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On intra-guild predation and cannibalism in *Orius insidiosus* and *Orius laevigatus* (Rhynchota Anthocoridae): laboratory experiments

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Abstract

The behaviour, intra-guild predation (IGP) and cannibalism (CANN), between two *Orius* species, *O. insidiosus* (exotic in Europe) and *O. laevigatus* (native) were investigated in small arena conditions. No differences were found in IGP between the two species. Regarding cannibalism, *O. laevigatus* seems more aggressive vs. 2nd instar nymphs compared to *O. insidiosus*. Adults of both species showed aggressive interactions more frequently vs. the young nymphs. No killing was observed between adults, and rarely between adult and 5th instar nymph. The IGP and CANN events were inversely correlated with abundance of food. For both species predation was never higher than cannibalism. *Orius insidiosus* releases in Europe may therefore produce negligible effects on the native *O. laevigatus*. The methods performed could be adopted as a first and simple standard bioassay to obtain data on risk assessment due to IGP.

Key words: *Orius insidiosus*, *Orius laevigatus*, Intra-guild predation, safety of biocontrol, cannibalism, exotic natural enemies.

Introduction

Up to now the potential negative effect of the introduction in Europe of *Orius insidiosus* (Say) (Rhynchota Heteroptera Anthocoridae), a predator species mass reared for biological control of thrips, has not been evaluated. The risk on non-target organisms due to the release and use of exotic macrobiological control agents is an issue which is becoming urgent to clarify (Hokkanen and Lynch, 1995; van Lenteren, 1997; van Lenteren and Loomans, 2000; Lynch *et al*., 2001).

Orius insidiosus is relatively easy to mass rear and it is applied in several IPM programmes in North America. It was introduced in North Europe from North America early in the nineties by European beneficial arthropods producers. This species was released against the exotic Western Flower Thrips, WFT: *Frankliniella occidentalis* (Perg.) (Thysanoptera Terebrantia Thripidae). This established insect pest is damaging several crops in Europe (Tommasini and Maini, 1995), particularly on protected crops in Italy (Tavella *et al*., 1994), The Netherlands (Disselvelt, 1997), Belgium (Sterk *et al*., 1997), Greece (Michelakis *et al*., 1997) and Malta (Mifsud, 1997).

At present in most European countries, the *Orius* species produced by biofactories is the native *O. laevigatus*. It is released on protected crops, due to the good (Tavella *et al*., 1996; Baraja *et al*., 1996; van der Blom *et al*., 1997) or partially good (Tommasini *et al*., 1997) results achieved against *F. occidentalis*. The lack of diapause, typical of some South European *O. laevigatus* strains, is also an important biological trait related to field efficacy (Tommasini and Nicoli, 1995; Tommasini and Nicoli, 1996).

Due to the importance to know the risk of exotic generalist predators on non target organisms and in particular on native beneficial arthropods, it is necessary to assess methods to evaluate such a possible risk. The risk could be higher when predators are living at the same trophic level. In several Mediterranean Countries greenhouses are simple plastic tunnels, open during the day with a possible spreading of arthropods on and from the crops. Intra-guild predation (IGP) and cannibalism (CANN) experiments regarding generalist exotic insect predators, imported and reared for biocontrol in plastic tunnels, have already been published (Lynch *et al*. 2001; Burgio *et al*., 2002). Since it is difficult to asses for intraspecific relationships among *Orius* sp. in the open field or in confined environments and to avoid the possibility of an establishment of a potential hazardous exotic species in the ecosystem we arranged laboratory experiments.

Taking the two polyphagous *Orius* species, *O. insidiosus* (exotic species) and *O. laevigatus* (native species), as a case study, the objectives of our experiments were: (i) to obtain data about the interactions such as IGP and CANN between these predatory bugs; (ii) to assess a first simple laboratory method to evaluate possible negative ecological effects of biocontrol.

Materials and methods

Five experiments on the behaviour (IGP or CANN events or contacts) of the two species *O. insidiosus* and *O. laevigatus* were carried out. Both predator species studied were supplied by European commercial insectaries, fed with frozen eggs of *Ephestia kueniella* (Zeller). Pod beans were provided as egg laying substrate.

Experiments 1-4 were carried out in an incubator set at 25±1°C, RH=70±10%, under continuous light. Predators were previously isolated and starved for 24 hours in

ventilated plastic tubes (150 cm³) with water. After the starvation period one *O. insidiosus* and one *O. laevigatus* were transferred in small arenas (glass Petri dishes: 8 cm diameter). Different amounts of frozen *E. kuehniella* eggs were glued on filter papers with water and offered to the pairs of predators: (i) no *E. kuehniella* eggs (0 Ek); (ii) 5 *E. kuehniella* eggs (5 Ek); (iii) 50 *E. kuehniella* eggs (50 Ek).

Experiments 1 to 4 were set up as detailed in table 1. When adults were compared all the possible combinations were set up (female vs. female of the same species for both species; female vs. male of the same species for both species; female of species 1 vs. female of species 2; male of species 1 vs. male of species 2; female of species 1 vs. male of species 2; female of species 2 vs. male of species 1). The adults used were 7-10 days old.

Each experiment was repeated 30 times, each one including two hours of direct observation, for a total of 720 hours. All interactions that occurred between the individuals were recorded. Observations were done on distinct interaction (event = contact between the two specimens) and exactly on aggressive interaction (no killing) and killing interaction.

Table 1. Description of the first four experiments. Thirty pairs of both *O. insidiosus* vs. *O. laevigatus* and *O. laevigatus* vs. *O. insidiosus*, were carried out for each experiment (n = 30).

Exp.	Combination
1	Adult vs. 5th instar nymph (N5)
2	Adult vs. 2nd instar nymph (N2)
3	5th instar nymph (N5) vs. 2nd instar nymph (N2)
4	Adult vs. Adult

The fifth experiment was undertaken using 4 combinations of *Orius* species/age by exposing an adult and a newly hatched nymph together in the same arena described above and leaving them for all the developing time from 1st instar nymph up to adult moult (i.e. the experiment lasting from 10 to 12 days in relation to data previously obtained by Tommasini and Nicoli, 1994). The rearing unit was set up as in the previous experiments but the photoperiod was set at 16 L: 8 D. *Ad libitum* food (frozen *E. kuehniella* eggs) glued on filter paper with water was supplied regularly. Observation recorded over one hour was carried out three times a day during all the experiment. Thirty replicates were done for each *Orius* species combinations, observing the behaviour of individuals for a total time of more than 3600 hours.

The data of experiments 1-4 were analysed by a non parametric correlation (Spearman test).

Frequencies of behaviours observed during all experiments were analysed by χ^2 test.

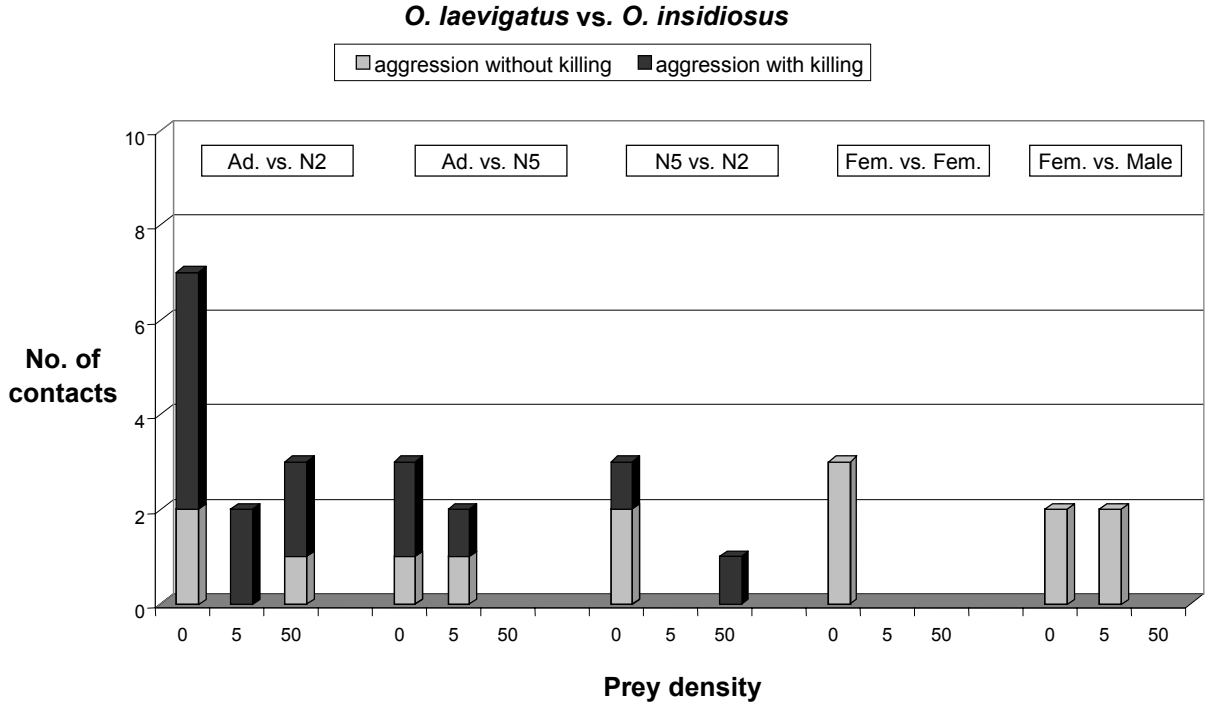
Results and discussion

From the data collected and summarised in table 2 and figures 1 and 2, three behaviours were defined: (i) AI, aggressive interaction (total aggressive interactions/total interaction); (ii) EA, efficacy of aggression (killing/aggressive interactions); (iii) PR, predation rate (killing/total interaction). No significant differences were observed in IGP, (i.e.: *O. insidiosus* vs. *O. laevigatus* and vice-versa), except in few cases reported in table 2. Generally no differences occurred on CANN, but adults of *O. laevigatus* appeared slightly more aggressive vs. 2nd instar nymphs of the same species compared to *O. insidiosus.*

Adults of both species showed aggressive interactions attacking the young nymphs more frequently. No killing events were recorded between adults for both *Orius* species and very few killings between adult vs. 5th instar nymphs. It is known that the predation capability of both *O. insidiosus* and *O. laevigatus* grows with the increase of the developmental stage up to the adult (Isenhour and Yeargan, 1981; Tommasini and Nicoli, 1993; 1994). The vulnerability of the youngest nymphs is confirmed by the results shown in figure 3. Mostly the first and the second instar nymphs were killed by the adult, but no differences were recorded (χ^2 test, $p<0.05$) on the 5th instar nymphs.

Table 2. O.i. = *O. insidiosus*, O.l. = *O. laevigatus*. **AI** aggressive interaction (total aggressive interactions/total); **EA** efficacy of aggression (killing/aggressive interaction); **PR** predation rate (killing/total).
When it is not specified no significant difference was found (χ^2 test, *p<0.05, [a] p=0.06).

		0 Ek			5 Ek			50 Ek		
		AI	EA	PR	AI	EA	PR	AI	EA	PR
Adult vs. N2	O.i. vs. O.l.	0.33	0.9	0.30	0.16	0.8	0.13	0.07	1	0.06
	O.l. vs. O.i.	0.23	0.71	0.16	0.07	1	0.06	0.1	0.66	0.06
	O.i. vs. O.i.	0.26	1	0.26	0	0	0	0	0	0
	O.l. vs. O.l.	0.33	1	0.33	0.23*	0.85	0.20*	0.1	0.66	0.06
N5 vs. N2	O.i. vs. O.l.	0.23	0.85	0.2	0.13	0.75	0.01	0	0	0
	O.l. vs. O.i.	0	0.66	0.07	0.07	0.50	0.03	0	0	0
	O.i. vs. O.i.	0.10	1	0.10	0.03	0	0	0	0	0
	O.l. vs. O.l.	0.20	1	0.20	0.10	1	0.10	0.16[a]	0.80	0.13



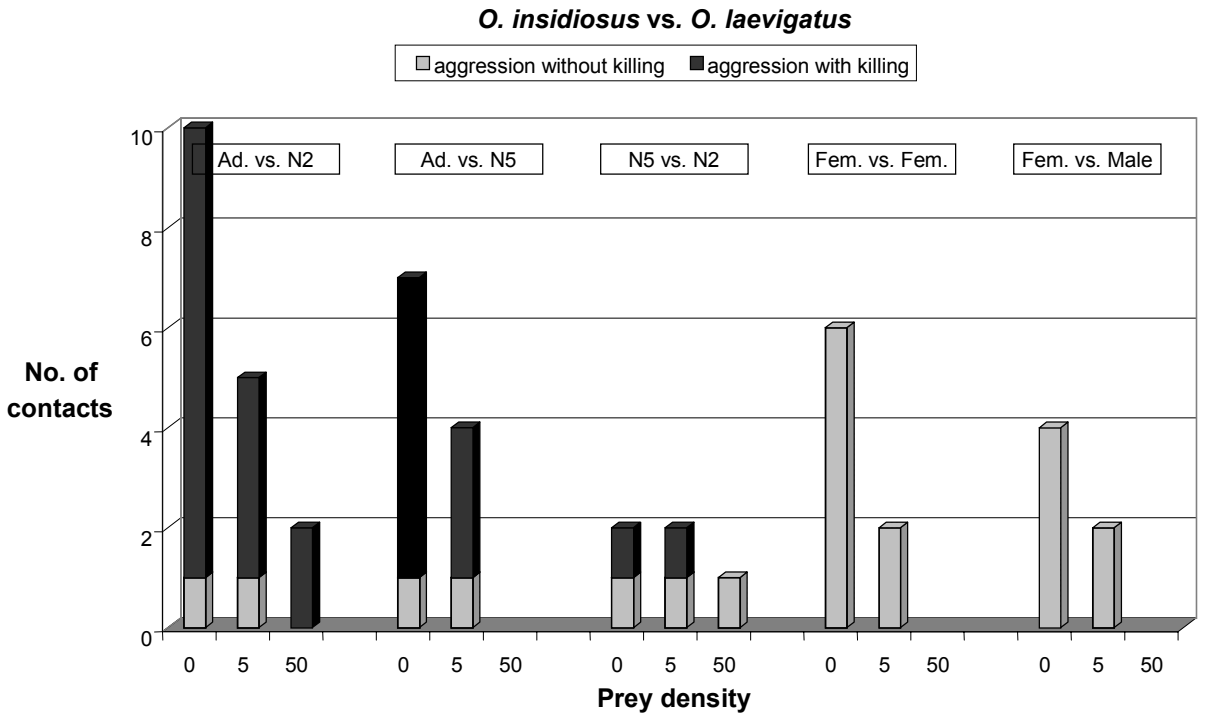


Figure 1. Number of aggressive interactions and killing by intra-guild predation (30 replicates). (Ad. = Adult female; N2 = 2nd instar nymph; N5 = 5th instar nymph; Fem. = Adult female; Male = Adult male.)

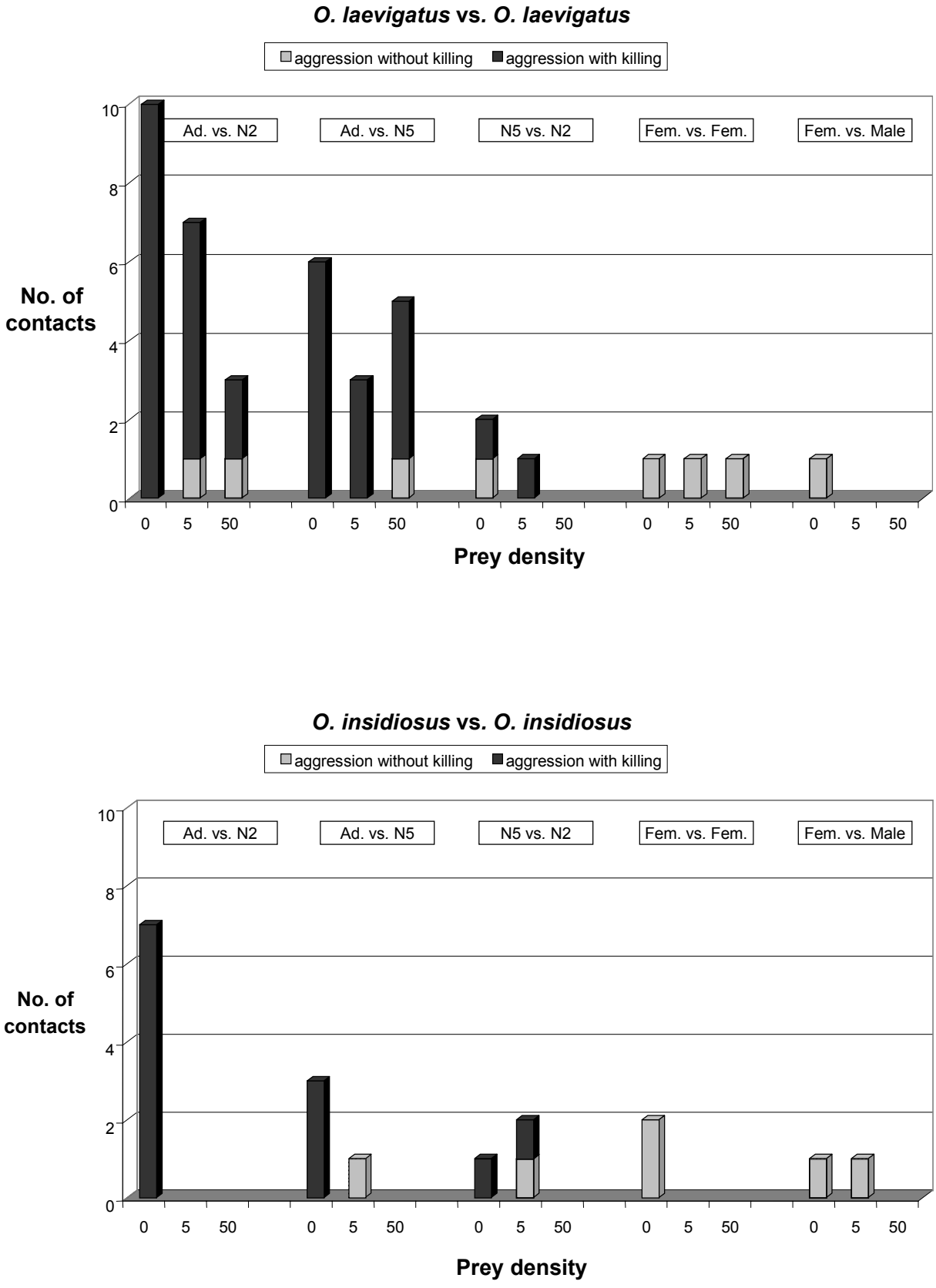


Figure 2. Number of aggressive interactions and killing by cannibalism (30 replicates). (Ad. = Adult female; N2 = 2nd instar nymph; N5 = 5th instar nymph; Fem. = Adult female; Male = Adult male).

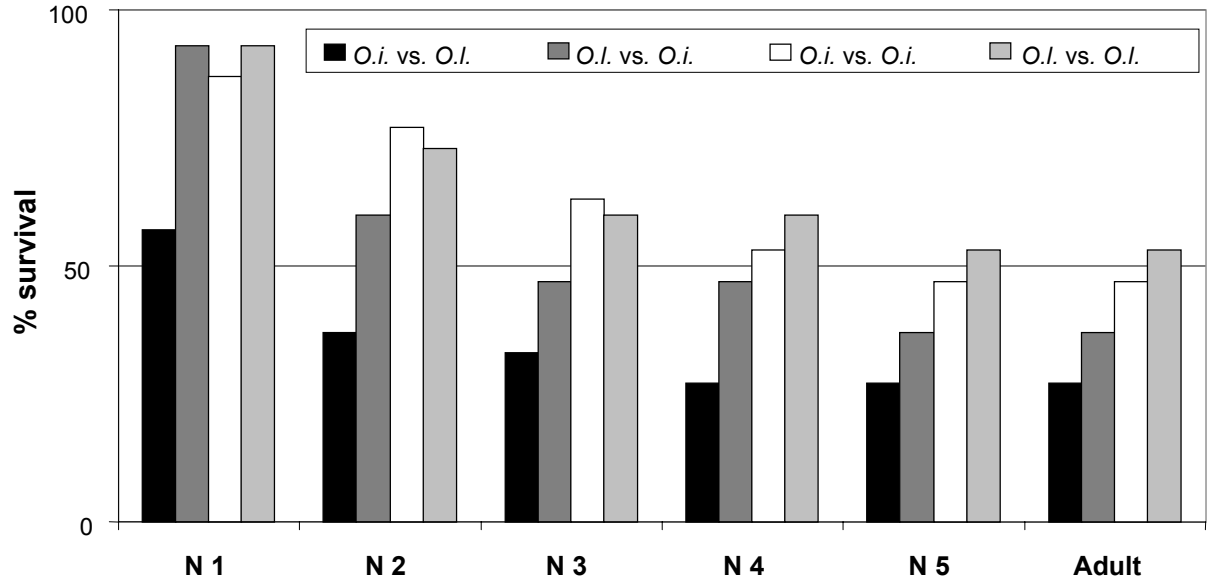


Figure 3. Percentage of surviving individuals after intra-guild predation and cannibalism due to adult vs. nymphs during their development time. No significant differences were recorded on the ratio of nymphs that reached adult stage. (O.i. = *O. insidiosus*; O.l. = *O. laevigatus*) (N1 = 1st, N2 = 2nd, N3 = 3rd, N4 = 4th, N5 = 5th instar nymphs).

The IGP and CANN events were in some cases inversely correlated with abundance of food for both species, as are also shown in figures 1 and 2 ($R = -0.78$, $P<0.001$ for adults vs. N2; $R = -0.63$, $P<0.05$ for N5 vs N2). Three significant non parametric correlations were also found between the amount of food supplied (*E. kuehniella* eggs) and the food eaten ($R=0.67$, $P< 0.01$, for adults vs. N2; $R = 0.57$, $P<0.01$, for N5 vs. N2; $R = 0.48$, $P<0.01$, for adults vs. adults). Isenhour and Yeargan (1981) found that in *O. insidiosus* the percentage of killed prey decreases when prey density increases, i.e. when the satiation of the predator is higher.

A low propensity for CANN and/or IGP events emerged in both native *O. laevigatus* and exotic *O. insidiosus*. Nevertheless, when no food was available, an increase in cannibalism/predation was recorded for both species, but predation was never higher than cannibalism for both species. Furthermore, no inter-specific mating attempts were observed during the experiment with adults, while 17.8% and 13.3% of intraspecific mating occurred in *O. laevigatus* and *O. insidiosus,* respectively. Knowledge on IGP and CANN events investigated in laboratory could need further experiments also in greenhouses, to evaluate these behaviours under more natural conditions than in a simple arena. Anyway, if the two *Orius* species could interact in a small arena, as our data demonstrate, we can assume that in semi-field or in open field trials, with many different preys, the IGP and CANN events could be difficult to evaluate. The strain of *O. insidiosus* used could have a reduced aggressiveness, due to continuous rearing in laboratory conditions and, in case of new introductions of this predator, it may be necessary re-investigate the IGP. Until now, it is known from a survey carried out in Italy (unpublished data) and in Spain (Riudavets and Castañè, 1994) that an establishment of *O. insidiosus* after seasonal inoculative releases was never found. Therefore the laboratory experiments performed can be a possible simple standard bioassay to obtain data to assess the risk on non-target insects due to importation of exotic predator species. The risk evaluation data obtained in our experiments show negligible possible effects of *O. insidiosus* releases in Europe on native *Orius* species.

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