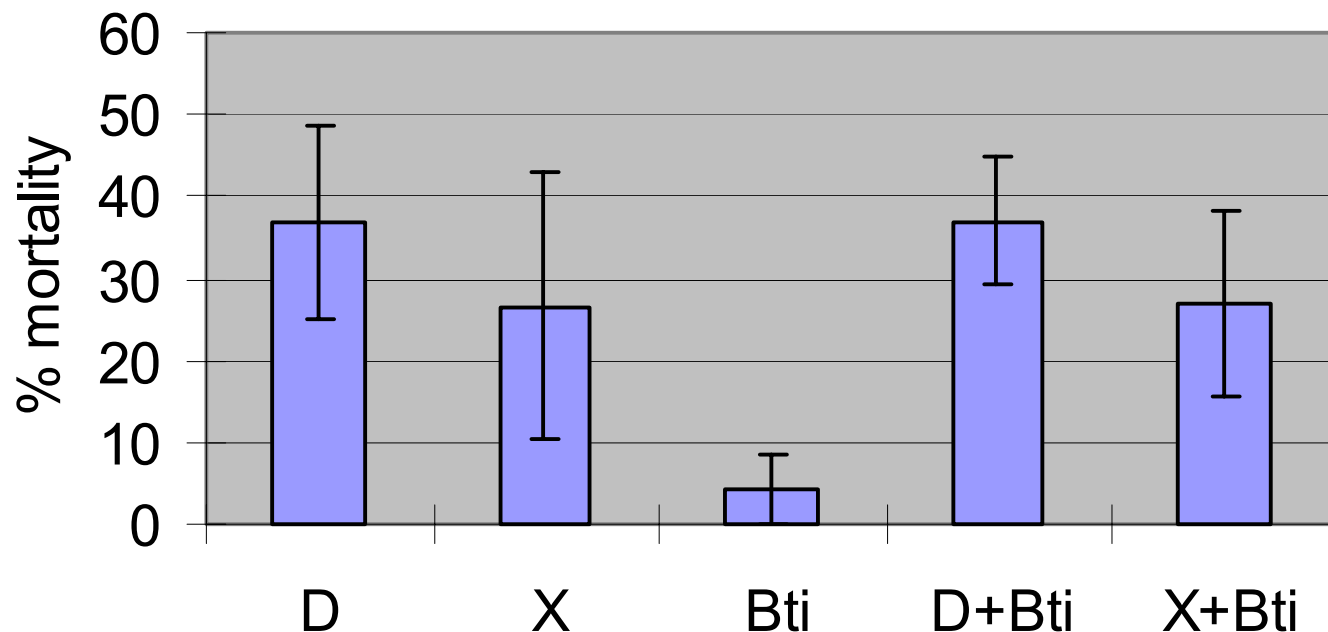
- calculate expected dead and living larvae assuming additive effect (Finney, 1971).
- test difference between expected and observed number of dead larvae (Fisher-test)
- significant difference → synergism or antagonism (Table 3).

Table 3 Criteria for the interaction (according to Finney, 1971)

difference between expected and observed number of dead larvae	relationship between expected and observed number of dead larvae (De : Do)	interaction between the components of combination
significant	De > Do	antagonism
significant	De < Do	synergism
not significant	--	additive effect

3. Result and Discussion

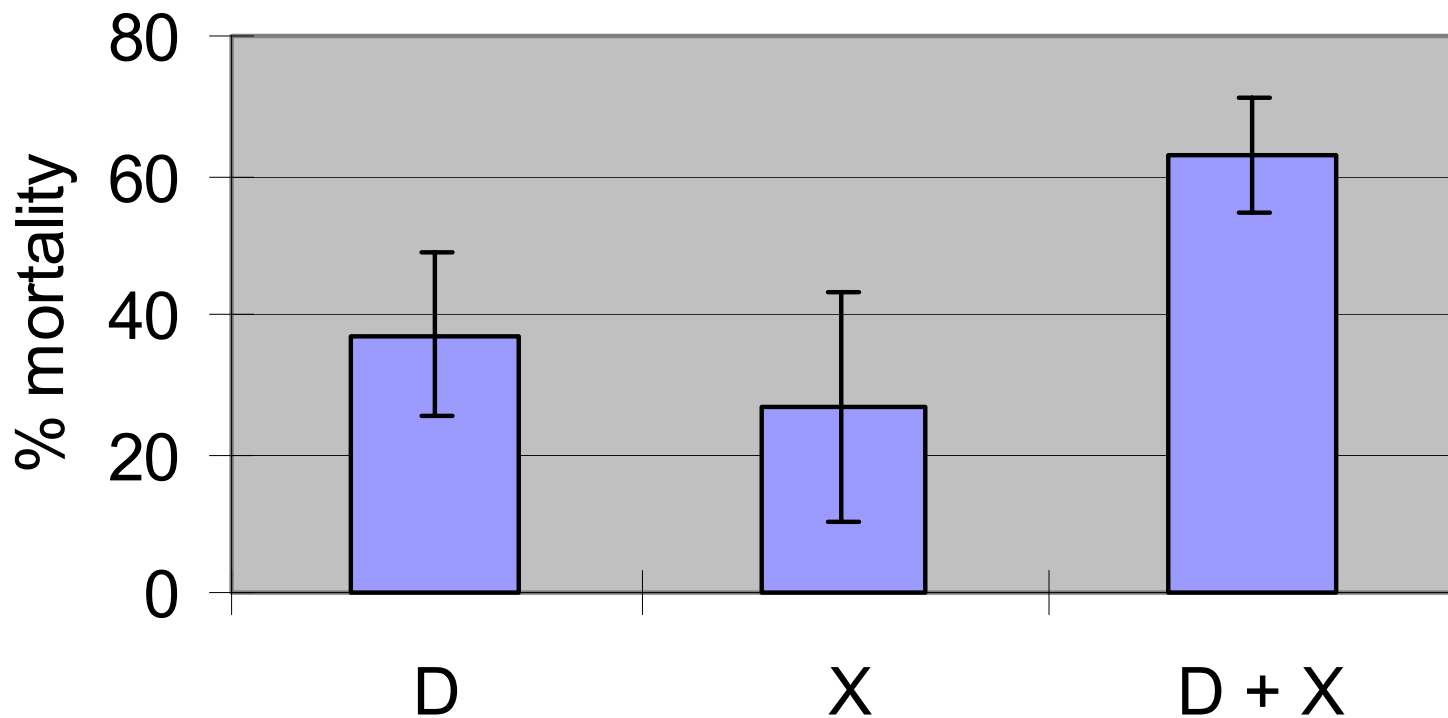
Does the Cyt1A protein of Bti also support toxicity of lepidopteran specific proteins?



Dipel (D), XenTari (X) and Biomük (Bti) at concentration of 20 ng/cm² each (n=3x24)

3. Result and Discussion

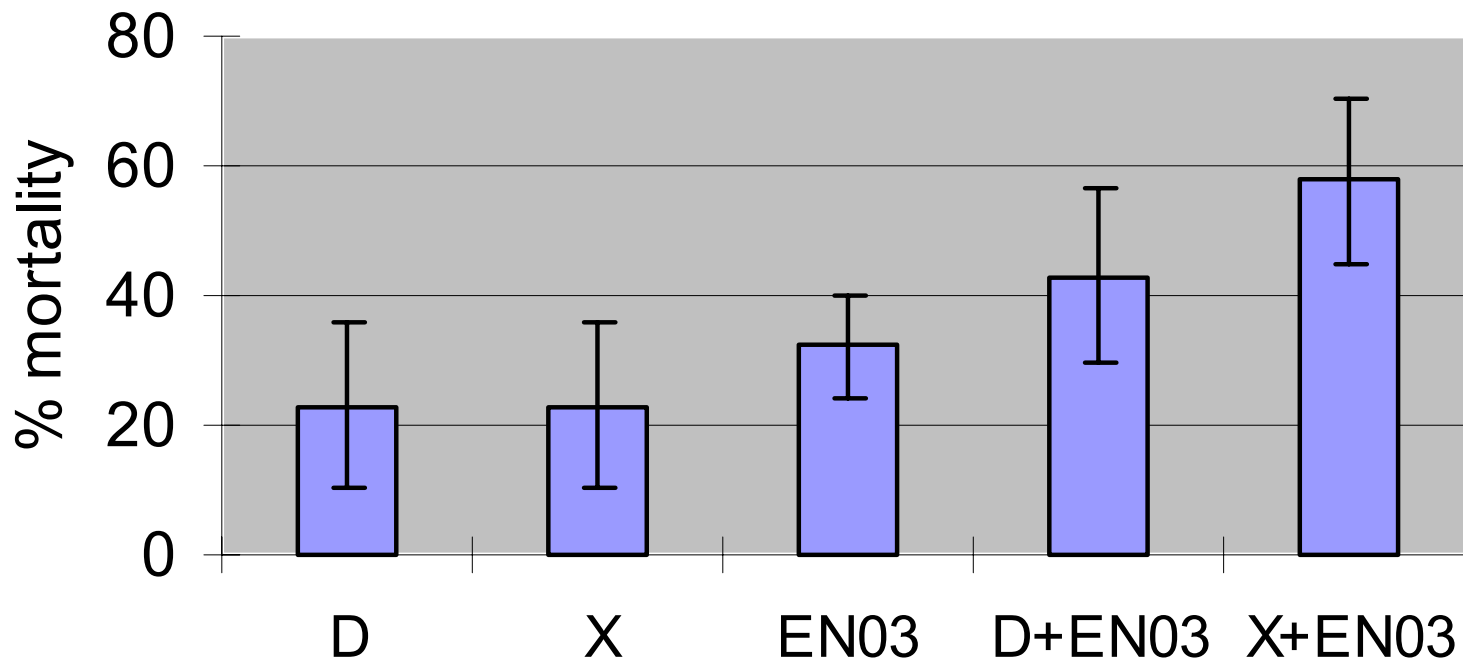
Is there a synergistic effect when combining Btk and Bta?



**Dipel (D) and XenTari (X)
at concentrations of 20 ng/cm² each (n = 3 x 24)**

3. Result and Discussion

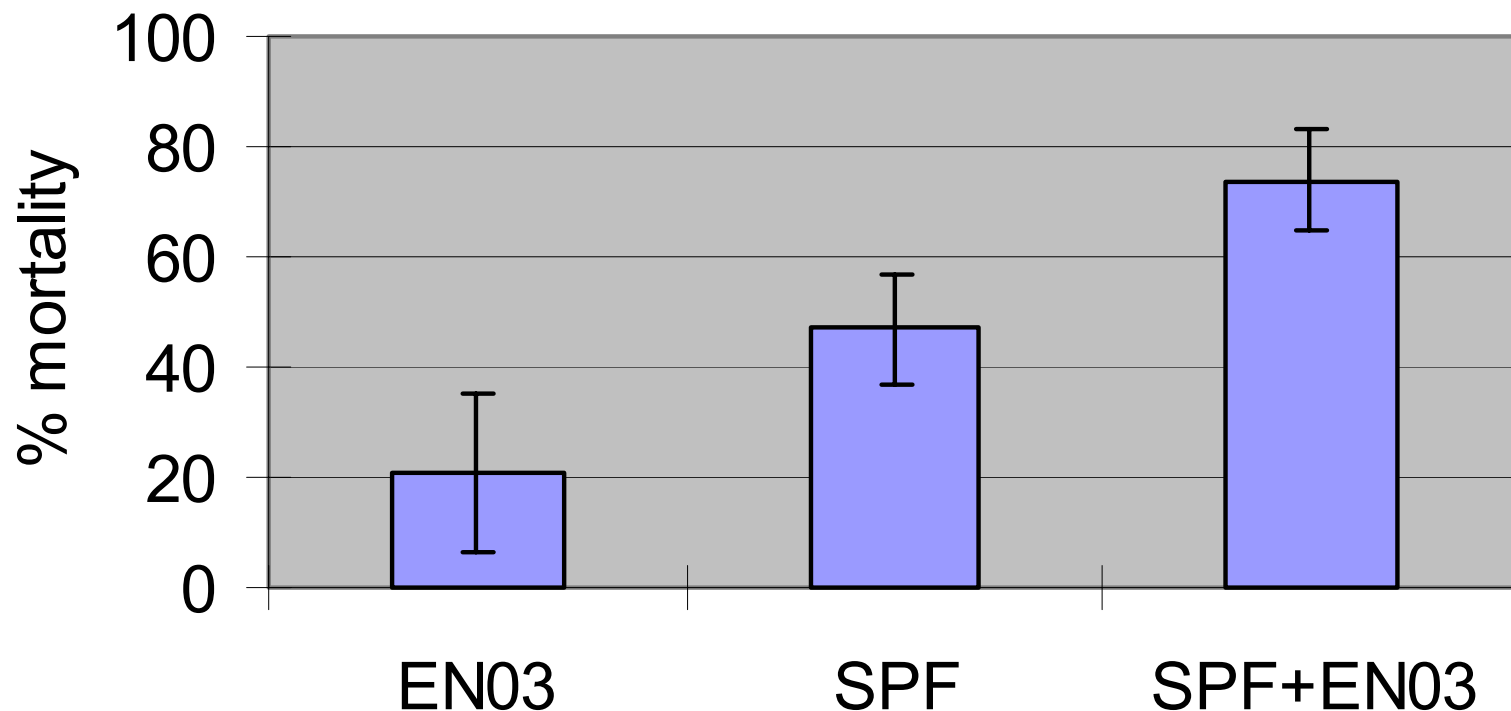
Are there synergistic effects if combining Bt and EPN?



Dipel (D) or XenTari (X) at 20 ng/cm² each,
EPN (EN03) at 5 IJs/L. (n = 3 x 24)

3. Result and Discussion

Is there a synergistic effect if combining SPF and EPN?



SPF: 0,3% Rimulgan and 0,3% Xanthan;

EPN: 3 IJs/L (n = 3 x 24)

3. Result and Discussion

Tab. 4: Interaction of combined agents against 3rd instar DBM

combination	Replicates	interaction			spray method
		additive	synergistic	antagonistic	
Dipel (20ng/cm ²) + Biomük (20ng/cm ²)	5	5			mixed
XenTari (20ng/cm ²) + Biomük(20ng/cm ²)	5	5			mixed
Dipel (20ng/cm ²) + XenTari (20ng/cm ²)	5	4	1		mixed
Dipel (20ng/cm ²) + XenTari (20ng/cm ²) + Biomük (20ng/cm ²)	4	3		1	mixed
Dipel (20ng/cm ²) + EN03 (5/L.)	4	3		1	one after another
XenTari (20ng/cm ²) + EN03 (5/L.)	4	4			one after another
SPF + EN03 (3/L.)	4	3	1		mixed
SPF + XenTari (10 ⁻⁵)	4	3	1		mixed
XenTari (10 ⁻⁵) + EN03 (3/L.)	4	3	1		mixed
SPF + EN03 (3/L.) + XenTari (10 ⁻⁵)	4	3	1		mixed

5 synergism (11.6%), 2 antagonism (4.7%), 36 additive effect (83.7%) of 43 Tests

4. Conclusion

- **mostly additive effects recorded with combinations of Bt and EPN against DBM**
- **no toxic effect was recorded by adding Bti to Btk or Bta**
- **mixed application of Bt and EPN is economically not feasible**
- **alternating applications of single agents are a powerful and reliable tool to avoid or retard development of Bt resistance in *P. xylostella***
- **Btk and Bta should also be used in alternating applications**

Acknowledgments

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DIABOLO

An integrative strategy for the sustainable control of diamondback moth (*Plutella xylostella*) by conservation of natural enemies and application of biocontrol agents