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Oviposition preference of *Anthocoris nemorum and A. nemoralis* (Heteroptera: Anthocoridae) for apple and pear.

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Abstract

Oviposition preference of *Anthocoris nemorum* and *Anthocoris nemoralis* for apple and pear was tested in the laboratory. *Anthocoris nemorum* laid significantly more eggs on apple (75%) than on pear, while *A. nemoralis* preferred pear (71%). Over 90% of *A. nemorum* eggs were laid at the leaf margins whereas eggs of *A. nemoralis* were most commonly found in the leaf centre, 5 mm or more inside the leaf. Both laid more eggs on the ventral than on the dorsal side of pear leaves (*A. nemorum* 63%, *A. nemoralis* 94%), and in the last two experiments *A. nemorum* laid significantly more eggs on the dorsal side of apple leaves. The females' choice of oviposition site is important for the later distribution of immatures in host plants. The oviposition preferences found correspond to the natural distribution of these predators in apple and pear orchards. The preference of *A. nemorum* for the leaf margins, and of *A. nemoralis* for the leaf centre as oviposition site,

supports earlier observations. Anthocorids did not display a preference for the dorsal or ventral side of a leaf *per se*. Oviposition may be affected by the pubescence of the leaves. While the dorsal side of a pear leaf is quite smooth, the ventral side of an apple leaf present the most pubescent oviposition surface. Most eggs were found between these two extremes. Preference for cut leaves to whole leaves could help *A. nemorum* to locate prey in a field situation as also suggested by its preference to oviposit on leaves with eggs of *Operophtera brumata*.

Introduction

Two of the most abundant predators in apple and pear orchards are *Anthocoris nemorum* (L.) and *Anthocoris nemoralis* (Fabricius) (Skanland, 1981; Solomon, 1982). Both have a wide range of hosts and habitats and oviposition occurs on various plants (Anderson, 1962a; Collyer, 1967; Scutareanu et al., 1994). *Anthocoris nemoralis* is mostly found on perennials, *A. nemorum* is found on both perennials and annuals (Anderson, 1962a). They are probably strictly predatory bugs, though they can take up plant sap. However, to *A. nemorum* the value seems only equal to that of water (Lauenstein, 1980a). Both are polyphagous predators preying on aphids, mites, psyllids and lepidopteran eggs and young larvae, and are considered to play an important role in controlling insect pests (Collyer, 1953; Collyer 1967; Hill, 1957; Solomon, 1982), *Anthocoris nemoralis* particularly in controlling pear psyllids (Fauvel et al., 1984; Solomon et al., 1989; Trapman & Blommers, 1992; Rieux et al., 1994; Scutareanu et al., 1999; Beninato & la Morella, 2000; Solomon et al., 2000).

A predator's choice of oviposition site may be affected by various factors including the presence of prey, prey dietary quality, prey preferences, cues of prey on the host plant as well as the plant itself. Several studies have documented variable dietary quality of preys. Where psyllids were tested they ranked over aphids and other prey. All studies involving *A. nemorum* demonstrated marked differences among aphid species tested (Lauenstein, 1977). The quality of *Myzus persicae* and whiteflies were equal to *A. nemorum* (Ekbom, 1981). Both *A. nemorum* and *A. nemoralis* were found to develop fastest and attain the highest adult weight when fed *Psylla mali* as compared to *Aphis fabae* and *Acyrthosiphon pisum* (Anderson, 1962b). *Anthocoris nemoralis* nymphs were found to develop faster on eggs of pear psylla than on eggs of *Ephestia kuhniella* and *Ceratitis capitata* (Fauvel et al., 1984). Though clear differences can be found in dietary quality, earlier studies have not shown preference for any particular prey. Thus Ruth & Dwumfour (1989) found that *A. nemorum* readily accepted different aphid species. However, in a recent study comparing preference for aphids of importance in greenhouse production (Meyling et al., 2002) both *A. nemorum* and *A. nemoralis* preferred *M. persicae* to *Macrosiphum euphorbiae*, *Aulacorthum solani* and *Aphis gossypii*. This result could have been affected by the fact that *M. persicae* nymphs were observed to be the least likely to move when attacked (Meyling et al., 2002). Earlier it was believed that anthocorids searched by tactile cues only (Lauenstein, 1980b). However, psylla infested pear trees have been shown to be attractive to anthocorids. Interestingly, laboratory reared *A. nemoralis* did not show such a preference before having experienced volatiles from damaged plants in the presence of food (Scutareanu et al., 1996; Drukker et al., 2000). Preferences for plants for oviposition have not been specifically tested, except for a comparison of pear and barley leaves to *A. nemorum* (Herard & Chen, 1985). However several studies have documented the distribution of anthocorids among various habitats and annual changes in distribution among habitats (Anderson, 1962a; Collyer, 1967; Fauvel, 1999).

The present study tested whether the two anthocorids would have any oviposition preference for apple or pear, as choice of oviposition choice can greatly influence the resulting densities of their offspring on a given host.

Materials and methods

Plant material

To assess the choice of *A. nemorum* and *A. nemoralis* between apple and pear leaves for oviposition, branches of pear (cv. Ovid) and apple (cv. Prima) were field collected immediately before the experiment at Rørrendegaard, an experimental orchard belonging to KVL. Branches were pruned to leave three fully developed healthy and undamaged leaves of 5-6 cm in length and 2-3 cm in width. Before being introduced to cages the pruned branches were gently shaken and thereafter carefully examined under stereomicroscope. Any remaining arthropods were removed with a fine paintbrush.

To assess oviposition choice in smaller cages between two single apples leaves either cut or undamaged, or with or without prey, branches were collected as described above, and individual healthy and undamaged leaves selected measuring 3-4 cm in length and 2-3 cm in width.

Insects *Anthocoris nemorum* females were field collected in and around orchards and up till the onset of the experiment they were kept in thermo cabinets providing L16:D8 with day temperature of 18±1 ^{o}C and night temperature of 12±1 ^{o}C in individual cages ('medicine cups'). Cages measured 3.5 cm in diameter at the base, 4 cm in diameter at the top and 4 cm in height, and had lids with ventilation holes. Water was provided on a 1 × 1 cm piece of gauze. A few *Anthocoris nemoralis* were field collected and tested in replicates one and two, the remaining were obtained from a laboratory rearing facility and then kept in the same way as *Anthocoris nemorum*. Excess of *Sitotroga cerealella* (Ol.) eggs were provided every second or third day until the onset of the experiment and at least for three days, to assure that all experimental animals were well fed. Eggs of *Operophtera brumata* L. were obtained from females collected on oak (*Quercus robur*) kindly provided by Dr. Vanbergen, CEC Banchory. Ten eggs were applied to each treated leaf with a wet paintbrush. Leaves were allowed to dry before use.

Cages Cages for comparing the oviposition on apple and pear were transparent plastic jars, 7 cm in diameter and 9 cm high. They were used upside-down. One branch of apple and one of pear were mounted in two individual, opposite holes equidistant from the centre and cage sides. Water was provided on a square piece of gauze on the cage floor between the branches. The ends of the branches reached down in a beaker with water. Each cage had a ventilation hole of 2 cm in diameter at the top. The ventilation hole was covered with filter paper.

The small cages used for individual rearing of anthocorids, as described above, were used to compare oviposition on single leaves either a whole or a wounded leaf, or a leaf with prey or one without prey. To provide water and support for the leaves the base of the cages were covered with 3% agar, into which the petiole could conveniently be stuck. For the comparison of oviposition on a whole or wounded leaf one leaf was inserted whole, and one leaf had approximately one quarter removed from the apical end with a scalpel immediately prior to the onset of the experiment. Leaves were so chosen that sizes of the two leaves were equivalent after the wounding of one leaf.

Method Three experiments tested the oviposition of *A. nemorum* given the choice of apple and pear. A fourth experiment with the same set-up included both *A. nemorum* and *A. nemoralis.* A few field collected *A. nemoralis* females were tested along the *A. nemorum* in the first and second experiments thus allowing a first comparison of responses with those of mass-reared *A. nemoralis*. All experiments were done between mid May and mid June (13 May, 21 May, 29 May and 23 June 2001) thus including a mixture of over wintering females and the first spring generation.

In small cages the effect of a healthy or a freshly wounded leaf on oviposition was compared. This experiment was replicated twice including both *A. nemorum* and *A. nemoralis* first both with leaves of apple or leaves of pear (26 June 2001, on apple *A. nemorum* n = 9, *A. nemoralis* n = 9 and on pear n = 8 and 10, respectively) second only with apple leaves (13 July 2001, *A. nemorum* n = 28, *A. nemoralis* n = 18). Finally an experiment assessed oviposition preference of *A. nemorum* for apple leaves with no prey and apple leaves with *Operopthera brumata* eggs (8 August, n = 20).

At the onset of an experiment one anthocorid female was carefully introduced into each cage, in the larger cages through the ventilation hole. Each experiment lasted 48 h, with L:D of 16:8 and temperature of 25 ± 2°C. Humidity within the cages was (mean ± s.d.) 89 ± 3% rh, ranging from 84-93% rh, enough to maintain the freshness of the plant material.

After removal of the anthocorids from the cages, plant material was examined for eggs under the stereomicroscope. For each egg, the eggs position was noted as leaf margin (< 5 mm from edge), leaf centre (> 5 mm from edge), leaf tip (< 5 mm from the tip), or petiole. For eggs laid on the leaf it was also noted whether they were laid on the dorsal or ventral side of the leaf. Finally eggs closer to each other than 5 mm were also recorded as a batch.

Data analysis All statistical analysis was carried out in SAS/STAT ver. 6.12 (SAS Institute 1990). Preferences within a species were analysed with a paired t-test (PROC MEANS on the difference between a pair of observations). Across species analyses were made using PROC MIXED with anthocorid individuals as a random effect for experiment four and for the experiment comparing preference for cut and uncut leaves. Replicates in which anthocorids did not lay any eggs during the 48 hours each experiment lasted were excluded from analyses.

Results

Oviposition on apple or pear The number of eggs laid by a female within or across experiments was not significantly different between *A. nemorum* (mean ± s.e. = 10.3 ± 0.8, n = 64) and *A. nemoralis* (8.1 ± 1.3, n = 26), nor between field collected and mass-reared *A. nemoralis* (8.5 ± 3.0, n = 8 and 7.9 ± 1.4, n = 18, respectively). Both species laid fewer eggs in experiment one (*A. nemorum* 7.5 ± 1.4, n = 16 and *A. nemoralis* 2.3 ± 1.9, n = 5). A total of six *A. nemorum* and five *A. nemoralis* laid no eggs and were excluded from the later analysis. Of these, three of each species were from experiment one, partly explaining the lower average number of eggs in this experiment. The remaining number of replicates in the experiments comparing oviposition on apple and pear were for *A. nemorum*: exp 1: n =13, exp 2: n = 14, exp 3: n= 13, exp 4: n= 18, and for *A. nemoralis*: exp 1: n = 2, exp 2: n= 4, exp 4: n = 16.

Both species often laid more eggs together (less than 5 mm apart) resulting in an average batch size of 1.5 ± 0.1 for *A. nemorum* and 1.6 ± 0.1 for *A. nemoralis* (p: n.s., both range: 1-4 eggs).

A clear difference in choice of plant for oviposition could be seen. *Anthocoris nemorum* laid 75% of its eggs on apple (exp 1, T = 3.1, P < 0.007, exp 2, T = 2.6, P < 0.02, exp 3, T = 4.3 P < 0.0004, exp 4: T = 5.9, P < 0.0001) and *A. nemoralis* 72% of its eggs on pear (exp 1, T = -0.7, P = 0.54, exp 2, T = -5.1, P < 0.02, exp 4, T = -2.6, P < 0.02) (Figure 1). In experiment four, where both anthocorid species were represented in almost equal numbers there was a significant effect of plant (F = 4.4, df = 1, P < 0.04) and the crossed effect of anthocorid species by plant on the number of eggs laid on either plant was highly significant (F = 37.2, df = 1, P < 0.0001) (n = 68).

Eggs of *A. nemorum* were predominantly found inserted in the leaf margins including the leaf tip on both apple and pear. Since there was no significant difference among experiments for pear, or for *A. nemoralis* on apple, these data were pooled for the T-test. Across experiments 93% of *A. nemorum* eggs were laid in leaf margins on apple.

Though there was a significant preference for leaf margin in all four experiments data for *A. nemorum* on apple could not be pooled (exp 1, T = 7.6, P < 0.0001, exp 2, T = 2.9, P < 0.02, exp 3, T = 7.5, P < 0.0001, exp 4, T = 6.2, P < 0.0001). Similarly *A. nemorum* laid 95% of its eggs in the leaf margin of pear (T = 6.0, P < 0.0001). Eggs of *A. nemoralis* were most commonly found in the leaf centre (5 mm or more from leaf margins) with 81% on apple (T = -2.8, P < 0.01) and 67% on pear, though only near significant on pear (T = -0.9, P = 0.07) (Figure 2). There was a highly significant effect of anthocorid species on the difference between numbers laid in margin and leaf centre in experiment four (F = 22.5, df = 1, P < 0.0001), a significant effect of plant (F = 9.9, df = 1, P < 0.003) and the crossed effect of anthocorid species by plant was highly significant (F = 19.1, df = 1, P < 0.0001) (n = 68), indicating that the level of preference for leaf margin and leaf centre depends on the interaction of the two factors.

Anthocoris nemorum laid 63% of its eggs on the ventral side of pear leaves (T = -2.0, P < 0.05) and *A. nemoralis* 83% (T = -3.7, P < 0.001). Since there was no significant difference among experiments for pear or for *A. nemoralis* on apple, data were pooled for the T-test. On apple *Anthocoris nemoralis* laid few eggs. Of these 52% were laid on the dorsal leaf side, showing no significant preference for leaf side in *A. nemoralis* on apple. Data for choice of leaf side by *A. nemorum* on apple could not be pooled. It laid significantly more eggs on the upper leaf side in experiments three and four (T = 3.1, P < 0.008 and T = 2.5, P < 0.02), but in experiments one and two no significant preference was found (Figure 3). Experiment four revealed a highly significant effect of plant (F = 25.5, df = 1, P < 0.0001), and a significant effect of species (F = 14.8, df = 1, P < 0.0003) (n = 68), but no significant crossed effect on the difference between numbers of eggs laid on the dorsal and ventral leaf sides, indicating that the choice of leaf side for oviposition on apple and pear does not interact with anthocorid species.

Oviposition preference assessed in small cages As data for the two experiments with cut versus uncut leaves were not significantly different they were pooled for statistical analysis. *Anthocoris nemorum* laid 63% of its eggs on cut apple leaves ($T = 2.0$, $P < 0.05$, n= 24), while the opposite was true for *A. nemoralis* which laid only 37% of its eggs on the cut apple leaves ($T = -2.1$, $P < 0.04$, n = 20). Few females laid eggs on pear leaves. While *A. nemoralis* also preferred uncut pear leaves ($T = -2.87$, $P < 0.04$, n = 5), preference for cut leaves was not significant for the few replicates with *A. nemorum* on pear ($T = 2.31$, $P = 0.15$, n = 3) (Figure 4). There was a significant crossed effect of anthocorid species by plant on the difference in eggs laid on cut and uncut leaves ($F = 12.27$, df = 1, $P < 0.001$, n = 52) and no significant main effects of anthocorid species or plant.

There was a near-significant preference of *A. nemorum* for oviposition on apple leaves with *O. brumata* eggs over clean leaves (mean with eggs ± s.e. = 5.1 ± 1.3, mean without eggs = 3.1 ± 0.5, $T = -2.2$, $P = 0.057$, n = 10).

Discussion

The present study demonstrated a clear preference for oviposition host plant for both anthocorid species and a highly significant difference between their host plant choices. Since the ability of moving to alternative plants is very limited for the wingless young, oviposition preference will greatly affect the resulting density of immatures on a given plant and thus the potential role of these predators in apple and pear orchards. Oviposition preference may be a major explanation why *A. nemorum* is more abundant in apple and *A. nemoralis* in pear (Skanland ,1981; Herard & Chen, 1985).

One other study assessed oviposition preference of *A. nemorum*: Herard & Chen (1985) found that young barley leaves were preferred over young pear leaves, except when prey (*C. pyri*) was offered on pear but not on barley. Batch size in this study was less for *A. nemorum* than the two to eight eggs mentioned by Sands (1957), who also noted that eggs of *A. nemoralis* were seldom laid in batches.

The observed preference of leaf margins of *A. nemorum* in contrast to *A. nemoralis* (Figure 2) corresponds to earlier records of their general oviposition pattern on different host plants (Hodgson & Aveling, 1988) and are in agreement with results from Elliott & Way (1968) who found that 95% of *A. nemorum* eggs were laid on the leaf margin (2mm from edge) (48.6%) or stipules (47.4%) of bean (*Vicia faba*). Search for prey by *A. nemorum* is also concentrated on leaf margins as found on *Brassica oleracea var. gongylodes* (90.4%), tobacco (67.3%) and bean *Phaceolus vulgaris* (65.1%) (Lauenstein, 1980b). On the contrary, *A. nemoralis* spend more time searching on the leaf centre. Thus Brunner & Burts (1975) observed that it searched the leaf midrib half its time and spent 71% of its time on the ventral side of pear leaves. Thus, the search pattern of *A. nemoralis* also seems to reflect its oviposition pattern. Overlap of oviposition and search for prey at the single leaf scale may present a potential risk of egg cannibalism at high predator densities. On a whole tree, however, oviposition sites and search for prey may be spatially separated.

No overall preference for dorsal or ventral leaf side *per se* was found but both species laid most eggs on the dorsal side of apple leaves and the ventral side of pear leaves, and in the two last experiments *A. nemorum* showed a significant preference for the dorsal side of apple leaves. While the ventral side of apple leaves is very hairy, and the dorsal side of pear leaves almost hairless, the dorsal side of apple leaves and the ventral side of pear leaves are intermediate in this respect. Thus it is possible that choice of oviposition site is affected by leaf surface structure. Possibly the difference between leaves sides on apple were more pronounced to anthocorids in the last two experiments, when leaves would tend to be slightly older. Warabieda et al. (1997) suggest that pubescence may protect spider mites (*Tetranychus urticae*) from predation. Possibly anthocorid females can protect their eggs by choosing oviposition sites with some pubescence, but on the other hand may prefer to search more smooth areas. Thus, according to Lauenstein (1980b), *A. nemorum* searched upper surfaces of leaves more thoroughly than under surfaces, most so on the smooth brassica, intermediate on tobacco, least so on bean.

The preference of *Anthocoris nemorum* to oviposit on cut apple leaves over whole leaves may be influenced by volatiles from the newly cut leaves. Such a preference could help *A. nemorum* to locate prey in the field. The fact that *A. nemoralis* preferred whole leaves to leaves with this particular damage may be that as this species mostly oviposits away from leaf margins it was in less direct contact with any leaf exudates from the cut leaf, and perhaps experienced a change in leaf quality as oviposition medium. Prey search of another anthocorids, *Orius tristicolor*, has earlier been found to increase when leaves were artificially damaged with a pin. Thus, bean leaves that prior to experiments had been exposed to plant feeding (thrips or spider mites) or artificial damage elicited increased searching, and resulting higher predation success (VanLaerhoven et al., 2000). Maybe the preference of *A. nemoralis*, which mostly preys upon psyllids and other insects which pierce, rather than chew, plant material, would have been different towards pierced rather than cut leaves.

Field collected leaves where preys are mechanically removed, as used in this study, may still hold volatiles related to preys or their feeding. The most common prey observed on collected leaves was *Psylla mali* on apple and a few aphids and psyllids on both apple and pear. From Dutch studies the attraction of *A. nemoralis* to volatiles from pear psylla-infested trees is well documented (Scutareanu et al., 1997; Drukker et al., 2000). This study suggests that *A. nemorum* prefer to oviposits near prey. Likewise, (Steer, 1929) observed that *A. nemorum* preferred to oviposit near spider mite colonies.

Anthocoris nemoralis is currently under evaluation for inoculative and/or inundative releases against pear psylla in various European countries, so far with variable results, often with initial success followed by dispersal away from the release area (Fauvel et al., 1984; Rieux et al., 1994; Beninato et al., 2000). Oviposition preference, presence of prey and/or plant related volatiles, may all affect the attraction to a plant, oviposition and later the retention of adult *A. nemorum* and *A. nemoralis* in orchards and thus the success of biological control.

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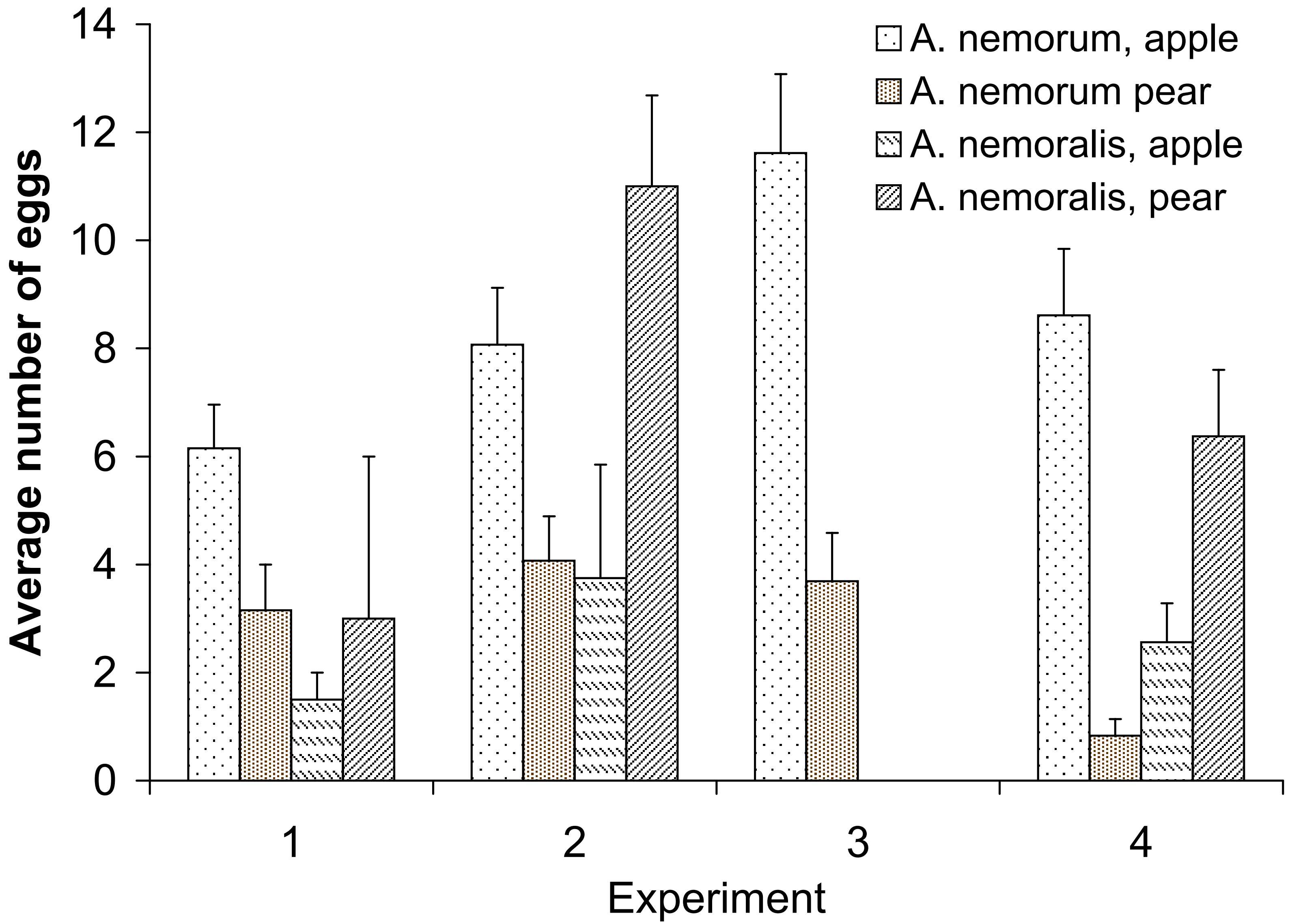
Figure 1. Oviposition preference of *Anthocoris nemorum* (exp 1: n =13, exp 2: n = 14, exp 3: n= 13, exp 4: n= 18) and *A. nemoralis (*exp 1: n = 2, exp 2: n= 4, exp 4: n = 16) for apple or pear (mean number of eggs + s.e.).

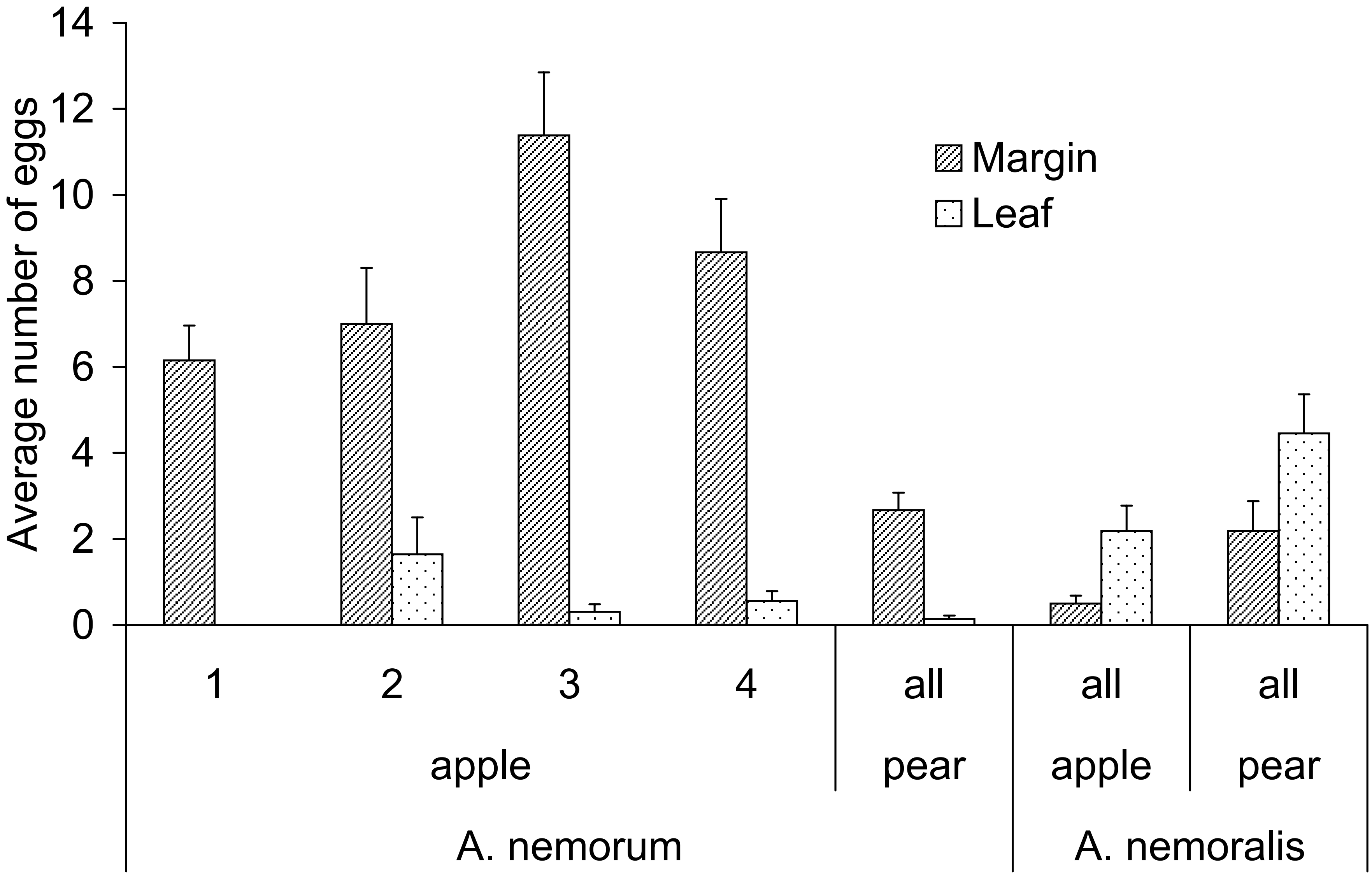
Figure 2. Oviposition preference of *Anthocoris nemorum* (n = 58) and *A. nemoralis* (n = 22) for leaf margin or leaf of apple and pear leaves (mean number of eggs + s.e.). Data for *A. nemorum* on apple could not be pooled and are presented for the individual experiments (exp 1: n =13, exp 2: n = 14, exp 3: n= 13, exp 4: n= 18). Remaining results were pooled and are presented across experiments.

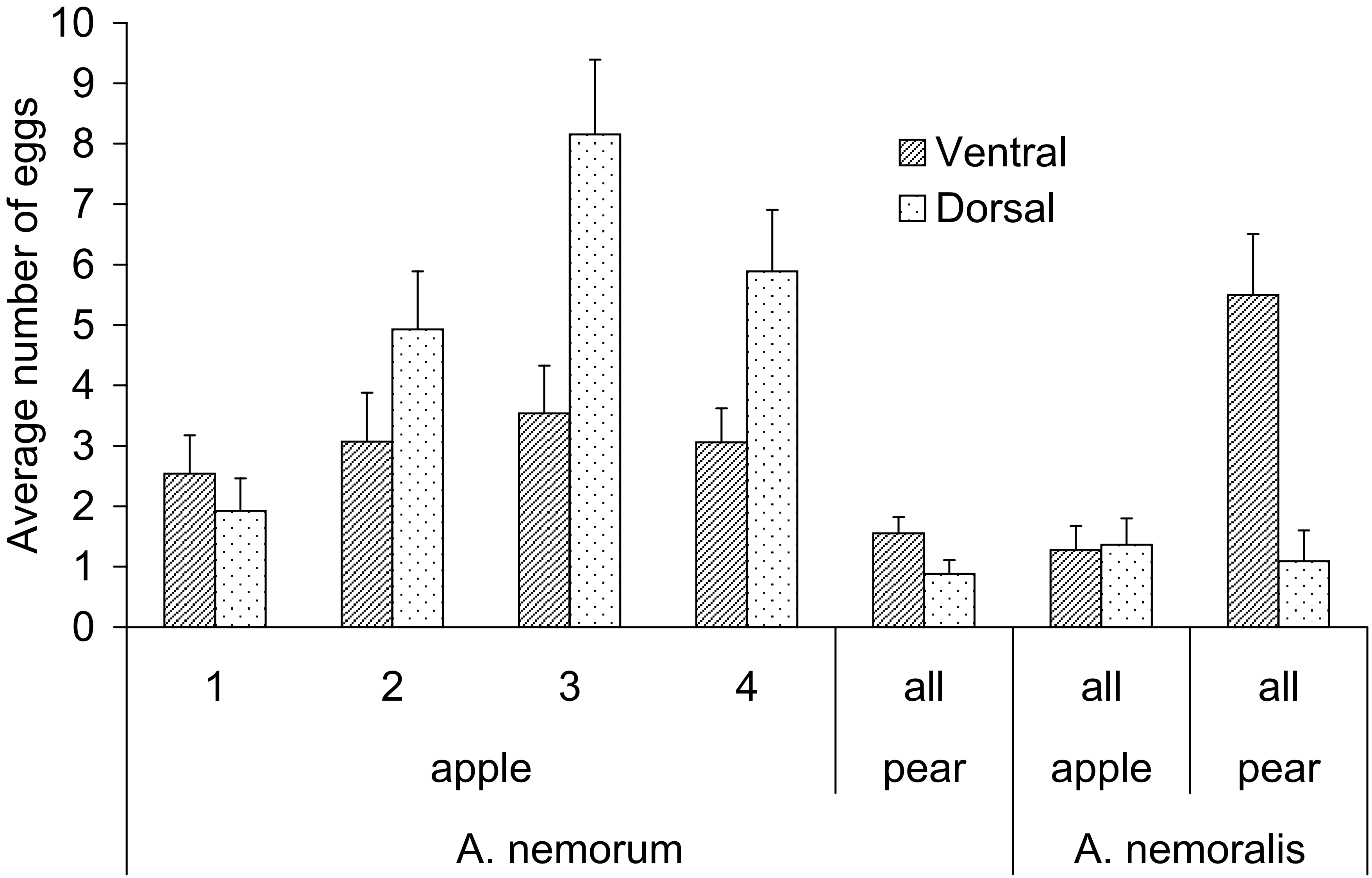
Figure 3. Oviposition preference of *Anthocoris nemorum* (n = 58) and *A. nemoralis* (n = 22) for dorsal or ventral leaf side of apple and pear leaves (mean number of eggs + s.e.). Data for *A. nemorum* on apple could not be pooled and are presented for the individual experiments (exp 1: n =13, exp 2: n = 14, exp 3: n= 13, exp 4: n= 18). Remaining results were pooled and are presented across experiments.

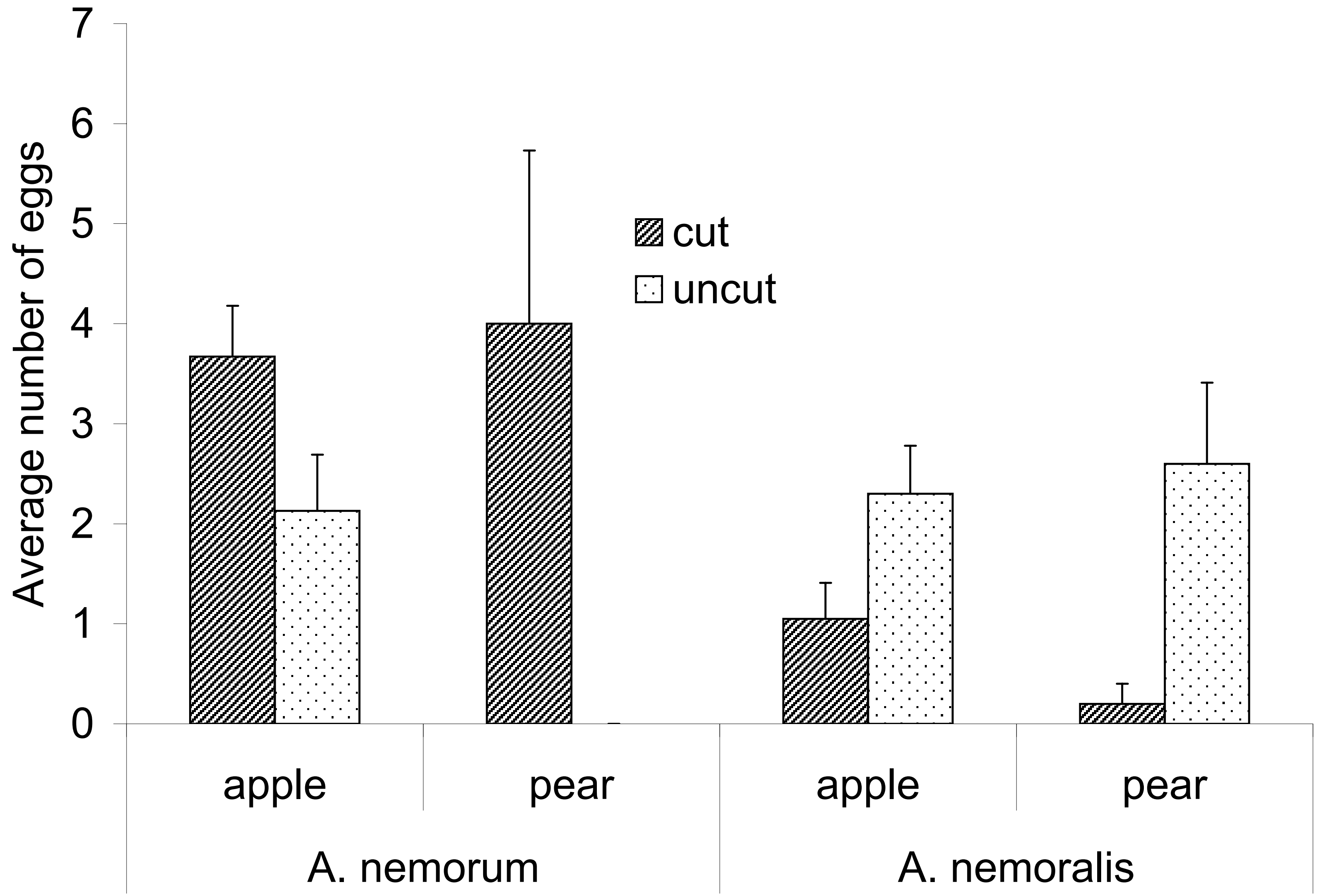
Figure 4. Oviposition preference of *Anthocoris nemorum* and *A. nemoralis* for cut or uncut leaves of apple (*A. nemorum*, n = 24, *A. nemoralis*, n = 20) or pear (*A. nemorum*, n = 3, *A. nemoralis*, n = 5) (mean number of eggs + s.e.).



Average number of eggs
14
12
10
8
6
4
2
0
1
2
3
4
Experiment
A. nemorum, apple
A. nemorum pear
A. nemoralis, apple
A. nemoralis, pear


Average number of eggs
14
12
10
8
6
4
2
0
Margin
Leaf
1
2
3
4
all
all
all
apple
pear
apple
pear
A. nemorum
A. nemoralis


Average number of eggs
0
1
2
3
4
5
6
7
8
9
10
Ventral
Dorsal
1
2
3
4
all
all
all
apple
pear
apple
pear
A. nemorum
A. nemoralis


7
6
5
4
3
2
1
0
Average number of eggs
cut
uncut
apple
pear
apple
pear
A. nemorum
A. nemoralis