

## ***Anthocoris nemorum* (Heteroptera: Anthocoridae) as predator of cabbage pests – voracity and prey preference**

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Laboratory experiments were performed with adult female *Anthocoris nemorum* (Linnaeus) (Heteroptera: Anthocoridae) at 20°C ± 1°C, L16:D8, 60–70% RH to determine voracity and preference on cabbage aphids (*Brevicoryne brassicae* L.) (Hemiptera: Aphididae), diamondback moth larvae (*Plutella xylostella* L.) (Lepidoptera: Plutellidae) and Western flower thrips (*Frankliniella occidentalis* Pergande) (Thysanoptera: Thripidae) (model species for cabbage thrips (*Thrips angusticeps* Uzel) (Thysanoptera: Thripidae)). When offered individually, *A. nemorum* readily accepted all three species with no significant differences in consumption. When aphids and moth larvae were offered simultaneously, *A. nemorum* showed preference for the latter (numbers eaten and biomass consumed). When aphids and thrips were offered together, *A. nemorum* preferred thrips in terms of numbers eaten but preferred aphids in terms of biomass consumed. The results showed that *A. nemorum* is a voracious predator of *B. brassicae*, *P. xylostella* and *F. occidentalis* and can therefore be considered as a potential candidate for biological control in cabbage.

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### **1. Introduction**

Fresh-market cabbage constitutes about 15% of the cultivated field-grown vegetables in Denmark (Danmarks Statistik 2003). Because of stringent market quality standards and EU stipulations, producers strive to maintain plants pest free and undamaged (Henriksen *et al.* 2003). Currently,

control of pests depends widely on insecticides (van Dijken *et al.* 1995, Cole 1997). However, it may prove difficult to rely solely on chemical control due to resistance development (e.g. Liu *et al.* 1982, Talekar & Shelton 1993), restricted availability of insecticides and difficulties in effectively targeting the pests (Brødsgaard 1994, Blackman & Eastop 2006).

Three important pest species in cabbage are cabbage aphid (*Brevicoryne brassicae* L.) (Homoptera: Aphididae), cabbage thrips (*Thrips angusticeps* Uzel) (Thysanoptera: Thripidae) and diamondback moth (*Plutella xylostella* L.) (Lepidoptera: Plutellidae). In Denmark the first two species are very common and occur in high numbers (Lewis 1973, Heie 2004) while the diamondback moth normally only occurs in scattered and moderate attacks. However, in 2007 there was a massive immigration of diamondback moths in Denmark and insecticide treatment was recommended by the Danish Agricultural Advisory Service (Landscentret 2007). With the climate changes foreseen as a result of global warming, this species may become more common in the future and since *P. xylostella* has acquired resistance towards many insecticides, including *Bacillus thuringiensis* (Tabashnik 1994), it may prove even more difficult to control the pest.

The polyphagous predator *Anthocoris nemorum* (L.) (Heteroptera: Anthocoridae) is common in Denmark (Sigsgaard 2005c, Sigsgaard *et al.* 2006) and a natural enemy of many aphid and thrips species (Hodgson & Aveling 1988, Jacobson 1991, Eilenberg *et al.* 2000). *A. nemorum* has been recorded as a predator occurring in field crop cabbage (Smith 1976, Herold & Stengel 1994), but studies of its potential contribution to pest control in cabbage have not been conducted earlier.

Nonetheless, *A. nemorum* has already received attention as a natural enemy of several pests including aphids, psyllids and thrips in orchards (Collyer 1967, Sigsgaard 2005a, Sigsgaard *et al.* 2006) and glasshouse crops (Jacobson 1991). In addition, it has been recorded to prey on some Lepidopteran species (Collyer 1967) and it can be reared on a diet with eggs of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) (Sigsgaard *et al.* 2006).

The aim of this study was to clarify whether *A. nemorum* is able to consume cabbage pests and consequently could be a potential candidate for biological control of aphids, thrips and diamondback moths in this crop. Second, we wanted to elucidate whether *A. nemorum* has any preference between the three pests.

In the present study two experiments were done to assess the above aims: 1) the voracity of *A.*

*nemorum* when offered *B. brassicae*, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and *P. xylostella* separately and 2) the prey preference of *A. nemorum* when offered *B. brassicae* vs. *F. occidentalis* and *B. brassicae* vs. *P. xylostella*.

*F. occidentalis* served as a model for *T. angusticeps* in the experiments since we were unable to obtain specimens of the latter at the time of the study. *F. occidentalis* is very similar in size, appearance and biology to *T. angusticeps* for the instars used in this study (Franssen & Huisman 1958, Lewis 1973) and it is assumed that results obtained apply for both thrips species.

## 2. Materials and methods

### 2.1. Origin and rearing of experimental animals

*A. nemorum* adults were collected from April to May 2007 by sweep-netting in herbaceous vegetation, hawthorn (*Crataegus laevigata* L.) and stinging nettle (*Urtica dioica* L.), of hedgerows in surrounding areas of the Research Centre Flakkebjerg, Faculty of Agricultural Sciences, Aarhus University in Southern Zealand, Slagelse, Denmark (55°18'53.17"N 11°23'42.66"E). *A. nemorum* was reared (modified after Parker (1981)) in Petri dishes (diameter 9 cm) provided with filter paper on the bottom and a piece of folded paper towel for hiding. Sugar water (2% solution) was provided and a piece of pilea leaf (*Pilea peperomioides* (Diels.) (Urticaceae)) served as a source of humidity and as a medium for oviposition. Both nymphs and adults were fed eggs of *S. cerealella* and *Myzus persicae* Sulzer (Hemiptera: Aphididae) twice a week. The nymphs were reared together until the 5<sup>th</sup> nymphal stage after which they were kept individually to prevent mating. Petri dishes were stacked in containers (approx. 8 dm<sup>3</sup>) with saturated salt water on the bottom for moisture stabilisation and placed in a climate cabinet at 22:12°C, L20:D4 h, 60–70% RH.

*F. occidentalis* was reared on sweet pepper (*Capsicum annuum* L.). *B. brassicae* nymphs were provided by Martin Hommes, Federal Biological Research Centre, Germany and reared on

curly kale (*Brassica oleracea* var. *acephala*). Both species were reared separately in greenhouse compartments in net-covered cages (85 × 75 × 68 cm), at 23°C and 70% RH. Eggs of *P. xylostella* were provided by Juergen Kroymann, Jena University, Institute for Chemical Ecology, Germany and reared on leaves of oilseed rape (*Brassica napus* ssp. *napus*) in containers (approx. 0.6 dm<sup>3</sup>) with a 62-mm mesh-covered hole in the lids to allow ventilation. The bottom was covered with water agar and 4 layers of paper towel. The cages were kept in the same condition as described above for *A. nemorum*. Emerging adults were moved to a net cage in the laboratory at room temperature (~20°C). The adults were provided with 10% sugar water with yellow fruit colour and oilseed rape plants for oviposition.

## 2.2. General conditions of predation experiments

All experiments were performed with adult female *A. nemorum* offspring from the field-collected insects. The females used were 1–7 days old and non-gravid to ensure similar size and voracity. The females were starved before the start of the experiment in Petri dishes (same dimensions and with the same content as described for the rearing) for 24 ± 1 hour, in a climate cabinet at 20 ± 1°C, L16:D8, 60–70% RH. The experiments were done in 9-cm Petri dishes. The prey was transferred to Petri dishes on a 3×3cm piece of curly kale leaf and was allowed to settle for 1 h before the starved *A. nemorum* female was introduced. The Petri dishes were sealed with parafilm and subsequently placed in the climate cabinet at the same conditions as above.

### 2.2.1. Voracity

The daily number of prey consumed by individual *A. nemorum* was determined in 24-h experiments with either 25 second instar *P. xylostella* (number of replications,  $n=28$ , i.e. tested with 28 individuals of *A. nemorum*), 25 second instar *B. brassicae* ( $n=32$ ) or 20 first instar *F. occidentalis* ( $n=16$ ). There were at least 5 controls (i.e. setups without predators) per tested prey species. *A. nemorum* was removed after 24 h and the number

of both dead and live prey was recorded. The number of prey given was based upon preliminary voracity trials done as part of MS thesis work (Simonsen 2008).

### 2.2.2. Preference

Preference was tested in pair-wise combinations in 4-h experiments, in climate cabinets at 20 ± 1°C, 60–70% RH and light during the 4-h period. Individual *A. nemorum* were presented with either 20 second instar *B. brassicae* vs. 20 second instar *P. xylostella* (number of replications,  $n=19$ , i.e. tested with 19 individuals of *A. nemorum*) or 20 second instar *B. brassicae* vs. 20 first instar *F. occidentalis* ( $n=14$ ). There were at least 5 controls (i.e. setups without predators) per tested prey species combination. The experimental time period was based upon preliminary voracity trials done as part of MS thesis work (Simonsen 2008). *A. nemorum* were removed after 4 h and the number of both dead and live prey was recorded.

### 2.2.3. Biomass

To calculate the biomass consumed by *A. nemorum* in the experiments, the three prey species were weighed using a scale of the type Sartorius MC5, with 1 µg accuracy. The prey species were transferred to the scale with a fine paintbrush and weighed either individually (*P. xylostella* (15 individuals), *B. brassicae* (20 individuals)) or in groups of 10 (*F. occidentalis* (60 individuals)).

## 2.3. Data analysis

Mortality was corrected for the mortality in the controls (Abbott 1925). In the voracity experiment, the control mortalities were 5.2 ± 3.8% (number of replications,  $n=6$ , i.e. tested with 6 individuals of *A. nemorum*) for *P. xylostella*, 2.3 ± 1.5 ( $n=7$ ) for *B. brassicae* and 14.0 ± 10.3% ( $n=5$ ) for *F. occidentalis*. In the preference experiment, the control mortalities in the combination aphids and thrips were 16.0 ± 8.1% ( $n=5$ ) for *B. brassicae* and 6.0 ± 1.9% ( $n=5$ ) for *F. occidentalis*; and in the combination of aphids and moth larvae the control mortalities were 3.0 ± 2.0% ( $n=5$ ) for

Table 1. Mean numbers ( $\pm$  S.E.) of prey eaten and mean biomass consumed (mg;  $\pm$  S.E.) by *Anthocoris nemorum* in 24 hours with 25 second instar *Plutella xylostella*, 25 second instar *Brevicoryne brassicae* or 20 first instar *Frankliniella occidentalis* offered individually. For the three combinations 28, 32 and 16 replications were carried out, respectively, with at least 5 controls per combination.

Prey species	<i>B. brassicae</i>	<i>P. xylostella</i>	<i>F. occidentalis</i>	F (df)	P
Number	15.8 $\pm$ 0.8	17.4 $\pm$ 1.2	12.8 $\pm$ 1.4	1.23 (2, 75)	0.3
Biomass	0.62 $\pm$ 0.03	1.05 $\pm$ 0.07	0.07 $\pm$ 0.009	87.92 (2, 75)	0.0001

*B. brassicae* and 5.0  $\pm$  5.0% ( $n=5$ ) for *P. xylostella*.

### 2.3.1. Voracity

As the data were count data and followed a binomial distribution, the data were transformed using  $\arcsin(\sqrt{\beta/\text{total prey density}})$  (Bibby *et al.* 2004). The data were analysed using one-way ANOVA (SAS Institute Inc. 2003) with prey type as a fixed factor to test for significant differences in numbers of prey eaten after 24 hours between the 3 prey species offered. The model parameters (prey type combinations) were compared using two tailed *t*-tests. The same model, without transformation, was used for biomass in order to see, if there were differences in biomass eaten after 24 hours. Boxcox plots were used to check for transformation validity.

### 2.3.2. Preference

Prey preferences exhibited by the predators were analysed by calculating Manly's Alfa index of prey preference (Manly *et al.* 1972). The index was calculated by the following formula, which takes food depletion into account:

$$\alpha_i = \frac{\ln((n_{i0} - r_i) / n_{i0})}{\sum_{j=1}^2 \ln((n_{j0} - r_j) / n_{j0})}, i = 1, 2; j = 1, 2 \quad (1)$$

where  $\alpha_i$  is the preference for prey type 1 or 2,  $n_{i0}$  and  $n_{j0}$  is the number of prey type 1 and prey type 2 present at the beginning of an experiment,  $r_i$  and  $r_j$  is the number of prey type 1 and prey type 2 remaining after experimentation. Thus, the value of  $\alpha_i$  will fall between 0 and 1, with a value significantly over 0.5 indicating preference. Manly's indices are compared between prey types using a

two-tailed paired *t*-test (SAS Institute Inc 2003). Differences are considered significant for  $P < 0.05$ .

In addition to the calculation of preference indices, data on mortality and biomass consumption were analysed by one-way ANOVA (SAS Institute Inc. 2003) to test for differences in 1) the number of *B. brassicae* killed in the two prey-combinations and 2) the total number of prey killed and total biomass consumed in the two prey combinations.

## 3. Results

### 3.1. Voracity

The average individual weight of *F. occidentalis* was (mean  $\pm$  S.E.) 0.0061  $\pm$  0.0004 mg; ( $n = 6$  weighings, total of 60 individuals for *F. occidentalis*). *B. brassicae* weighed 0.0402  $\pm$  0.0032 mg ( $n = 20$ ) and *P. xylostella* 0.0601  $\pm$  0.0040 mg ( $n = 15$ ). The experiment with prey species given individually revealed that *A. nemorum* readily accepted all three prey species. The total number of killed prey did not differ significantly for the three prey types with approximately 13–17 prey individuals killed (Table 1). However, the biomass consumed was significantly different for the three prey types (Table 1).

### 3.2. Preference

The preference experiment revealed that *A. nemorum* had a significant preference for *P. xylostella* to *B. brassicae* both in terms of numbers killed and biomass consumed (Table 2). In the combination with *B. brassicae* and *F. occidentalis* *A. nemorum* showed a significant preference for *F. occidentalis* in terms of numbers of prey killed

Table 2. Preference of *Anthocoris nemorum* between 20 second instar *Brevicoryne brassicae* and 20 second instar *Plutella xylostella* in terms of mean number ( $\pm$  S.E.) of prey killed and mean consumed biomass (mg;  $\pm$  S.E.) in 4 hours. \*Data corrected with Abbott's mortality factor. The experiment was replicated 19 times with at least 5 controls.

	<i>B. brassicae</i>	<i>P. xylostella</i>	<i>P</i> (df)
Prey killed*	4.89 $\pm$ 0.8	7.37 $\pm$ 0.6	0.0001 (1, 18)
Biomass eaten*	0.19 $\pm$ 0.03	0.44 $\pm$ 0.04	0.0001 (1, 18)
Manly's $\alpha$ , prey killed	0.37 $\pm$ 0.1	0.63 $\pm$ 0.1	0.02
Manly's $\alpha$ , biomass eaten	0.30 $\pm$ 0.04	0.70 $\pm$ 0.04	0.0001

Table 3. Preference of *Anthocoris nemorum* between 20 second instar *Brevicoryne brassicae* and 20 first instar *Frankliniella occidentalis* in terms of mean number ( $\pm$  S.E.) of prey killed and mean biomass consumed (mg;  $\pm$  S.E.) in 4 hours. \*Data corrected with Abbott's mortality factor. The experiment was replicated 14 times with at least 5 controls.

	<i>B. brassicae</i>	<i>F. occidentalis</i>	<i>P</i> (df)
Prey killed*	6.45 $\pm$ 1.3	8.30 $\pm$ 0.8	0.0001 (1, 18)
Biomass eaten*	0.25 $\pm$ 0.05	0.05 $\pm$ 0	0.0001 (1, 18)
Manly's $\alpha$ , prey killed	0.40 $\pm$ 0.1	0.60 $\pm$ 0.1	0.03
Manly's $\alpha$ , biomass eaten	0.74 $\pm$ 0.1	0.26 $\pm$ 0.1	0.0002

but a preference for *B. brassicae* in terms of biomass consumed (Table 3).

The prey type offered together with *B. brassicae* did not have any significant influence on the number of aphids killed or aphid biomass consumed by *A. nemorum* ( $P = 0.37$ ;  $df = 1$  and  $P = 0.28$ ;  $df = 1$ ).

The total prey consumption by *A. nemorum* in the two combinations was significantly different with more prey individuals killed ( $P = 0.0001$ ) in the combination with *B. brassicae* and *F. occidentalis* (mean number  $\pm$  S.E.: 14.75  $\pm$  2.1) compared to the combination with *B. brassicae* and *P. xylostella* (mean number  $\pm$  S.E.: 12.26  $\pm$  1.45) but with a lower (mean mg  $\pm$  S.E.: 0.30  $\pm$  0.1) biomass consumption in the first combination compared to the second combination (mean mg  $\pm$  S.E.: 0.64  $\pm$  0.1) ( $P = 0.0001$ ).

## 4. Discussion

### 4.1. Voracity

The study confirmed that *A. nemorum* can be considered as a potential candidate for biological

control in cabbage. It was able to prey upon the two important cabbage pests *P. xylostella* and *B. brassicae* as well as on *F. occidentalis*, indicating that *A. nemorum* is likely to be able to prey on the cabbage thrips. Further studies are needed to verify our assumption of similarity between *F. occidentalis* and *T. angusticeps* in terms of the expression of the predatory characteristics (voracity, preference) that they elicit in *A. nemorum* through their defence or escape behaviour and dietary quality.

The number of prey killed did not differ significantly for the three prey types indicating a prey handling plateau of *A. nemorum* of about 15 prey individuals per day in accordance with results reported by Meyling et al. (2003). Satiation was not the limiting factor in prey consumption as the biomass consumed differed significantly between the three offered prey species reflecting the rather large differences in size of the prey species.

*A. nemorum* was able to prey upon adult stages of both *B. brassicae* and *F. occidentalis* (Simonsen 2008) confirming similar observations by Campbell (1977) (on *Phorodon humuli* (Homoptera: Aphididae)) and Jacobson (1991) (on *F. occidentalis*). However, *A. nemorum* was unable

to prey upon *P. xylostella* beyond the 2<sup>nd</sup> larval stage (pers. obs. in preliminary trials in MS thesis work (Simonsen 2008)). This could perhaps be attributed, in part, to the fact that older larvae are able to move away from the predator (no longer being confined in their mining holes) and, in part, to the rather violent defensive movements of larvae when attacked by *A. nemorum* (M.-L.R. Simonsen & C.N. Bang, pers. obs.). With respect to *P. xylostella*, the biocontrol potential of *A. nemorum* will thus be restricted to the young stages (egg to 2<sup>nd</sup> instar). Although the possibility of predation on eggs of *P. xylostella* was not directly examined in this study, it is considered likely, due to the fact that *A. nemorum* can feed on eggs and larvae of other Lepidopteran species (Collyer 1967, Sigsgaard 2004, Sigsgaard *et al.* 2006).

#### 4.2. Preference

In the combination with *B. brassicae* and *P. xylostella*, *A. nemorum* showed preference for *P. xylostella* both in numbers of prey killed and biomass consumed. In the combination with *B. brassicae* and *F. occidentalis*, the predator preferred *F. occidentalis* in terms of numbers of prey killed but preferred *B. brassicae* in terms of biomass consumed. Previous studies on the preference of *A. nemorum* include examples both of no preference (Dempster 1963, Herard & Chen 1985, Meyling *et al.* 2004) and of preference to specific prey species (Meyling *et al.* 2003, Sigsgaard, 2005b).

*A. nemorum* fed on all prey offered in these preference trials. However, levels of preference found in these experiments could have an implication on predation in the field. *A. nemorum* has been characterised as a “hit and run” predator and would probably be more effective at controlling pests with scattered spatial distribution (Björkman *et al.* 2003) such as both *P. xylostella* and *F. occidentalis* rather than *B. brassicae*, which appears in groups.

To be able to predict the prey consumption of a target prey, such as *B. brassicae*, *A. nemorum*'s preference for pests present in cabbage should be known, which may require inclusion of additional cabbage pests.

The experiments show that *A. nemorum* can consume some cabbage pests and consequently

may be considered as a potential candidate for biological control of *B. brassicae*, *P. xylostella* and *F. occidentalis*, and likely also *T. angusticeps*. Additional investigations on e.g. temperature dependent predation, survival of predator around and within cabbage fields and preference in relation to other important pests in cabbage are, however, needed to further elucidate the predatory potential.

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