Density-Dependent Response of the Pea Aphid (Hemiptera: Aphididae) to Imidacloprid

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Abstract  A study was conducted to determine the influence of initial population density on the effects of pesticides on pea aphid, Acyrthosiphon pisum (Harris), populations. Three initial starting densities of pea aphids (147, 295 and 590 aphids per m²) were exposed to no pesticide or imidacloprid at rates of 1 or 5 g a.i./ha on broad bean plants, Vicia faba L., in a greenhouse. Ten days later, population size was assessed. In general, higher initial aphid population density resulted in a higher final population density for all imidacloprid concentrations. However, population growth rates for populations started with the highest density (590 aphids per m²) were significantly lower than those with initial densities of 147 and 295 aphids per m². This was due to a relative reduction in population number. Populations begun with 147 aphids per m² were 50% lower after exposure to the highest concentration of imidacloprid, whereas the populations begun with 295 and 590 aphids per m² were 42 and 25% of the starting population size, respectively. Therefore, the pesticide actually had a greater impact on the population started with the highest density. This can be explained by a synergistic effect of the pesticide and crowding. The lower growth rate observed in the population started with the highest density was probably due to crowding, whereby aphids approached the carrying capacity and were stressed. Even though these populations were reduced, final density was still sufficiently high to limit resources. These results indicate that the response of organisms to stress is influenced by population density at the start of a stressful event, such as a pesticide exposure. Therefore, different experimental designs may result in different outcomes and starting population densities must be carefully considered when designing population-level toxicological experiments.

Key Words  population growth rate, starting population density, pea aphid, imidacloprid, stress

The evaluation of pesticide effects at the population-level rather than the individual-level has been recommended because it takes into account the loss of individuals due to toxicant-induced mortality as well as the effects that multiple sublethal effects may have on survivors (Stark and Banks 2003). This approach, which is sometimes called demographic toxicology, usually involves the development of life tables and is often followed by modeling (Stark and Banks 2003). Demographic toxicology is slowly being adopted by agencies that regulate pesticide use such as the U.S. EPA (Applied Biomathematics, Inc. & Woodlot Alternatives, Inc. 2003). Addi-

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tionally this approach is being used more often within the scientific community as indicated by the number of studies using demographic toxicological approaches being published in the peer review scientific literature (Stark and Banks 2003). However, there remain limitations to the use of population growth rate for estimation of population level effects. One problem with this method is that in the majority of published studies, small groups of individuals, often well below the carrying capacity, are exposed to toxicants. Because the starting population size is the same among groups, it is impossible to determine whether starting population density has an impact on the outcome of toxicant exposure.

Several authors have argued that the initial population density may influence population response to toxicants (Sibly 1999, Forbes et al. 2001). For example, results of experimental work on blowflies (Moe et al. 2002), polychaetes (Linke-Gamenick et al. 1999), copepods (Sibly et al. 2002), rotifers (Sarma et al. 2001), midges (Hooper et al. 2003a) and enchytraeids (Kramarz et al. 2005) have shown that starting population density may have an influence on population level susceptibility.

The objective of this study was to determine whether starting population density affected population susceptibility to pesticide exposure. We used the pea aphid, *Acyrthosiphon pisum* (Harris), as a model organism for this study. The pea aphid is a significant pest species worldwide and is ideal for population-level studies because it has a short generation time and high reproduction rate. Additionally, there is a large body of literature on the biology of this species as well as several published population-level toxicological studies (e.g., Stark and Wennnergren 1995, Walthall and Stark 1997, Wennnergren and Stark 2000, Laskowski 2001).

**Materials and Methods**

Pea aphids were obtained from a laboratory culture maintained at the Washington State University, Puyallup Research and Extension Center. Aphids were reared on potted broad bean plants, *Vicia faba* L., in an environmental chamber at 20°C and a photoperiod of 16:8 h (light:dark).

The insecticide used in this study was imidacloprid (Provado® 1.6 F, 17.4% imidacloprid) (Bayer CropScience, Research Triangle Park, NC). Imidacloprid, a systemic insecticide, was applied as a soil drench to Fava bean plants grown in plastic pots filled with 50 g dry weight of potting soil. Before plants were potted, the soil was oven-dried at 105°C for 24 h, and the water-holding capacity was measured gravimetrically. The soil was watered with distilled water, and one bean plant per pot was planted. After 4 wks, when the plants were approx. 22 cm high, imidacloprid was applied at a rate of either 1 or 5 g ai/ha. Deionized-distilled water was applied to pots containing the control plants. Throughout the experiment, the potted plants remained in plastic trays filled with tap water.

Five replicates of each treatment were established in a greenhouse. Each replicate consisted of 5 plants each in separate pots but with leaf contact among the plants. Additionally, pots were connected by cardboard at the base to minimize aphid loss due to dropping and to allow for migration among plants during the experiment. Each replication was enclosed in a sleeve made from netting to prevent aphid escape. Adult aphids were introduced to the plants 4 d after the application of imidacloprid. Aphids were introduced to the middle plant of each group of five. Three initial densities (low, medium and high) were evaluated in this study. Thus the initial numbers of aphids used in each treatment were 147, 295 and 590 per m², respectively. Ten days (one generation time) after aphid introduction, the plants were cut and the aphids were counted to obtain the final density of aphids.
The endpoints of interest in this study were final population density (number of individual aphids) and the instantaneous rate of population increase ($r_i$) (Walthall and Stark 1997). The instantaneous rate of increase is a direct measure of population growth rate similar to the intrinsic rate of increase developed with life tables.

The following equation was used to generate instantaneous rates of increase:

$$
\ln\left(\frac{N_f}{N_0}\right) = r_i t
$$

where $N_0$ and $N_f$ are the population sizes at the start and at the end of experiment respectively, and $t$ is time in days (Walthall and Stark 1997, Sibly 1999).

**Statistical analysis.** All data (population number and population growth rate) were checked for normality (the chi-square test) and homogeneity of variance (the Cochran's test) (Snedecor and Cochran 1989). The Grubbs' test (Snedecor and Cochran 1989) was performed to identify potential outlier (StatPoint 2006).

To obtain linear dependence of $r_i$ on imidacloprid concentrations, the independent variable was reciprocally transformed to: $1/(\text{imidacloprid concentration} + 1)$. Differences in regression lines between the initial densities (grouping variable) were detected by comparing regression parameters with "dummy" variables (see Statgraphics software package, Manugistics Inc. for further details).

**Results**

Population density and population growth rate data fit a normal distribution ($P > 0.05$), and the variances were not statistically different ($P > 0.05$). Furthermore, the Grubbs’ test did not reveal any statistically significant outliers ($P > 0.05$). The relationship between population density and imidacloprid concentration and between population growth rate and imidacloprid concentration each fit the linear regression model with $r^2$ values ranging from 0.46-0.58 and 0.40-0.71 for density and growth rate, respectively (Figs. 1, 2).

Population density was significantly affected by exposure to imidacloprid in a concentration-dependent manner ($P < 0.0001$) (Fig. 1). Results showed that aphid population response to toxicological stress is a function of initial aphid density. At the end of the experiment, the final population size was related to the initial population density. The higher the initial density ($N_0$) was, the higher the final aphid population density ($N_f$) was for all imidacloprid concentrations (Fig. 1). A comparison of regression line analysis for population density revealed differences between intercepts and slopes ($P < 0.0001$) (Fig. 1). A pairwise comparison of population density illustrated that the parameters of regression lines for all studied densities differed from each other (intercepts: $P < 0.001$, slopes: $P < 0.001$) (Fig. 1).

Although populations with the highest starting density ($N_0 = 16$) had the highest final densities even after exposure to the highest imidacloprid concentration, population growth rate was significantly lower in these populations (Fig. 2). Population growth rates for populations with an initial density of 590 per m$^2$ were significantly lower than those with initial densities of 147 and 295 aphids per m$^2$ (Fig. 2). In general, the higher the initial population resulted in the greater the relative reduction in population number. Populations started with 147 aphids per m$^2$ were 50% lower after exposure to the highest concentration of imidacloprid whereas the populations started with 295 and 590 aphids per m$^2$ were 42 and 25% of the starting population size, respectively. Therefore, the pesticide actually had a greater impact on the population started with the highest density.
Fig. 1. Relationship between aphids' population number ($N_0$) and imidacloprid treatment (g a.i. imidacloprid ha$^{-1}$) for three initial densities.
Fig. 2. Relationship between aphids' instantaneous growth rate ($r_i$) and imidacloprid treatment (g a.i. imidacloprid ha$^{-1}$) for three initial densities.
A comparison of regression line analysis for population growth rates revealed differences between intercepts ($P < 0.0001$, Fig. 2) as well as slopes ($P < 0.02$; Fig. 2). A pairwise comparison illustrated that the parameters of regression lines for the two lower densities (147 and 295 aphids per m$^2$) did not differ from each other (intercepts: $P > 0.4$, slopes: $P > 0.2$; Fig. 2). In contrast, the regression line for the initial highest density of 590 aphids per m$^2$ was statistically different from both of the lower starting densities, 147 aphids per m$^2$ (intercepts: $P < 0.0005$, slopes: $P < 0.04$; Fig. 2) and 295 aphids per m$^2$ (intercepts: $P < 0.0003$, slopes: $P < 0.01$; Fig. 2).

**Discussion**

The results of this study indicate that population response to stressors, such as pesticides, is influenced by the initial size of the population being exposed. Imidacloprid actually had a greater impact on the population started with the highest density. The lower growth rate observed in the population started with the highest density was probably due to crowding, and although these populations were reduced, they were still sufficiently high enough to limit resources. Additionally, crowding results in stress to individuals resulting in less offspring being produced. Our results corroborate those of other studies. For example, toxicant effects were intensified when resources became scarce due to overcrowding for cladocerans (Chandini 1986) and rotifers (Cecchin and Snell 1999). In this scenario, populations are already stressed due to overcrowding, and the pesticide may be exerting not only mortality but sublethal effects. This results in a greater than expected reduction in numbers. In contrast, toxicant effects have been found to be less than expected when initial population density was high (Postma et al. 1994, Sibly 1999, Barata et al. 2002, Hooper et al. 2003b, Game-Flores et al. 2004). In this scenario, populations are reduced by a pesticide but no sublethal effects occur. When the population is reduced, more resources are available to survivors, and they produce more offspring than expected (Stark et al. 1997).

Our results indicate that the response of organisms to stress is influenced by population density at the beginning of a stressful event, such as a pesticide exposure. Also, there appears to be no predictable pattern as to the outcome of ecotoxicological studies conducted with high initial densities. Therefore, different experimental designs may result in different outcomes and starting population densities must be carefully considered when designing population-level toxicoological experiments.

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