Can Leek Interfere With Bean Plant–Bean Fly Interaction? Test of Ecological Pest Management in Mixed Cropping

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J. Econ. Entomol. 102(3): 999–1008 (2009)

ABSTRACT Effects of volatile odors from leek, *Allium porum* L., on the behavior of bean fly, *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae), were tested in laboratory olfactometer bioassays. Aqueous and solvent extracts (dichloromethane and methanol) of leek were repellent to adult flies. Whole leek plants were repellent and prevented attraction to the host plant, beans. Beans that had been exposed to volatiles from living leek plants for 7 d became repellent to the fly. Leek and several other crops were tested in field experiments to identify candidate crops for a mixed cropping system to minimize bean fly attack in beans, *Phaseolus vulgaris* L. In a wet season field experiment, mixed cropping of bean with leek or three other vegetable crops did not significantly reduce bean fly infestation or infection with *Fusarium oxysporum* Schltdl. compared with a mono crop, but significantly reduced plant death caused by both agents combined. In two dry season field experiments, mixed cropping of beans with leek significantly reduced adult bean fly settling, emergence, and death of bean plants compared with a mono crop. Bean yield per row was ~150% higher for the mixed crop, and economic returns were approximately Sri Lankan Rs. 180,000/ha, higher than for the mono crop. For the mono crop, the farmer had a monetary loss, which would become a small profit only if the costs of family labor are excluded. The study is an example of the first steps toward development of sustainable plant protection in a subsistence system.

KEY WORDS olfactometry, *Ophiomyia phaseoli*, intercropping, plant protection, *Allium porum*

Bean fly, *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae), is a stem-boring fly that attacks economically important legume crops including beans, soybeans, snap beans, and cowpea in tropical and subtropical regions of the world. Eggs are laid on the upper side of leaves, and the larva mines toward the lower parts of the stem. Attack is usually more severe during the seedling stage of the crop and generally leads to plant death, whereas older plants are also attacked but tend to tolerate the damage (Talekar 1990). In wet regions or seasons, attack by bean fly may be associated with increased plant infection by the fungal pathogen *Fusarium oxysporum* Schltdl., and the two are sometimes regarded as a single plant mortality agent (Singh and Yadav 2002).

Bean fly control is mostly dependent on pesticides, applied as seed dressing or foliar sprays (Anon 1997). Cultural control methods, such as avoidance of cultivation during high populations, mulching with cut weeds or straw, earthing up soil in ridges around the base of the plants, and crop rotation have also been recommended (Subasinghe and Amarasena 1983). Attempts to control the pest using resistant varieties (Singh et al. 1998) and biological control (Waterhouse 1998, Kudagamage 1998) have only been partially successful. A recent survey of Sri Lankan farmers showed they rely heavily on pesticides for controlling pests, especially in vegetable crops (Glinwood et al. 2008), and favor conventional broad spectrum agents because of their comparatively low price, wide availability, and persuasive marketing. Furthermore, most of the farmers surveyed did not follow safety guidelines, and threats both to human health and to the environment are increasing among the farming community. These hazards, and other problems associated with pesticide use, mean there is an urgent need for ecological pest management.

Mixed-species cropping can increase on-farm productivity, improve soil fertility, and suppresses pests or diseases (Matteson et al. 1984, Vandermeer 1989, Morales 2002, Sharma and Ram 2004), and selection of suitable crop combinations is the main criterion for establishing a viable mixed cropping system. Sound knowledge of the chemical interactions between plants and insects is needed to develop suitable crop combinations that minimize pest infestations. Field studies often give inconsistent results because of variation in biotic factors (Karel 1991, Letourneau 1995); therefore, rapid laboratory techniques based on

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0022-0493/09/0999–1008$04.00/0

chemical ecology are helpful in identifying suitable plant combinations and elucidating the mechanisms involved in pest suppression. Existing knowledge can also be used to target plant groups with proven biological activity. For example, species of the genus *Allium* have shown repellency against a wide range of arthropods including moths (Landolt et al. 1999), cockroaches (Scheffler and Dombrowski 1993), spider mites (Dabrowski and Seredynska 2007), and aphids (Amarawardana et al. 2007). Some of the thiosulfinates present in *Allium* species have shown biological activity against insects (Bayer et al. 1989, Wagner et al. 1990, Sendl et al. 1992). Propane thiosulfinates released by damaged leek were found to be attractive to leek moth, *Acrolepiopsis assectella* (Zeller), which uses leek as a host, but repellent to *Ephestia kuehniella* Zeller, which does not (Rouz and Thibout 1988, Auger et al. 1989). However, when selecting candidate crops for mixed cropping, care must be taken to ensure that the system gives a satisfactory economic return compared with that of the mono crop.

The aim of this study was to test the potential of mixed cropping as an ecological bean fly management method for small scale farmers in Sri Lanka. The approach taken was to investigate the possible interference of nonhost crop plant volatiles on olfactometric orientation of the fly in the laboratory, followed by mixed cropping with promising plants in field trials.

Materials and Methods

Insects and Plants. Bean fly cultures for laboratory experiments were initiated at HORDI, Peradeniya, Sri Lanka, and SLU, Uppsala, Sweden, using pupae collected from infested bean *Phaseolus vulgaris* L. plants at Peradeniya. Cultures on potted bean seedlings (cultivar KWG) were maintained under prevailing environmental conditions in glass houses at HORDI (temperature 26-32°C, 12:12-h L:D natural light) and under controlled conditions in a glasshouse at SLU (temperature 22-25°C, 12:12-h L:D maintained with growth lamps). Plants used in experiments were grown under the same conditions as bean fly cultures but in separate glasshouse chambers.

Plant Extracts. Extracts of leek, *Allium porrum* (variety: large long summer, Holland), were prepared using dichloromethane and methanol as solvents. Material from dried whole plants (120 g) was ground to powder using a mechanical grinder and extracted with dichloromethane (700 ml) for two consecutive 24-h periods in a reciprocal shaker. Solvents in the filtrate were removed in a vacuum rotary evaporator at 40°C to obtain the dichloromethane extract (1.2 g). The residue was re-extracted with methanol (700 ml) using the same procedure as above to obtain the methanol extract (7.5 g).

Exposure of Bean Plants to Volatiles From Neighboring Leek Plants. Exposure of bean plants to volatiles from living, whole leek plants was made using clear Perspex exposure cages (Pettersson et al. 1999, Glinwood et al. 2004), divided into two separate chambers (each 10 by 10 by 40 cm), connected by an opening (7 cm diameter) in the dividing wall. Air entered the forward chamber through an opening in the cage wall (7 cm diameter) and passed over a leek plant before entering the rear chamber containing the bean plant to be exposed, from where it was extracted through a tube attached to a vacuum tank and vented outside the room by an electric fan. Pots were placed in petri dishes to prevent interaction through roots and watered through an automated water drop system, with a single 2-min delivery occurring daily at 0800 hours (2 h into the photophase) that delivered a total of 35 ml to each pot.

In each separate exposure cage, a pot containing a single bean plant was exposed to a single leek plant. Five or six such cages were used, and an equal number of cages with beans but without leek plants were used as controls. Control and treatment cages were placed alternately on a bench in a glasshouse at 18-22°C, with a L-12:D-12 light cycle. Bean plants were exposed to leeks for a period of 7 d and offered as a stimulus in olfactometer bioassays.

Olfactometer Bioassays. Initial olfactometer screening at HORDI (K.A.N.P.B., unpublished data) had indicated that leek, brinjal, *Solanum macrocarpan* L., and kankun, *Ipomoea aquatica* Forsk., plants were repellent to adult bean flies. After considering the availability and economic value of these as crops and the results of initial field tests, leek was selected for further laboratory study

A four-arm olfactometer (Glinwood et al. 2004) was used to test bean fly responses to plant odor sources. It consisted of an enclosed Perspex arena (12 cm diameter) with a central chamber and four side arms. Air was drawn from the center of the olfactometer using a water-driven vacuum pump, establishing discrete air currents in the side arms (air flow was 180 ml/min). The odor source was introduced to one of the side arms as a leaf in a glass tube, chemical in a microcapillary tube held inside a 5-ml plastic syringe, or a potted plant(s) in a Perspex exposure cage (described above), connected to one side arm of the olfactometer with Teflon tubing; the remaining three arms serving as controls. All olfactometer arms contained moist filter paper to compensate for differences in humidity.

Dichloromethane and methanol extracts of leek were introduced in a microcapillary tube as 2 μl of 50,000 ppm in acetone and aqueous extract of leek as 2 μl of the extract (10 g/5 ml water). Whole, live bean plants were sprayed with a 50,000-ppm solution of dichloromethane extract of leek (1 g extract dissolved in 2 ml acetone, 0.5 ml Teepol, made up to 50 ml with water) until run off using an atomizer, and allowed to dry before placed into a Perspex exposure cage. Control plants were sprayed with a similar amount of the above solution, lacking leek extract.

Experiments were carried out between 0900 and 1300 hours in a laboratory at a temperature of 20 ± 2°C, with a 40-W incandescent bulb 50 cm above the olfactometers. One bean fly adult was introduced into the olfactometer, and its position was recorded every 3 min for 30 min. If an insect did not move between

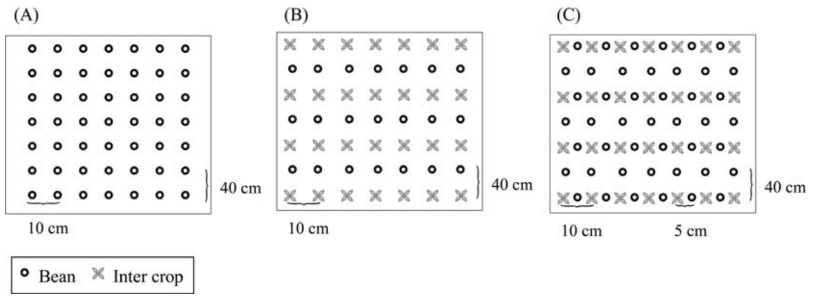

Fig. 1. Layout of plots in field experiments with mixed cropping of beans and other crops (not to scale): (A) bean mono crop in experiments 1, 2, and 3, (B) mixed cropping of beans and other crops in field experiment 1, and (C) mixed cropping of beans and leek in field experiments 2 and 3 (beans within mixed crop rows were removed before harvest). Plot sizes were 4 by 3 m in experiment 1 and 4 by 5 m in experiments 2 and 3.

three consecutive observations (was motionless), the replicate was discarded and a new one started with a fresh insect. The observation frequency method has been used to show attraction and repellence in a variety of insects (Glinwood and Pettersson 2000, Glinwood et al. 2003, Ninkovic and Pettersson 2003) and gives a reliable measure irrespective of whether the behavior is characterized by frequent short visits or few long visits in the olfactometer arm. Before experiments, preliminary studies showed that the movement and behavior of *O. phaseoli* made it suitable for the use of this method.

Bean flies used in the experiments were one-day old adults, which were isolated from the host plant 30 min before use. When both sexes were tested, equal numbers of males and females were used in all experiments. Each experiment was replicated 15-32 times using a fresh adult fly for each replicate. The number of visits to the odor-treated arm was compared with the mean of control arms using a Wilcoxon matched pairs test in the Statistica statistical package (StatSoft, Tulsa, OK).

For tests of combined volatiles, bean plants were contained in separate cages to leek plants, and the Teflon air intake lines to the olfactometer combined using a T-connector before connection to the olfactometer. To obtain oviposited/feeding damaged bean plants, potted beans were exposed to one pair (male and female) of bean flies for 24 h. Oviposition/feeding punctures were noted on the exposed plants before use.

Field Experiments. Based on preliminary olfactometer tests (not reported), three crop plants that showed repellent effects to bean fly (leek, brinjal, and kankun) and one that showed no effect (red onion, *Allium cepa* L.) were selected for field trials of mixed cropping with beans (wet season field experiment 1; November 2003 to March 2004). Based on the results, the most promising intercrop, leek, was subsequently tested in two further dry season field experiments (field experiments 2 and 3: May–September 2005 and 2007, respectively).

Field Experiment 1. The experiment was carried out at HORDI, Sri Lanka (80°36′ E and 7°16′ N) from November 2003 to March 2004, which is a wet season in this region. The experiment was arranged in a randomized complete block design (RCBD) with six replicates. Crops were established in rows within 4 by 3-m plots (Fig. 1A and B), separated by unplanted 0.5-m borders. In mixed cropping plots, the planting dates of different crops were adjusted to give sufficient foliage at the time of bean germination. All crops were sown at the standard spacing recommended by the Sri Lankan Department of Agriculture (DOA). Row spacing in bean mono crop and mixed plots of bean-kankun, bean-leek, and bean-red onion were 40 cm, with 10 cm between plants. In bean-brinjal, the row spacing was 60 cm, with 60 cm between brinjal plants (spacing between bean plants was still 10 cm). All other agronomic practices were according to DOA recommendations. There were thus five treatments: (1) mono cropped beans (variety: Top crop), (2) beans mixed with kankun (variety: Thai), (3) beans with brinjal (variety: Padagoda), (4) beans with leek (variety: Large long summer), and (5) beans with red onion (variety: Vaedalum).

The number of adult bean flies settled on all bean plants in each plot was recorded at weekly intervals, starting 5 d after planting bean seeds. Observations were made on the same day each week between 0700 and 0900 hours, because it has been shown that adult settling and oviposition peak during early morning (Talekar 1990). Adult bean flies were observed on the upper surfaces of leaves and, because they tended to walk rather than fly if disturbed, could be accurately counted by a careful observer. Dead bean plants were uprooted on two occasions (1 wk apart) and dissected for bean fly infestation and *Fusarium oxysporum* infection. Bean fly infestation was scored when pupae were found beneath the epidermis of the lower part of the dead stems. *Fusarium* infection was scored when decaying of the epidermis, turning it dark brown, was observed. Samples of infested stems were incubated to confirm fungal identity from the spores formed. Stems with bean fly pupae were kept separately until the emergence of adults, and the number of adults emerging was recorded. To account for differences in the total number of bean plants in mono crop and mixed crop plots, experimental observations were converted to value per row of bean plants.

Table 1. Mean (± SEM) no. of visits made by bean fly adults to odors of extracts, live plants, bean plants exposed to leek volatiles, or bean plants fed/oviposited by the fly in olfactometer bioassays

Experiment	Treatment mean	Control mean	n	Z^a	P^a
Bean leaves versus blank	6.59 (3.65)	1.15 (1.92)	32	4.1	0.007
Leek (CH_2Cl_2) ext. versus blank (♂)	0.40 (1.05)	2.66 (0.93)	15	2.9	<0.0001
Leek (CH_2Cl_2) ext. versus blank (♀)	0.24 (0.43)	2.43 (0.78)	25	4.2	0.0009
Leek (MeOH) ext. versus blank (♀)	0.37 (0.49)	2.75 (0.46)	24	4.2	<0.0001
Leek aqueous extract versus blank	0.88 (1.32)	2.27 (0.64)	18	2.7	0.01
Bean sprayed with leek versus bean	0.37 (0.65)	7.68 (2.45)	32	4.8	<0.0001
Bean plant versus blank	5.43 (1.79)	1.81 (1.30)	32	4.7	<0.0001
Leek plant versus blank	1.31 (0.27)	4.32 (0.54)	16	3.4	0.0007
Leek plant versus bean plant	1.00 (0.22)	6.94 (0.49)	16	3.5	0.0004
Leek + bean plant versus bean plant	0.62 (0.22)	5.69 (0.60)	16	3.4	0.0005
Exp. bean plant versus bean plant	0.75 (0.19)	6.81 (0.34)	16	3.5	0.0004
Oviposited bean plant versus bean (♀)	1.46 (1.58)	5.03 (2.93)	32	4.1	<0.0001

Equal numbers of male and female flies were tested, except where stated in parentheses.
[a] Wilcoxon matched pairs test.
ext, extract; Exp, exposed (to volatiles from leek).

Field Experiment 2. The experiment was carried out at HORDI, Sri Lanka, ≈500 m away from the location of field experiment 1, from May to September 2005, which is a dry season in this region. The experiment was arranged in a RCBD with 10 replicates. Crops were established in rows within 4.5 by 5.0-m plots (Fig. 1A and C), separated by unplanted 0.5-m borders. Spacing between bean and leek rows was 40 cm, with 10 cm between plants. However, in this trial, a modification was made to examine the effect of bean-leek distance on the protective value of leek. As such, in the mixed crop plots, bean plants were grown within the rows of leek, 5 cm away from neighboring leek plants (Fig. 1C). These bean plants, however, were manually removed after the experimental observations had been made (1 mo after sowing) and before harvesting and are not included in the yield. There were thus three treatments: (1) beans in mono crop; (2) mixed crop—beans 40 cm from leek; and (3) mixed crop—beans 5 cm from leek.

The number of bean flies settled, pupae in bean plants, and *Fusarium* infestation (although *Fusarium* was not detected in this field) were determined as described above for experiment 1. In addition to the number of bean flies, the number of parasitoids that emerged was recorded. Apart from bean fly, no other pupae were found inhabiting the stems; therefore, the two unidentified species of hymenoptera and one unidentified dipteran that emerged were assumed to be parasitic on bean fly. As in experiment 1, observations were converted to value per row of bean plants.

After harvest, yields data were recorded, and an economic analysis (Trumble et al. 1994) for each experimental treatment was carried out to estimate the relative economic return of the cropping systems to farmers based on wholesale market prices in Sri Lanka (beans: Sri Lankan rupees [Rs.] 38.30/kg as of June 2005, leeks: Rs. 37.65/kg as of August 2005) when the respective harvests were made (Hector Kobbekaduwa Agrarian Research and Training Institute, Colombo 7, Sri Lanka).

Field Experiment 3. The experiment was carried out at HORDI, Sri Lanka, at the same location as field experiment 2 during the same period in 2007 using the same methods as for field experiment 2, except that 12 replicates were performed instead of 10. The calculations for economic return (Trumble et al. 1994) were based on average wholesale market prices for beans in June 2007 (Rs. 77.00/kg) and leeks in August 2007 (Rs. 38.50) in Kandy district, Sri Lanka (Hector Kobbekaduwa Agrarian Research and Training Institute, Colombo 7, Sri Lanka).

Statistical Analyses of Field Experiments. Data were not normally distributed; however, after transformation by $\log n + 1$ (where n is the data value), they were deemed acceptable for analysis by analysis of variance (ANOVA). Repeated-measures ANOVA was used to compare measurements of adult settling made repeatedly at weekly intervals in the same plots during the experimental period, followed by one-way ANOVA analysis of adult settlement on each weekly sample occasion (Ninkovic et al. 2002). Overall, mean dead plants, adults emerged, and natural enemies were analyzed by one-way ANOVA. In cases where ANOVA returned a significant value of P (<0.05), a Tukey test was applied. Bean yields from bean mono cropping and mixed cropping were compared using one-way ANOVA. All statistical analyses were performed using the Statistica statistical package (StatSoft 2005).

Results

Olfactometer Bioassays. Olfactory responses of adult bean flies to the odors of plants and plant extracts are presented in Table 1. Solvent and aqueous extracts of leek were repellent, and odor of bean plant sprayed with extract of leek was less preferred than odor of untreated bean. Odor of bean plant was attractive to bean flies, whereas the odor of leek was repellent. Combined odor of bean and leek was less preferred compared with the odor of bean plant alone. Odor of bean plant that had been exposed to volatiles from leek for 7 d was significantly less preferred than unexposed bean. Odor of beans that had been previously ovipos-

Table 2. Infestation levels (means ± SEM) of bean fly and *F. oxysporum* on beans in mono cropping and mixed-cropping with kankun, leek, brinjal, and red onion in field experiment 1

Cropping system	Adult settlement (adults/row)	Dead plants infested with pupae/ row	Adults emerged/row	Dead plants infected with *Fusarium*/row
Bean mono	3.32 (0.35)	25.46 (2.86)	92.68 (39.7)	25.47 (3.01)
Bean-leek	3.70 (0.37)	13.73 (2.21)	31.05 (4.65)	19.78 (2.75)
Bean-kankun	4.10 (0.44)	20.55 (2.84)	33.55 (8.25)	22.02 (1.95)
Bean-brinjal	3.17 (0.42)	16.67 (3.50)	39.10 (10.8)	21.00 (2.22)
Bean-red onion	4.88 (0.39)	25.45 (4.37)	45.67 (13.4)	23.61 (4.81)
ANOVA *F*	1.69	2.28	1.56	0.42
ANOVA *P*	0.19	0.096	0.22	0.79

ited/fed by bean fly was significantly less preferred than odor of unexposed bean.

Field Experiment 1. Repeated-measures ANOVA on mean adult settlement per row during the experiment showed no significant differences between treatments (ANOVA, $F = 1.73$; df = 4,25; $P = 0.18$), significant differences between sample occasion (ANOVA, $F = 199$; df = 3,75; $P < 0.0001$), but no significant treatment × sample occasion interaction (ANOVA, $F = 1.0$; df = 12,75; $P = 0.42$). Adult settlement on each weekly sampling occasion was analyzed, and there were no significant differences between treatments in week 1 (ANOVA, $F = 2.1$; df = 4,20; $P = 0.12$), week 2 (ANOVA, $F = 1.7$; df = 4,20; $P = 0.19$), and week 3 (ANOVA, $F = 0.5$; df = 4,20; $P = 0.74$). There were significant differences in week 4 (ANOVA, $F = 3.2$; df = 4,20; $P = 0.03$); however, a Tukey test showed marginal significance in the difference between bean mono crop and bean-red onion ($P = 0.05$) and marginal nonsignificance between bean-kankun and bean-red onion ($P = 0.08$).

There were no significant differences between treatments in terms of number of adults emerging or number of dead plants with *Fusarium* (Table 2). The number of dead plants infested with bean fly was lower in bean-leek plots compared with the mono crop, but the difference was not significant ($P = 0.096$). There were significant differences between treatments in the total number of dead plants per row caused by bean fly and/or *Fusarium* (bean wilt complex; (Fig. 2; ANOVA, $F = 3.8$; df = 4,20; $P = 0.01$). The number of dead plants was significantly lower in bean-leek plots than in the mono crop (Tukey test, $P = 0.01$).

Field Experiment 2. Repeated-measures ANOVA on mean adult settlement per row during the experiment showed significant differences between treatments (ANOVA, $F = 726$; df = 2,27; $P < 0.0001$) and sample occasion (ANOVA, $F = 199$; df = 4,108; $P < 0.0001$), with a significant treatment × sample occasion interaction (ANOVA, $F = 31.7$; df = 8,108; $P < 0.0001$). Settlement in bean-leek 5 cm was significantly lower than either bean-leek 40 cm or

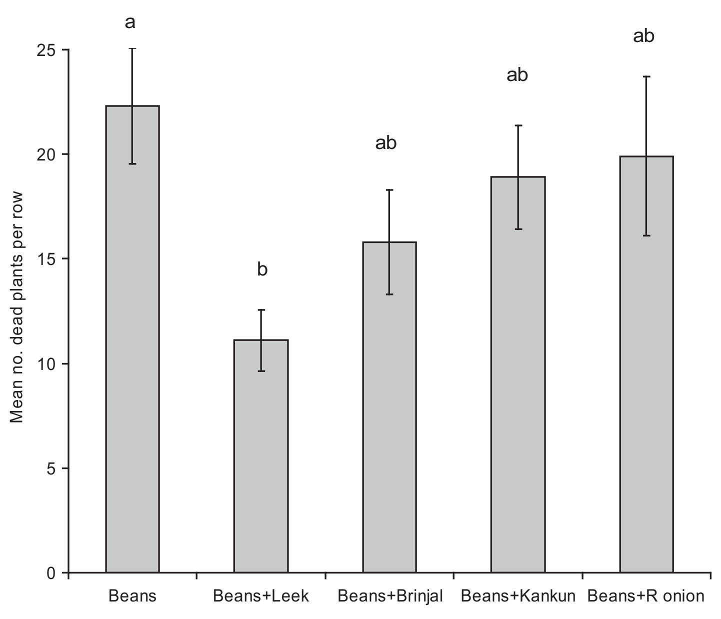

Fig. 2. Mean (±SEM) number of dead bean plants per row caused by either bean fly, *Ophiomyia phaseoli,* and/or wilt, *Fusarium oxysporum* (wilt complex), in plots with bean mono crop or mixed cropping in field experiment 1. Bars followed by different letters are significantly different (Tukey test).

Table 3. Infestation levels (means ± SEM) of bean fly and emergence of bean fly natural enemies in beans in mono cropping and mixed-cropping with leek (leek plants either 5 or 40 cm away from beans) in field experiments 2 and 3

Cropping system	Adult settlement adults/row	Dead plants infected with pupae/row	Adults emerged/row	Natural enemies emerged/row
Field trial 2				
Beans	19.2 (1.7) a	4.23 (0.6) a	4.33 (0.8) a	0.22 (0.5) a
Bean-leek 40 cm	15.5 (1.1) a	1.13 (0.2) b	1.11 (0.3) b	0.03 (0.02) b
Bean-leek 5 cm	0.81 (0.2) b	1.21 (0.1) b	1.24 (0.2) b	0.27 (0.1) a
ANOVA *F*	726	24.14	12.64	3.35
ANOVA *P*	<0.0001	<0.0001	<0.0001	0.046
Field trial 3				
Beans	1.56 (0.09) a	13.5 (1.4) a	12.3 (2.1) a	1.13 (0.35) a
Bean-leek 40 cm	1.16 (0.04) b	7.8 (0.71) b	6.5 (1.2) ab	0.45 (0.19) ab
Bean-leek 5 cm	0.34 (0.04) c	7.5 (0.53) b	5.0 (0.7) b	0.20 (0.07) b
ANOVA *F*	171	11.1	5.94	3.72
ANOVA *P*	<0.0001	0.0002	0.006	0.03

Means followed by different letters in each column are significantly different (Tukey test).

bean mono crop, which were not significantly different from each other (Tukey test; Table 3). Adult settlement on each sampling occasion and the results of Tukey tests are shown in Fig. 3. There were significant differences between treatments in week 1 (ANOVA, $F = 45.2$; df = 2,27; $P < 0.0001$), week 2 (ANOVA, $F = 125$;

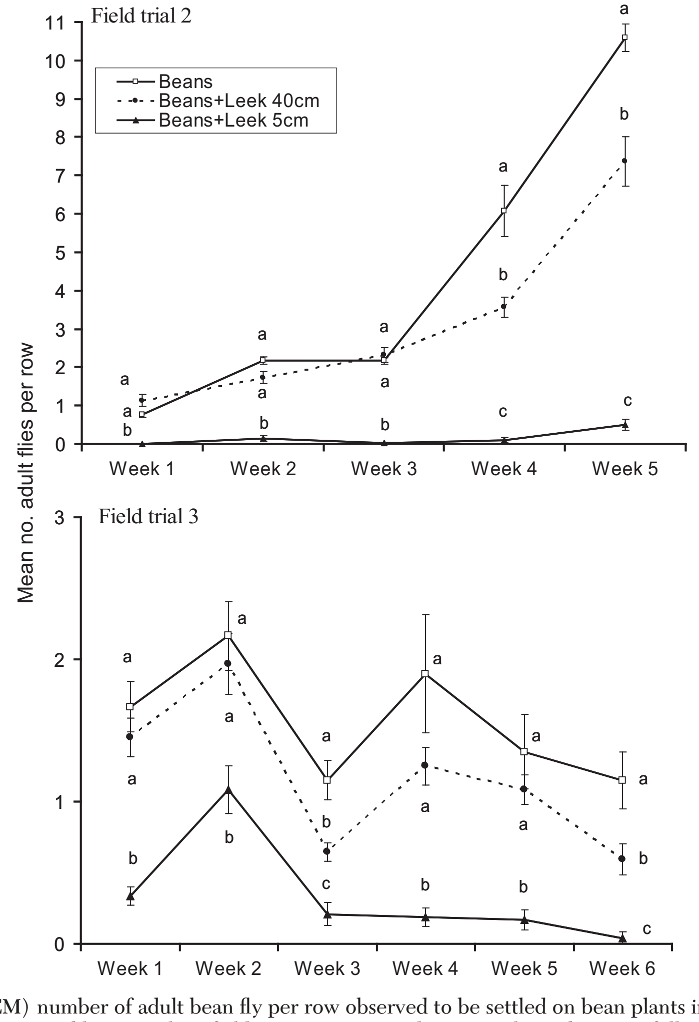

Fig. 3. Mean (±SEM) number of adult bean fly per row observed to be settled on bean plants in plots with bean mono crop or mixed cropping at weekly intervals in field experiments 2 and 3. On each week, points followed different letters are significantly different (Tukey test).

Table 4. Yields (mean ± SEM) and economic returns from bean mono cropping and bean-leek mixed cropping

Cropping system	Yield (kg/plot)	Bean yield (kg/row)	Income (Rs/ha)
Field trial 2			
Mono crop	4.1 (0.2)	0.58 (0.05) a	69706
Mixed crop—beans	2.7 (0.1)	0.90 (0.03) b	45922
Mixed crop—leek	14.1 (2.1)		235689
Mixed crop—total	16.8 (1.5)		281611
Field trial 3			
Mono crop	1.5 (0.2)	0.21 (0.02) a	51282
Mixed crop—beans	0.9 (0.1)	0.32 (0.02) b	30800
Mixed crop—leek	15.2 (0.8)		259836
Mixed crop—total	16.1 (0.8)		290636

Economic returns are calculated based on yields and average market prices during 2005 (field trial 2) and in the month of harvest in 2007 (field trial 3).

Means followed by different letters are significantly different (ANOVA). Means followed by no letter were not analyzed.

Rs, Sri Lankan rupees.

df = 2,27; $P < 0.0001$), week 3 (ANOVA, $F = 293$; df = 2,27; $P < 0.001$), week 4 (ANOVA, $F = 196$; df = 2,27; $P <$ 0.0001), and week 5 (ANOVA, $F = 260$; df = 2,27; $P <$ 0.0001). Settlement was always significantly lower in bean-leek 5 cm than in the other treatments.

There were significant differences between treatments in the number of dead bean plants infested with bean fly pupae per row (ANOVA, $F = 24.1$; df = 2,27; $P < 0.0001$); the number of dead plants was significantly higher in bean mono crop than in the other treatments (Tukey test; Table 3). There were significant differences between treatments in the number of adult bean flies emerged per row (ANOVA, $F = 12.6$; df = 2,27; $P = 0.0001$); the number of adults emerged was significantly higher in bean mono crop, than in the other treatments (Tukey test; Table 3). There were significant differences between treatments in the number of natural enemies emerged per row (ANOVA, $F = 3.5$; df = 2,27; $P = 0.04$); the number of natural enemies was significantly lower in bean-leek 40 cm than in the other two treatments (Tukey test; Table 3).

Mean yield of beans per row (calculated by dividing the total bean yield by the number of rows of beans present) was significantly higher in bean-leek plots (ANOVA, $F = 30.4$; df = 1,18; $P < 0.0001$; Table 4). The economic return (Sri Lankan rupees per hectare; Table 4) was almost three times greater from bean-leek than from the mono crop because of the high market value of leeks.

Field Experiment 3. Repeated-measures ANOVA on mean adult settlement per row during the experiment showed significant differences between treatments (ANOVA, $F = 171$; df = 2,33; $P < 0.0001$) and sample occasion (ANOVA, $F = 22.1$; df = 5,165; $P <$ 0.0001), with no significant treatment × sample occasion interaction (ANOVA, $F = 1.4$; df = 10,165; $P =$ 0.21). Settlement in bean-leek 40 cm was significantly lower than bean mono crop, and settlement in bean-leek 5 cm was significantly lower still (Tukey test; Table 3). Adult settlement on each sampling occasion and the results of Tukey tests are shown in Fig. 3. There were significant differences between treatments in week 1 (ANOVA, $F = 40.7$; df = 2,33; $P < 0.0001$), week 2 (ANOVA, $F = 9.2$; df = 2,33; $P = 0.0006$), week 3 (ANOVA, $F = 25.5$; df = 2,33; $P <$ 0.0001), week 4 (ANOVA, $F = 19.4$; df = 2,33; $P <$ 0.0001), week 5 (ANOVA, $F = 23.3$; df = 2,33; $P < 0.0001$), and week 6 (ANOVA, $F = 30.8$; df = 2,33; $P < 0.0001$) Settlement was always significantly lower in bean-leek 5 cm than in the other treatments.

There were significant differences between treatments in the number of dead bean plants infested with bean fly pupae per row (ANOVA, $F = 11.1$; df = 2,33; $P = 0.0002$); the number of dead plants was significantly higher in bean mono crop than in the other treatments (Tukey test; Table 3). There were significant differences between treatments in the number of adult bean flies emerged per row (ANOVA, $F = 5.90$; df = 2,33; $P = 0.006$); the number of adults emerged was significantly higher in bean mono crop than in the other treatments (Tukey test; Table 3). There were significant differences between treatments in the number of natural enemies emerged per row (ANOVA, $F = 3.72$; df = 2,33; $P = 0.03$); the number of natural enemies was significantly lower in bean-leek 40 cm than in bean-leek 5 cm (Tukey test; Table 3).

Mean yield of beans per row was significantly higher in bean-leek plots (ANOVA, $F = 5.39$; df = 1,22; $P =$ 0.029; Table 4). The economic return (Sri Lankan rupees per hectare; Table 4) was higher in the mixed crop in both field trials compared with the mono crop. The lower yield of bean in field trial 3 was caused by the prevalence of bean yellowing syndrome that affected bean yields in the Kandy region and contributed to higher prices for beans at that time. Affected bean plants show profuse flowering but little pod formation. Unlike the field experiment, which was conducted without insecticides, farmers would be able to reduce the impact of the syndrome, suspected to be caused by insect toxins, by the use of insecticides.

Discussion

Volatiles from leek can interfere with bean plant–bean fly interaction, and leeks can protect beans from the pest when grown in mixed cropping. Olfactometry suggests that the interference mechanism may be related to repellence of the fly by nonhost volatiles, and field data show that the presence of leeks reduces adult settlement and thereby infestation. An economic analysis showed that the bean-leek cropping system also allows the farmer to increase both bean yields and the profitability of the land. Solvent extracts of leek repelled bean fly and appeared to disrupt olfactory orientation when sprayed on the host plant. Aqueous extracts of leek were also repellent to the fly. It may be possible for farmers to prepare crude, aqueous extracts of these plants for use as a botanical repellent/antifeedant; however, this requires further testing in the laboratory and field, especially with regard to its economic viability.

Volatiles from leek were repellent to flies and inhibited attraction to bean odor. Electrophysiological recordings from Agromyzid antennae have shown that flies can detect a range of volatile compounds associated with beans but also nonhost plants (Zhao and Kang 2003). Repellency of bean plants exposed to leek volatiles for several days can be attributed to two possible mechanisms. First, leek volatiles may adhere to the surface of bean plants, and be subsequently released into the atmosphere, interfering with host location by the fly. A second explanation is that the bean plants changed their chemistry or physiology in response to exposure to leek volatiles. It has been shown that plants can become less acceptable to herbivores after exposure to undamaged neighbors (Ninkovic et al. 2002, Glinwood et al. 2004). Either of these mechanisms, along with direct repellency and odor masking, would contribute to the beneficial effects of mixed cropping in the field.

However, there are other factors to consider in the natural environment. Apart from direct competition, beans and leeks may interact through chemicals, and this may occur both above and below ground. Laboratory experiments are needed to assess to what extent these two plants can affect each other through exchange of chemical substances. Furthermore, the protective effects of leeks in the field may be wholly or partly caused by disruption of visual cues or crop architecture (Finch and Collier 2000). Thus, although there is no direct evidence for volatile repellence or interference in the field, this study shows the value of laboratory olfactory screening for selection of intercrops.

Bean fly and *Fusarium* commonly co-occur in tropical and subtropical regions and are regarded as a single plant mortality agent, described as wilt complex (Singh and Yadav 2002). Herbivore damage has been shown to facilitate entry of fungal pathogens into their host plants (Gatch and Munkvold 2002), and herbivores may also vector the pathogen (Sobek and Munkvold 1999). In the case of bean fly, damage at the base of the stem caused by larvae may provide an entry point for *Fusarium.* In this study, mixed cropping of bean with leek appeared to significantly reduce the overall number of dead bean plants caused by the wilt complex.

A beneficial effect of leeks was more clearly supported by the two subsequent field experiments, which gave comparable results. Although adult settling appeared to be more greatly reduced when beans were 5 cm from leeks, mixed cropping reduced the incidence of dead bean plants and the emergence of adult flies irrespective of planting distance. Therefore, it may not be necessary to grow beans and leeks closer together to achieve repellence. In fact it may be detrimental, because experience has shown that when the two plants are planted at distances of around 5 cm apart, growth and yield of bean can be retarded (K.A.N.P.B., unpublished data). These results suggest that mixed cropping with leek protected beans from attack by bean fly. Intercropping with maize has been found to reduce infestation of *Ophiomyia phaseoli* in beans; counts of bean fly larvae and pupae were significantly lower in mixed stands than in pure stands, and increasing densities of plants in general also led to reduced fly incidence (Karel 1991).

Mixed cropping with leeks was more effective in field experiments 2 and 3 than in experiment 1. One reason may be the differences in climatic conditions. Experiment 1 took place during the regular wet season in this region of Sri Lanka, whereas experiments 2 and 3 took place during the dry season. The damp climate during experiment 1 favored *Fusarium*, which itself may have influenced bean–bean fly interactions more strongly than that the presence of leeks. Climatic conditions may directly influence the fly itself, affecting its host location and acceptance behavior. Thus, it is possible that the mixed cropping strategy may be effective only during the dry season. Fly populations varied in magnitude between trials, but this is usual for the Kandy region where numbers vary from season to season depending on weather patterns and the availability of hosts as determined by farmer cropping patterns.

There was evidence of reduction in the incidence of natural enemies in bean-leek plots. This may be an indication of a negative impact of mixed cropping on the natural enemies of the target pest. Mixed cropping can have unpredictable effects on herbivore natural enemies; it can provide alternative host plants and refuge that helps to sustain their populations but may also interfere with host habitat location by specialized natural enemies (Andow 1991, Landis et al. 2000). Further study is needed to ascertain whether bean-leek cropping has a negative impact on other insects in the system; however, these results show the importance of considering trophic level effects in mixed-cropping studies.

An economic analysis of the potential benefits of the bean-leek system showed that, although the income from the sale of beans alone is reduced in mixed cropping plots, the overall income is much greater than from mono cropping, because of the higher yield of leeks. In the Sri Lankan upcountry (the central region of the country >1,000 m in elevation), farmers already intercrop leeks with other vegetables. Although the results suggest that it may be economically advantageous for the farmer to grow leeks as a mono crop rather than the mixed crop, demand for leeks is limited because they are considered a high value niche product. The intercropping system would allow the farmer to gain an extra profit from this crop while simultaneously allowing more secure production of beans, an important component of the diet. Importantly, protecting beans in this way may reduce the farmers' reliance on insecticides, which will be beneficial for their economy and their health.

The cost of cultivation for the respective seasons was determined using data published by the Sri Lankan Department of Agriculture (Anon 2006, 2008). The cost of production for the mixed crop was between 11 and 13% more than that of the mono crop. This reflects the additional costs of maintaining the nursery and of transplanting leeks 2 wk before sowing beans so that the repellent effect is active during the most susceptible growth stage of bean and of maintaining the plot for a further 2 mo after bean harvest until leeks are harvested.

The economic analysis showed that the farmer makes a loss when growing bean as a mono crop, although a notional profit is made because the cost of family labor is overlooked in the mainly small-scale vegetable cultivation in Sri Lanka. The value of family labor was at Sri Lankan Rs. 39,100 and 41,202 per hectare for the mono crop and the mixed crop, respectively, in 2005. The figures for 2007 are, respectively, Sri Lankan Rs. 55,522 and 58,507 per hectare. If these costs are excluded, the farmer would have made a small profit per hectare of Rs. 20,270 on the mono crop during 2005. This became a marginal profit of Rs. 677 in 2007 largely because of the low yields caused by the prevalence of bean yellowing syndrome during that season.

Mixed cropping has potential as an ecological control method for bean fly. The combination of plants is economically viable because both crops give good monetary returns to the user, fulfilling an important requirement for the success of the mixed cropping approach. Although further study of the optimal species ratios, planting conditions, and effects of climate are necessary before the system could be recommended to growers, this study showed a first step for attaining sustainable pest control in a subsistence farming system.

Acknowledgments

We thank D. Bandara and K. Weerasuriya for assistance. This work was supported financially by the VR-Sida Asian Links Program, Sweden. R.G. and V.N. were supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) and Mistra through the PlantComMistra program.

References Cited

Amarawardana, L., P. Bandara, V. Kumar, J. Pettersson, V. Ninkovic, and R. Glinwood. 2007. Olfactory response of *Myzus persicae* (Homoptera: Aphididae) to volatiles from leek and chives -potential for intercropping with sweet pepper. Acta Agric. Scand. B 57: 87-91.

Andow, D. A. 1991. Vegetational diversity and arthropod population response. Annu. Rev. Entomol. 36: 561-586.

Anon. 1997. Pesticide recommendations. Department of Agriculture, Peradeniya, Sri Lanka.

Anon. 2006. Cost of cultivation of agricultural crops 2005 yala. Department of Agriculture, Peradeniya, Sri Lanka.

Anon. 2008. Cost of cultivation of agricultural crops 2007 yala. Department of Agriculture, Peradeniya, Sri Lanka.

Auger, J., C. Lecomte, and E. Thibout. 1989. Leek odor analysis by gas chromatography and identification of the most active substance for the leek moth, *Acrolepiopsis assectella.* J. Chem. Ecol. 15: 1847-1854.

Bayer, T., W. Breu, O. Seligmann, V. Wray, and H. Wagner. 1989. Biologically active thiosulphinates and α-sulphinyldisulfides from *Allium cepa.* Phytochemistry 28: 2373-2377.

Dabrowski, Z. T., and U. Seredynska. 2007. Characterisation of the two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) response to aqueous extracts from selected plant species. J. Plant Protect. Res. 47: 113-124.

Finch, S., and R. H. Collier. 2000. Host plant selection by insects—a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomol. Exp. Appl. 96: 91-102.

Gatch, E. W., and G. P. Munkvold. 2002. Fungal species composition in maize stalks in relation to European corn borer injury and transgenic insect protection. Plant Dis. 86: 1156-1162.

Glinwood, R. T., and J. Pettersson. 2000. Movement by mating females of a host alternating aphid: a response to leaf fall. Oikos 90: 43-49.

Glinwood, R. T., J. Pettersson, S. Kularatne, E. Ahmed, and V. Kumar. 2003. Female European tarnished plant bugs, *Lygus rugulipennis* (Heteroptera: Miridae), are attracted to odours from conspecific females. Acta Agric. Scand. A Anim. Sci. 53: 29-32.

Glinwood, R., V. Ninkovic, J. Pettersson, and E. Ahmed. 2004. Barley exposed to aerial allelopathy from thistles (*Cirsium* spp.) becomes less acceptable to aphids. Ecol. Entomol. 29: 188-195.

Glinwood, R., M. Birkett, N. S. Kumar, P. Bandara, V. Ninkovic, and J. Pettersson. 2008. Sustainable plant protection for increased food security in a changing climate. Currents (Uppsala) 44-45: 29-33.

Karel, A. K. 1991. Effect of plant populations and intercropping on the population patterns of bean flies on common beans. Environ. Entomol. 20: 354-357.

Kudagamage, C. 1998. Present status of horticultural research in Sri Lanka. *In* World Conference on Horticultural Research (WCHR), 17-20 June 1998, Rome, Italy.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45: 175-201.

Landolt, P. J., R. W. Hofstetter, and L. L. Biddick. 1999. Plant essential oils as arrestants and repellents for neonate larvae of the codling moth (Lepidoptera: Tortricidae). Environ. Entomol. 28: 954-960.

Letourneau, D. K. 1995. Associational susceptibility effects of cropping pattern and fertilizer on Malawian bean fly levels. Ecol. Applic. Ecol. Soc. Am. 5: 823-829.

Matteson, P. C., M. A. Altieri, and W. C. Gagné. 1984. Modification of small farmer practices for better pest management. Annu. Rev. Entomol. 29: 382-402.

Morales, H. 2002. Pest management in traditional tropical agroecosystems: lessons for pest prevention research and extension. Integrat. Pest Manag. Rev. 7: 145-167.

Ninkovic, V., and J. Pettersson. 2003. Searching behaviour of sevenspotted ladybird, *Coccinella septempunctata*—effects of plant-plant odour interaction. Oikos 100: 65-70.

Ninkovic, V., U. Olsson, and J. Pettersson. 2002. Mixing barley cultivars affects aphid host plant acceptance in field experiments. Entomol. Exp. Appl. 102: 177-182.

Pettersson, J., V. Ninkovic, and E. Ahmed. 1999. Volatiles from different barley cultivars affect aphid acceptance of neighbouring plants. Acta Agric. Scand. Sect. B 49: 152-157.

Rouz, H., and E. Thibout. 1988. Analyse on olfactometer de l'attraction des larves d'*Acrolepiopsis assectella* par des substances allelochimiques. Entomol. Exp. Appl. 47: 231-237.

Scheffler, I., and M. Dombrowski. 1993. Behavioural responses of *Blattella germanica* L. (Orthopt., Blattellidae) induced by repellent plant extracts. J. Appl. Entomol. 115: 499-505.

Sendl, A., M. Schliack, R. Löser, F. Stanislaus, and H. Wagner. 1992. Inhibition of cholesterol synthesis in vitro by extracts and isolated compounds prepared from garlic and wild garlic. Atherosclerosis 94: 79-95.

Sharma, S. S., and P. Ram. 2004. Methods to study effects of cropping systems on insect pests, pp. 118-147. *In* S. S. Narwal, R. Singh, and R. K. Walia. Research methods in allelopathy. Scientific Publishers, Jodhpur, India.

Singh, J., J. S. Bhalla, K. S. Brar, T. S. Dhillon, M. Singh, and J. Singh. 1998. Evaluation of some pea varieties against stem fly, *O. phaseoli.* J. Insect Sci. 11: 58-59.

Singh, P. P., and R. P. Yadav. 2002. Effect of intercrops on the plant mortality caused by stem fly, *Ophiomyia phaseoli* Tryon and wilt complex (*Fusarium* spp.). J. Appl. Zool. Res. 13: 208-209.

Sobek, E. A., and G. P. Munkvold. 1999. European corn borer (Lepidoptera: Pyralidae) as vectors of *Fusarium moniliforme,* causing kernel rot and symptomless infection of maize kernels. J. Econ. Entomol. 92: 503-509.

StatSoft. (2005). STATISTICA (data analysis software system), version 7.1. (www.statsoft.com).

Subasinghe, S.M.C., and J. Amarasena. 1983. Seasonality and control of the bean fly, *Ophiomyia phaseoli* (Tryon) (Dip; Agromysidae). Trop. Agric. 139: 75-84.

Talekar, N. S. 1990. Agromyzid flies of food legumes in the tropics, AVRDC, Taiwan. Wiley Eastern, New Delhi, India.

Trumble, J. T., W. G. Carson, and K. K. White. 1994. Economic Analysis of a *Bacillus thuringiensis*-based integrated pest management programme in fresh-market tomatoes. J. Econ. Entomol. 87: 1463-1469.

Vandermeer, J. 1989. The ecology of intercropping. Cambridge University Press, Cambridge, United Kingdom.

Wagner, H., W. Dorsch, T. Bayer, W. Breu, and F. Willer. 1990. Antiasthmatic effects of onion: Inhibition of 5-lipoxygenase and cyclooxygenase in vitro by thiosulfinates and 'cepaenes' Prostag. Leukotr. Ess. 39: 59-62.

Waterhouse, D. F. 1998. Biological control of insect pests: Southeast Asian prospects. ACIAR Monogr. 51: 235-236.

Zhao, Y. X., and L. Kang. 2003. Olfactory responses of the leafminer *Liriomyza sativae* (Dipt., Agromyzidae) to the odours of host and non-host plants. J. Appl. Entomol. 127: 80-84.

Received 3 November 2008; accepted 28 February 2009.