# Effect of the Application of an Organophosphate Pesticide (Fenitrothion) on Foraging Behavior of Ants

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**Abstract :** Organophosphate pesticides inhibit cholinesterase. It is likely that application of organophosphate pesticides affect behavior of arthropods. This study aimed to find changes in foraging behavior of ants due to application of fenitrothion, one of the widely used organophosphate pesticides. Foraging activity (FA) of ants was observed using bait cards in a pesticide sprayed pine stand and in an unsprayed stand before and after aerial application of fenitrothion in 2003 and 2004. Ant abundance and species richness of ants were also monitored using pitfall traps during the activity season in 2003 and 2004. There was not a significant decrease in abundance and species richness after the application of fenitrothion. However, FA of an ant, *Paratrechina flavipes* (Smith), which was abundant enough to be statistically compared, was depressed from 2 hours to 10 days after application of the pesticide. FA was fully recovered at day 14 in 2003, and was partially recovered at day 18 and fully at day 31 in 2004. FA of other ant species also decreased significantly during the FA depression period of *P. flavipes*. On the bait cards, workers of the species responded dully to baits during the FA depression period. Despite the decline in activity, alertness of *P. flavipes* to other species did not decrease even during the FA depression period.

Key words: insecticide, competition, foraging behavior, aerial spray, Paratrechina flavipes

# Introduction

Development of the first organic pesticides (DDT) in 1874 contributed to an increase in food production and to a decline in epidemics of human diseases vectored by insects (Freedman 1989). Despite the astonishing achievements on pest control, DDT has long persistence time in ecosystem and caused environmental contamination. To overcome these problems, various organic pesticides such as lindane, cyclodienes, organophosphates, and carbamates had been developed. Organophosphate pesticides are used as insecticides, acaricides, and nematicides. They have a high toxicity to arthropods but a short persistence time in the environment (Freedman 1989, Fukuto 1961). These pesticides are also highly toxic to nontarget arthropods (EXTOXNET 2004), and a variety of studies on their impacts on nontarget arthropods have been carried out. However, most of these studies focused changes of arthropod abundance and richness caused by pesticides. The toxicity of organophosphate pesticide is due to the inhibition of cholinesterase (Fukuto 1961, Nath and Kumar 1999, Villatte and Bachmann 2002), which kills animals at higher dosage and which likely changes various behaviors at lower dosage. However, changes of behaviors of surviving arthropods are unexplored except for a few studies (Perfecto 1990, Murphy and Croft 1990) which document changes of ant foraging activity after pesticide application.

Ants are a good model to study the change of behaviors because of their abundance, diversity, ease in observation, and wide season of activity (Hölldobler and Wilson 1990, Agosti *et al.*, 2000). The baiting method was widely used to study foraging patterns, territorial behavior, and competition among ants (Halley and Burd 2004, Morrison 2002, Feller 1987). This tool may also be effectively used for detecting changes in foraging, feeding and competition behaviors after pesticide application. Ants were used to study nontarget impacts of pesticides in a few studies, but these were aimed to determine changes in abundance and richness of ant communities caused by pesticide application. The results of these studies do not agree (Chong *et al.*, 2007); some showed significant impacts of pesticide but most did not (Matlock and Cruz 2003).

In my previous work, I did not find any significant non-target impacts on ants in fenitrothion-sprayed pine stands (Kwon *et al.*, 2005, Kwon 2008). The aim of the present study is to determine whether foraging behavior

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of ants is changed by pesticide application. I predicted that, because the nerve system of ants is disturbed by the inhibitory action of fenitrothion on cholinesterase, ants attracted to baits would decrease because smell and/or communication between workers are affected, and that they would dully respond to baits. In addition, ants may be less alert to ants of other species, which may lead to a decrease of interspecies competition in feeding behavior. Changes of abundance and species richness of ant communities were monitored using pitfall traps during activity seasons of the study years.

#### Materials and Methods

#### 1. Study sites and treatment

The study was conducted in Haman, Gyeongsangnamdo (35° 15' N, 128° 30' E), in southern Korea. The two study sites, pesticide-sprayed and unsprayed, were selected from the secondary pine forests of approximately 30 years of age. The sprayed and unsprayed sites were 1.6 km apart. Japanese red pine, Pinus densiflora, was most abundant, followed by Pinus thunbergii. Shrubs and herbs were well developed. More details on the sites are shown in Kwon (2008). The insecticide aerial sprays were conducted three times in 2003 (6, 16 and 28 June), and one in 2004 (6 June). The dose of the insecticide (fenitrothion 50% EC) was 0.5 kg a.i.. The aerial spray was done early in the morning (05:30-08:00 a.m.) from a Korea Forest Service helicopter (Bell-206L3). Flying speed was 30-35 knot/h and height above the canopy was 5-10 m (KFRI, 1996). During the study period, the average of temperature in the study area was 14.8°C in 2003 and 15.7°C in 2004 (KMA 2008). Annual precipitation was 2,036 mm in 2003, and 1,827 mm in 2004.

#### 2. Ant survey

Foraging activities (FA) of ants were surveyed using 25 bait cards, which were set on the ground in a line at an interval of 5 m. Strawboard (11.5×16 cm²) was used as bait cards and small pieces of tuna (10-20 g) were put on the center of the card as bait. Ants on the cards were counted 10, 20, and 30 minutes later after the cards were set up. Behaviors of ants on cards were classified as foraging or feeding. Attacks and killed ants were also recorded. Each card was observed for about 10 seconds. The FA surveys were performed on sunny days.

Surveys were conducted on six study days in 2003; 5 June 2003 (-1 day; one day before the first pesticide spray), 6 June (3 h, 6 h after the first pesticide spray), 10 June (4 days after the first pesticide spray), 26 June (10 d after the second spray day), and 14 July (14 days after the third spray day). Surveys were conducted on eight days in 2004; 6 June (-4 days; 4 days before the spray),

10 June (2 h after the spray), 11 June (1 days after the spray), 12 June (2days after the spray), 13 June (3 days after the spray), 16 June (6 days after the spray), 28 June (18 days after the spray), and 11 July (31 days after the spray). The surveys were usually conducted for 2 hours from 14:00 to 16:00 except the survey at the 2 and 3 h surveys on 5 June 2003 and 10 June 2004 respectively, which were conducted from 10:00 to 12:00. The survey at the unsprayed site was conducted 1 hour after the survey at the sprayed site, but was not conducted on 5 and 10 June 2003. During the surveys, temperature was recorded using a maximum-minimum thermometer. Mean of temperature during the surveys was 28.8±1.4°C (SD) in 2003, and 29.3±1.3°C in 2004.

To monitor changes in abundance and species richness during the activity season, pitfall traps made of plastic bottles of 292 cm<sup>3</sup> volume (depth 6.5 cm, mouth diameter 8.5 cm, bottom diameter 6.5 cm) were used from May to October in the study years. Ten pitfall traps were filled one third with polyethylene glycol and were set up in a line with 5 m between them. The pitfall traps were changed monthly on most season except in June they were changed biweekly.

#### 3. Ant identification

During the FA survey, all ants were identified to species except the genus of *Crematogaster*, which were identified as *Crematogaster osakensis* or *C.* spp.; *C. matsumurai*, *C. vagula*, and *C. teranishi*, were identified as *C.* spp because they were difficult to identify in the field. Ants specimens collected in pitfall traps were identified to species. The specimens were deposited at the Forest Ecology Laboratory of Korea Forest Research Institute.

#### 4. Data analysis

The number of individuals and species of ants collected in ten pitfall traps was represented as abundance and species richness, respectively, on each sampling day. Descriptive analysis was applied on abundance and species richness, because there was no replication of data. Among the 12 ant species observed on bait cards, Paratrechina flavipes was the only one abundant enough to be tested statistically. Therefore, only FA of P. flavipes was examined in this study. The number of P. flavipes individuals observed on bait cards was logtransformed [ln (N+1)] to reduce variance and was compared using one-way ANOVA of three observations (i.e. 10, 20, 30 minutes) followed by multiple comparison of Neuman Keuls. In addition, the data of the sprayed site were compared with data of the unsprayed site using onetailed t-test because the number of ants was expected to decrease because of the pesticide spray. When the null hypothesis was rejected, the impact of pesticide was

assumed to affect FA of ants. On the basis of this decision, all study times at the sprayed site were grouped as the 'Depressed' time with significant impact of pesticide on behavior change and 'Normal' time with non-significant impact. All study times at the unsprayed site were grouped as 'Normal' time. Frequencies (i.e., number of observed surveys) of other ant species except P. flavipes were compared between the 'Depressed' and 'Nomal' times to find the impact of pesticides on FA of the species. To find the effect of pesticides and interspecific competition on FA of P. flavipes, the proportion of ants of P. flavipes participating in feeding behavior with the absence of other ant species were compared with those in the presence of other species between 'Depressed' and 'Normal' times. The proportions were arcsine-transformed for normalization, and were analyzed using two-way ANOVA with two factors, pesticide ('Depressed' or 'Normal') and other species (presence or absence), followed by a posteriori Keuls-Newman multiple comparison. All analyses were conducted using Statistica (ver. 6.1).

#### Results

Twenty three ant species were collected in pitfall traps at the sprayed and unsprayed sites, and about half that number of species was observed on the bait cards (Table 1). Attractiveness to bait was significantly different among ant species. Ten-fold or more ants of *P. flavipes* were observed on bait cards than collected in pitfall traps. However, although *Pachycondyla javana* was one of most abundant species in pitfall traps, only one ant was

Table 1. The number of ants observed on bait cards or collected in pitfall traps in the sprayed and unsprayed pine stands.

Species	Sprayed		Unsprayed	
	Bait	Pitfall	Bait	Pitfall
Aphaenogaster japonica		24	1	
Camponotus japonicus	148	132	302	541
Crematogaster osakensis	5	7		
Crematogaster spp.	2	27	18	13
Dolichoderus itoi				1
Formica japonica		1	1	13
Lasius japonicus		25		
Messor aciculatus				1
Monomorium chinense	91	39	155	234
Myrmecina nipponica		1		
Pachycondyla chinensis		1		2
Pachycondyla javana		150	1	196
Paratrechina flavipes	10,486	1,163	14,252	1,081
Pheidole fervida	6	35		9
Ponera japonica		3		1
Pristomyrmex pungens	58	158		414
Proceratium itoi		1		1
Solenopsis japonica		39		9
Strumigenys lewisi		18		11
Tapinoma melanocephalun	n			1
Technomyrmex gibbosus				4
Tetramorium tsuhimae			19	32
Vollenhovia emeryi		13	1	12
No. of Species	7	18	9	19
No. of Individuals	10,796	1,837	14,750	2,576

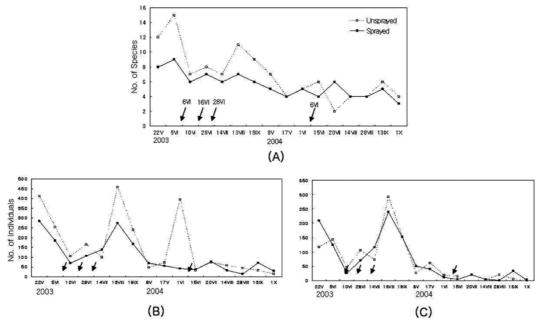


Figure 1. Ants collected in pitfall traps; number of species (A), number of total ants (B), and number of *Paratrechina flavipes* ants (C). Arrows indicate dates of pesticide application.

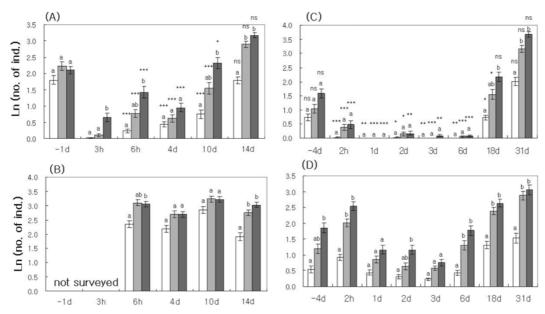


Figure 2. Number (ln N+1) of *Paratrechina flavipes* ants observed at the bait cards at the sprayed site in 2003 (A), unsprayed site in 2003 (B), sprayed site in 2004 (C), unsprayed site in 2004 (D). Error bars indicate one SE. Different captions above bars mean significant difference of means among three observations (i.e. 10 minutes, 20 minutes, and 30 minutes). Asterisks and ns in the sprayed stand represent significant and insignificant difference between the sprayed and unsprayed stands, respectively. \*: p < 0.05, \*\*: p < 0.01, \*\*\*: p < 0.001.

attracted to bait (Table 1). Figure 1 shows seasonal changes in abundance and species richness at the sprayed and unsprayed sites from May 2003 to September 2004. Compared to the pre-spray periods, no difference was found in species richness and abundance of ants at the sprayed site. Furthermore, the change of abundance of *P. flavipes* at the sprayed site was synchronous with that of the unsprayed site before and after the pesticide spray in 2003 and 2004. These results strongly suggest that fenitrothion did not significantly change abundance or species richness of ants.

However, the pesticide spray significantly affected FA of P. flavipes ants (Figure 2). Compared to the mean of number (log-transformed) of P. flavipes on the day before the first pesticide spray in 2003, the figure abruptly decreased 3 h after the spray. When compared with that of the unsprayed site, the figures of the sprayed site were significantly lower from 6 h to 10 d after the spray. At 14 d, however, the mean number of P. flavipes at the sprayed site did not differ from the mean number at the unsprayed site, representing the recovery of FA. A similar ephemeral depression and recovery of FA of P. flavipes was also recognized in 2004. When mean numbers were compared between the sprayed and unsprayed sites at 4 d before the pesticide spray in 2004, there was no difference. However, the mean number of ants at the sprayed site was consistently lower from 2 h to 6 d, showing a depression of FA during this period.

The mean number of ants at the sprayed site increased greatly at 18 d in 2004. The mean number of ants at the

third observations (30 minutes) was not lower than that of the unsprayed site. However, the mean number of ants at the first (10 minutes) and second (20 minutes) observations were significantly lower than that of the unsprayed site. It is likely that ants at the sprayed site respond more slowly to baits even at 18 d after the pesticide spray. Hence, ants at 18 d in 2004 did not seem to be fully recovered at the sprayed site, implying a remaining inhibition of cholinesterase. In addition, the dull response to bait was ascertained by a decrease of ants which were participating in feeding behavior on the 'Depressed' times (0.463±0.297 (SD), n=874 in 'Normal' times, 0.298±0.315, n=168 in 'Depressed' times;

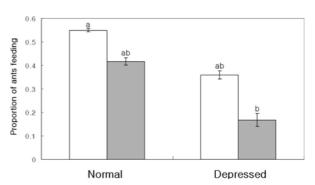


Figure 3. Proportion (arcsine-transformed) of *Paratrechina flavipes* ants feeding on baits among total *P. flavipes* ants on bait cards. The open and dark bars show the means in the presence and the absence of other ant species, respectively. Error bars indicate one SE. The categories 'Normal' and 'Depressed' are explained in text. Different captions above bars mean significant differences of means among the groups.

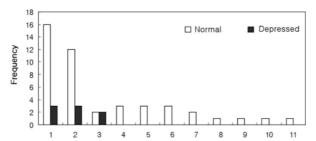


Figure 4. Frequency (number of observed surveys) of ant species except *Paratrechina flavipes* occurring on bait cards. The numbers indicate the following ant species; 1: *Camponotus japonicus*, 2: *Monomorium chinense*, 3: *Pristomyrmex pungens*, 4: *Pheidole fervida*, 5: *Crematogaster* spp., 6: *Tetramorium tsushimae*, 7: *Pachycondyla javana*, 8: *Aphaenogaster japonica*, 9: *Formica japonica*, 10: *Vollenhovia emeryi*, and 11: *Crematogaster osakensis*. The categories 'Normal' and 'Depressed' are explained in text.

Mann Whitney test, U=50548, p<0.001). When ants of other species were present on bait cards, the proportions of *P. flavipes* ants showing feeding behavior also significantly decreased (0.447 $\pm$ 0.309, n=905 in absence of other species, 0.367 $\pm$ 0.277, n=137 in presence of other species; Mann Whitney test, U=52342, p<0.01). The interspecies inhibitions on feeding behavior were shown in both 'Normal' and 'Depressed' times (Figure 3), showing no significant interactive effects of pesticide and competition (two-way ANOVA, F=0.095, df=1, p=0.758). This illustrates that pesticide did not decrease the alertness of ants to other species.

Depression of FA in the 'Depressed' times of *P. flavipes* also occurred in other ant species (Figure 4). In Camponotus japonicus, which were dominant in abundance and behavior, only 11 of 148 ants observed on bait cards at the sprayed site were counted in the 'Depressed' times in 2 h, 10d in 2003, and 18 d in 2004 (Table 1). Similarly, in Monomorium chinense, only 18 of 97 ants attracted to baits at the sprayed site were found during 'Depressed' times in 4 d, 10 d in 2003 and 18 d in 2004. Other species such as Crematogaster osakensis, C. spp., Formica japonica, Pachycondyla javana, Pheidole fervida, Tetramorium tushimae, and Vollenhovia emeryi, were only attracted to baits in the 'Normal' times at the sprayed site and at the unsprayed site. Compared with other species, more Pristomyrmex pungens ants were attracted to baits in 'Depressed' times. At the sprayed site, 14 ants of this species were observed in 'Depressed' times (4d, 10d in 2003), while 44 ants were observed during 'Normal' times (16 d in 2003, 31 d in 2004).

#### Discussion

Despite non-significant impacts of fenitrothion sprayed on abundance and species richness of ant communities, the surviving ants showed depressed behavior as expected. Although a similar recovery process of FA of *P. flavipes* was observed in both 2003 and 2004, the recovery rate and the strength of depression were different between two years. *P. flavipes* was fully recovered at 14 d after pesticide spray in 2003, but only partially recovered at 18 d in 2004. Rainfalls differed between two years. Rainfall in 2003 was 372 mm during the 14 d, while that in 2004 was 289 mm during the 18 d. Rainy days were 13 d in 2003 and 9 d in 2004 during the periods. Foraging activity of ants decrease in rainy days, so contacts of ants with pesticide would decrease. Furthermore, rain removed pesticide sprayed on foliage and litter in the sprayed pine stand (Kim *et al.*, 2005).

P. flavipes, the main species in present study, is abundant in forests in most regions of South Korea, especially in southern part (Kwon, unpublished). This small ant species is monogynous species and is endemic to north-eastern Asia. They live in forest and grassland in small colonies (JAID 2008, Ichnose 1986). Although P. flavipes was most abundant in pitfall traps and on bait cards in present study, the species was not dominant over other species in competition for foods. On a bait card, P. flavipes monopolized bait during the first observation, but they were replaced by workers of Pristomyrmex pungense. The two species attacked each other around baits. When workers of P. flavipes contacted with workers of Componotus japonicus, they were sometimes killed by the big ants. However, P. flavipes frequently attacked C. japonicus, so C. japonicus also avoided the bait occupied by many workers of P. flavipes. Workers of *P. flavipes* were alert even to the presence of smallest ants, Monomorium chinense. Workers of P. flavipes feeding on baits significantly decreased in the presence of the small ants. Thus, all ant species avoided contact with other ant species on bait cards. The well-known hierarchical system of ant communities (Feller 1987, Cerdá et al. 1998, Basu 1997, LeBrun 2005) was not apparent among ant species at the study sites, since dominant and subordinate ant species were not clearly divided. Conspecific attacks were also observed among workers of P. flavipes. The strong tension among ants due to competition for foods may lead to persistence of alertness to other ants under the dullness caused by pesticide.

Other studies using baiting methods also indicated significant impacts of pesticide application on ant fauna. In the study using the baiting technique, ant foraging activity decreased significantly after application of chlorpyrifos, another organophosphate pesticide (Perfecto 1990). The decrease led to depressed predation on pests by ants and the resurgence of pests. However, it is possible that some portion of the depressed predation was caused by the decreased foraging activity of surviving ants rather

than decreased foragers, as indicated by the author. Murphy and Croft (1990) showed that ants attracted to bait boards decreased more after carbaryl application, compared with ants collected at drop traps. They attributed the great decrease to a change in behavior rather than to killed ants. Most of other studies using pitfall traps did not report the significant impacts of pesticides. Chong et al. (2007) showed that ants in vineyards were not influenced by application of chlorpyrifos. They documented that ants should not be considered indicators for assessment of pesticide impacts because worker abundance may be a poor indicator of chemical pressures. The nonsignificant impacts of pesticides on ants were also reported in other studies using pitfall traps (Catangui et al., 1996, Kwon et al., 2005, Matlock and DE RA Cruz 2003, Wang et al., 2000). Thus, conclusions for impacts of pesticides on ants may be dependent on survey methods.

The results of present study uncovered a neglected phenomena, the decreased activity of surviving ants caused by cholinesterase inhibition of pesticides. In the few studies using baiting methods, the number of ants attracted to baits was assumed to represent the number of ants (e.g. Murphy and Croft 1990, Perfecto 1990) but the number of ants attracted to bait does not have a strong relationship to the number of existing ants, as shown in present study. The decrease in behavior activity leads secondarily to a change of arthropod communities in pesticide sprayed forests. It is likely that colonyrelated behaviors, such as nursing and cleaning nests, would be inhibited during the short term pesticide-inhibitory period, which temporarily decreases production of ants. The decreased activity of ants would also lower ant pressure on other arthropods. Activity of other arthropods may also temporarily decrease. The effect of pesticides on activity of surviving arthropods also changes the influences of arthropods on plants, fungi and bacteria. Rapid recovery from the dullness caused by pesticides may be important for the continuous survival of arthropods in pesticide sprayed ecosystems. Hence, the behavior change due to pesticide should be regarded as nontarget impacts of pesticides.

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