

Undersowing cruciferous vegetables with clover: the effect of sowing time on flea beetles and diamondback moth

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Abstract

The effects of undersowing cruciferous vegetables with clover on the population of flea beetles (*Phyllotreta* spp.; Coleoptera Chrysomelidae) and the diamondback moth [*Plutella xylostella* (L.); Lepidoptera Plutellidae] were tested, as well as its impact on the crop yield. In 2003, a spring and summer field experiment was conducted, each involving cabbage and cauliflower, at the Experimental Farm of the University of Padua, Italy. Cabbage and cauliflower were undersown with clover, which was seeded 9 days before the crop transplanting, simultaneously with transplanting, and 9 days after transplanting, and were tested against those raised on bare soil as a control. The 37% and 27% fewer flea beetles (Spring and Summer experiment, respectively) were found on cabbage and cauliflower undersown with clover seeded simultaneously with transplanting compared to those undersown with clover seeded after transplanting. In contrast, the diamondback moth density did not vary among the different undersowing treatments in either season. The effect of the undersowing on the crop yield was estimated by comparing the mean weight of the harvested crop heads for each treatment. For cabbage, the mean head weight was reduced in both seasons in plots where clover was seeded simultaneously with transplanting. For cauliflower, the mean head weight did not differ significantly among the different treatments during the Summer experiment. Results show that while sowing clover before or during transplanting of cruciferous vegetables reduces infestation by some but not all economic pests, there may also be a negative impact on crop yield. It is therefore suggested that clover should be sown after transplanting to achieve a smaller reduction in pests but no reduction in crop yield.

Key words: cabbage, cauliflower, crop yield, *Phyllotreta*, *Plutella xylostella*, intercropping, *Trifolium*.

Introduction

Crop production increasingly involves intensive agricultural practices based on machinery and pesticides, allowing cultivation of vast areas dominated by single crop environments. These intensive practices lead to the dominance of monoculture systems comprising of a single crop species or even a single genotype. In contrast, diversified cropping systems or polyculture systems involve growing two or more plant species on the same piece of land at the same time, and takes different forms depending on the arrangement of plant species. In this context, the term polyculture applies to intercropping, undersowing or cover cropping, mixed cropping (Andow, 1991a).

Intercropping is an ancient cropping system practiced in the tropics for reasons of maximising the output from the limited land resources as well as for various horticultural purposes (Perrin and Phillips, 1978). Diversified cropping systems often support lower phytophage densities compared to monoculture systems (Perrin and Phillips, 1978; Andow, 1991a, 1991b; Finch and Kienegger, 1997; Skovgård and Päts, 1997; Schoonhoven *et al.*, 1998).

Some hypotheses were developed to explain the low numbers of insects found in polyculture systems. It was found that polyculture systems affect phytophagous insect through saturating the environment with a mixture of plant odours (Tahavanainen and Root, 1972), or through emitting repellent chemicals (Uvah and Coaker, 1984), or through hiding of the resource as implied by the resource concentration hypothesis (Root, 1973), which predicts that specialist phytophages are more

likely to find host plants that grow in dense or nearly pure stand. Moreover, polyculture systems constitute favourable habitats to natural enemies, predicting that complex environments sustain a greater diversity of phytophages and relatively stable populations of generalist predators and parasitoids (Root, 1973). These systems provide alternative food sources (prey and hosts), nectar and extrafloral nectaries for adult enemies, as well as shelter sites during adverse conditions (Schoonhoven *et al.*, 1998).

The response of phytophagous insects to diversified cropping systems of a crop varies with geographic locations (Vandermeer, 1989). Research work on the response to diversified cropping systems has come out with various results. For instance, intercropping was associated with low abundance of the carrot rust fly (Uvah and Coaker, 1984; Rämert and Ekbohm, 1996), cabbage aphid (Tukahirwa and Coaker, 1982; Finch and Kienegger, 1997; Bukovinszky *et al.*, 2003), pod-sucking bugs of soybean (Sastawa *et al.*, 2004), the diamondback moth and the large white cabbage butterfly (Finch and Kienegger, 1997), the cabbage flea beetles (Andow *et al.*, 1986) and the onion thrips (den Belder *et al.*, 2000). Maguire (1984) found higher numbers of eggs and larvae of the small white cabbage butterfly on collards surrounded by tomatoes.

Cruciferous crops are grown in various cropping systems in tropical and temperate regions, and are attacked by a complex of specialist and generalist insect pests (Hooks and Johnson, 2003). Flea beetles, *Phyllotreta* spp. (Coleoptera Chrysomelidae), represent an important group, because they inflict severe damage to seedlings, particularly when the cotyledons are present.

Adults feed on the leaves, creating small, round pits “shot holes” (Al-Doghairi, 1999) that cause most of the damage to the crop, while larvae are root-feeders and do not cause economic damage. There are several species of flea beetles associated with cruciferous crops, of which the most common species found on cruciferous vegetables in northern Italy is *Phyllotreta cruciferae* (Goeze) (Dalla Montà *et al.*, 2005). Adult flea beetles overwinter in weeds or plant debris and emerge from overwintering sites in early spring and this coincides with the seedling stage of the host plants. *P. cruciferae* often feed in groups, responding to a male-produced aggregation pheromone (Peng *et al.*, 1999), and can cause severe damage to young seedlings.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera Plutellidae), is an economically important specialist pest on crucifers. The diamondback moth has become the most destructive insect of crucifers throughout the world and the annual cost for managing it is estimated to be U.S. \$ 1 billion (Talekar and Shelton, 1993). It feeds on the foliage of cruciferous plants from the seedling stage to harvest, and can greatly reduce the quality and yield of the crop. The continuous use of synthetic insecticides has led to the development of resistance in the diamondback moth. Moreover, it was the first insect reported to develop resistance to the formulations of *Bacillus thuringiensis* (Berliner) applied in the field (Talekar and Shelton, 1993; Kirsch and Schmutterer, 1988). Its ability to migrate over long distances enables it to successfully colonise new areas. This study investigates the effects of undersowing cruciferous vegetables (cabbage, *Brassica oleracea* L. var. *capitata* L. and cauliflower, *Brassica oleracea* L. var. *botrytis* L.) with clover (*Trifolium* spp.), in particular the time of introducing the undersown clover, on the 1) population densities of flea beetles (*Phyllotreta* spp.) and the diamondback moth (*Plutella xylostella*), and 2) on the final yield of both crops.

Materials and methods

Experimental design

Two consecutive field experiments were carried out at the Experimental Farm of the University of Padua, Italy: one during the spring (April 9 to July 3, 2003) (Spring experiment) and the other during the summer (August 4 to October 29, 2003) (Summer experiment). The field is located in the vicinity of Agripolis campus, about 12 km south-east of Padua (9 m a.s.l.; 45°21'N, 11°57'E), and the soil was ploughed, disc-harrowed and levelled.

The experiments followed a split-plot design, and were laid out in three blocks; each block was divided into four plots and each plot was further subdivided into two sub-plots of 5 x 8 m (Spring experiment) and 4.5 x 7 m (Summer experiment). Blocks and sub-plots were separated by bare soil borders of 3 m and 1.5 m, respectively.

Approximately 6-week old seedlings of cabbage, *B. oleracea* var. *capitata* cultivar Capehorn, and cauliflower *B. oleracea* var. *botrytis* cultivar Arizona, (Spring experiment) and cultivar Fremont (Summer ex-

periment) were obtained from a commercial producer. For the undersown crop, we used two species of clover because of their different adaptation to seasonal climate: the white clover (*Trifolium repens* L., cultivar Haifa) in spring and alessandrino clover (*Trifolium alexandrinum* L.) in summer (Stella and Kókény, 1985).

Based on timing of seeding the clover, the four treatments for each crop type were as follows: a) seeding clover 9 days before transplanting cabbage and cauliflower; b) seeding clover simultaneously with transplanting the crops; c) seeding clover 9 days after transplanting the crops; and d) transplanting the crops on bare soil (control). Cabbage and cauliflower were systematically assigned to the sub-plots, whereas the treatments were randomly assigned to the plots.

Clover was manually seeded at a rate of 20 kg/ha. Cabbage and cauliflower were mechanically transplanted in rows with plant spacing of 70 cm between rows and 48 cm within rows on the 18th of April 2003 (Spring experiment) and the 12th of August 2003 (Summer experiment). On the average, 113 and 81 plants per plot of each crop were maintained in spring and summer, respectively. The control plots as well as the borders between the blocks and sub-plots were weeded throughout the experimental period. No chemical fertiliser was applied. The vertical height of plants used in the experiments were measured toward the end of the test. The ground coverage by clover was also visually estimated.

Data collection

Visual count of the flea adults beetles and diamondback moth (larvae and pupae) was carried out on 15 and 10 (Spring and Summer experiment, respectively) randomly selected plants in each sub-plot. Different plants were surveyed on each date of counting for the first half of each experimental season and repeated selection of some plants was performed thereafter. The flea beetles were counted twice a week whereas the diamondback moth was counted once a week. During counting of the flea beetles, sampled plants were approached quietly to avoid casting shade on the plants and disturbing the beetles. A mirror was used to aid in counting the flea beetles hidden in the parts of the plant that were difficult to see (Andow *et al.*, 1986).

Crop yield assessment

Because of delayed flowering, cauliflower did not produce sufficient heads by the end of the Spring experiment (July 3, 2003), and only cabbage yield was assessed, by weighing the heads of 15 randomly selected cabbage plants from each sub-plot. At the end of the Summer experiment (October 29, 2003), all plants in all sub-plots (excluding border ones) were cut at the ground level. Out of these, the heads of 15 randomly selected plants from each plot of cabbage and cauliflower were weighed.

Statistical analysis

For each insect species, the mean percentage of infested plants was plotted against the days after transplanting. For flea beetles, the plotted data revealed two

distinct periods of abundance in both seasons; 0-41 and 41-73 days after transplanting. The corresponding area under the curve for each plot was then calculated by summing the areas of successive trapeziums (two periods for flea beetles) in both seasons (B. Ekbom, personal communication). This area was calculated using the equation $[(y_2+y_1)/2*(t_2-t_1)]$, where y_2 and y_1 are the percentages of infested plants corresponding to the days after transplanting t_2 and t_1 , respectively, in each trapezium. The resulting areas were then divided by the number of days in the time period to derive an average of interpolated values, which was then statistically analysed in an ANOVA. Flea beetle abundance on specific date, and the data on crop yield, were tested in an ANOVA. The Tukey test was implemented for mean separation. In the Summer experiment, the treatment of seeding clover 9 days before transplanting was excluded from the analysis because of poor establishment of the clover. All the analyses were carried out using STATISTICA 6.0 (StatSoft Italia, 2001). Data on the area under the curve for the first period of flea beetle abundance during spring were square root-transformed. In all other cases, ANOVA assumptions were met and all the variables were left non-transformed.

Results

Flea beetles (*Phyllotreta* spp.)

In both experiments, the plots of cabbage and cauliflower undersown with clover suffered a significantly lower percentage of infestation by flea beetles compared to the bare soil plots. Effects of treatments on the percentage of plants infested by flea beetles were not detected in the first period (0-41 days after transplanting) of the Spring experiment as evidenced by the analysis of the area under the curve ($F_{3,16} = 1.11$, $P = 0.38$, figure 1). In this period, although there were few beetles, cabbage has suffered significantly high percentage of infestation compared to cauliflower ($F_{1,16} = 5.17$, $P = 0.04$).

During the second period of beetle abundance (41-73 days after transplanting), however, significant differences were detected in the infestation of the crops ($F_{3,16} = 9.65$, $P < 0.01$). Significantly small area under the curve corresponded to the treatment of seeding the clover before and simultaneously with transplanting (table 1). The control treatment, however, corresponded to significantly large area under the curve. Moreover, during this second period the beetles showed no preference for one crop to the other

($F_{1,16} = 0.97$, $P = 0.34$) as evidenced by the percentage of infestation.

We viewed this abundance of flea beetles by analysing their numbers 55 days after transplanting. During that specific date, numbers of the beetles were significantly low on crops with clover seeded before and during transplanting compared to those on crops with clover seeded before transplanting and those on bare soil (figure 2).

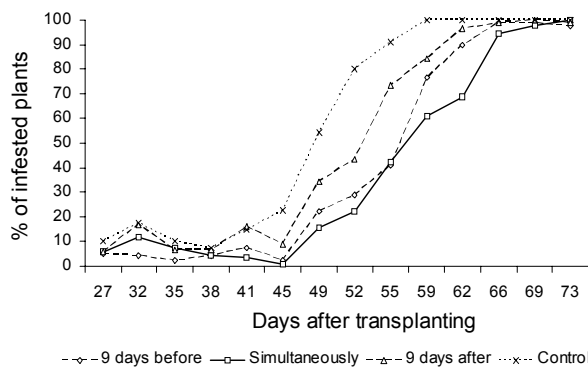


Figure 1. The effect of undersowing with clover, seeded at different times relative to transplanting of cabbage and cauliflower, on percentage of plants attacked by flea beetles during spring 2003.

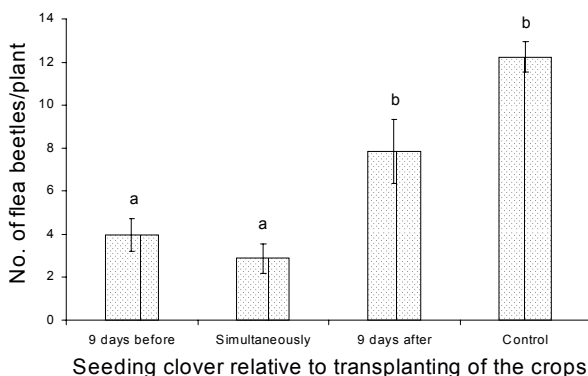


Figure 2. Mean numbers (\pm S.E.) of adult flea beetles per plant on 55 days after transplanting on plots of cabbage and cauliflower undersown with clover seeded at different times relative to transplanting of the crops during spring 2003. Different letters imply significant differences in the pairwise comparison of means (Tukey test, $P < 0.05$).

Table 1. Summary statistic (mean \pm SE) for percentage of cabbage and cauliflower infested by flea beetles. The method used to calculate the area under the curve of figures 1 and 3, is given in the Materials and Methods – Statistical analysis. Different letters indicate significant differences in pairwise comparison of means (Tukey test, $P < 0.05$). dat = days after transplanting.

Treatment	Spring		Summer	
	Average area under the curve (0-41 dat)	Average area under the curve (41-73 dat)	Average area under the curve (0-41 dat)	Average area under the curve (41-73 dat)
9 days before	15.1 \pm 5.8	200.5 \pm 9.1 ab	-	-
Simultaneously	25.3 \pm 10.1	178.5 \pm 18.0 a	303.0 \pm 6.2	106.8 \pm 8.8 a
9 days after	36.3 \pm 14.2	233.7 \pm 13.3 b	313.1 \pm 6.9	164.0 \pm 8.6 b
Control	42.5 \pm 15.3	247.4 \pm 7.1 c	312.4 \pm 7.8	211.5 \pm 16.6 c

In the Summer experiment, a high percentage of infestation in all plots was detected at the beginning of the experiment which declined toward the end of the experiment. The first period (0-41 days after transplanting) have witnessed a high incidence of the beetles when treatment effects have not been detected ($F_{2,12} = 1.07$, $P = 0.37$, figure 3). In this period, significantly higher percentage of infestation was detected on cauliflower compared to cabbage ($F_{1,12} = 12.35$, $P < 0.01$).

In the second period (41-73 days after transplanting), simultaneous seeding of clover with transplanting corresponded to significantly small area under the curve compared to that of seeding the clover after transplanting and the control treatment (table 1). In this period, however, the beetles showed no preference for one crop to the other ($F_{1,12} = 0.04$, $P = 0.84$).

The diamondback moth (*Plutella xylostella*)

Undersowing cabbage and cauliflower with clover did not result in a reduction in the population density of the diamondback moth, estimated as the percentage of infested plants during spring (figure 4) and summer. During both seasons, the different undersowing treatments were associated with similar rates of infestation. In the Summer experiment, however, very low number of diamondback moth was detected in all treatments (data of Summer experiment are not presented).

Analysing the area under the curve of each treatment in spring did not reveal significant differences ($F_{3,16} = 0.46$, $P = 0.7$, mean 427.3, SE 23.4). The moth did not show preference for one crop to the other ($F_{1,16} = 1.32$, $P = 0.27$) as evidenced by the percentage of infestation.

Crop yield

The weight of cabbage heads was significantly reduced when the clover was seeded before and simultaneously with transplanting in spring and when simultaneously seeded with transplanting in summer, but not when seeded after transplanting in both seasons (table 2). No significant differences were detected in the weight of cauliflower heads as a result of undersowing with clover in summer (table 2).

Discussion

Our results showed that undersowing cruciferous vegetables with clover reduced infestation by flea beetles but had no effect on infestation by diamondback moth. Undersowing with clover also reduced the marketable

weight of cabbage. These results, although preliminary and related to small-size plots, support earlier findings that different insect pests respond differently to diversified cropping systems (Andow, 1991b; Held *et al.*, 2003; Hooks and Johnson, 2003). The undersown clover may hinder the movement of beetles, rendering the crops unfavourable to them. The reduced colonisation by flea beetles (Tahavanainen and Root, 1972) and the reduced tenure time of the beetles (Garcia and Altieri, 1992) in undersown plots could thus explain their lower abundance.

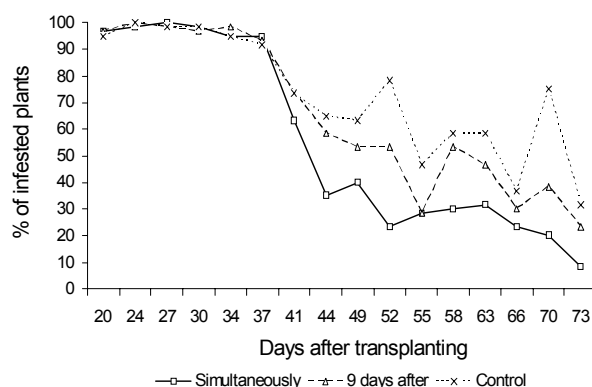


Figure 3. The effect of undersowing with clover, seeded at different times relative to transplanting of cabbage and cauliflower, on the percentage of plants infested by flea beetles during summer 2003.

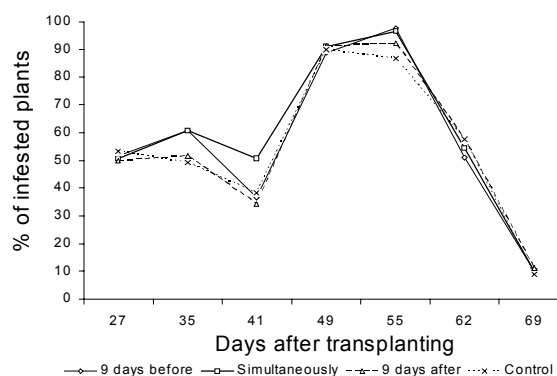


Figure 4. The effect of undersowing with clover, seeded at different times relative to transplanting of cabbage and cauliflower, on the percentage of plants infested by the diamondback moth during spring 2003.

Table 2. Mean (\pm SE) head weight of cabbage and cauliflower undersown with clover seeded on different dates relative to transplanting of the crops during spring and summer 2003. Different letters indicate significant differences in pairwise comparison of means (Tukey test, $P < 0.05$).

Treatment	Spring	Summer	
	Cabbage Mean weight (g)	Cabbage Mean weight (g)	Cauliflower Mean weight (g)
9 days before	639.7 \pm 21.7 a	-	-
Simultaneously	629.2 \pm 16.1 a	727.8 \pm 47.5 a	680.9 \pm 65.7
9 days after	716.2 \pm 21.1 b	970.3 \pm 48.2 b	666.2 \pm 55.0
Control	772.7 \pm 26.5 b	927.1 \pm 50.2 b	653.1 \pm 58.1

During the Spring experiment, the vulnerable seedling stage of the crops coincided with a low abundance of the flea beetles as reflected by the low percentage of infested plants. The low density of the flea beetles in the beginning of Spring experiment may have been due to the slow build-up of the population after emerging from overwintering sites. Later, when the incidence of the flea beetles increased during the late crop developmental stages, the established crops could be able to withstand flea beetle injury, as observed by Gavloski and Lamb (2000) for other crucifers. In contrast, the high density of the flea beetles during the Summer experiment coincided with the seedling stage of the crops. The dynamics of flea beetles presents some degree of complications since summer is the main growing season for cruciferous vegetables in the region (Giulianelli, 1954). As the beetles remain active throughout the spring and summer on cruciferous crops and weeds, young seedlings can suffer severe attack early in the summer growing season, during which time the clover, in our experiment, was too young to offer protection to the young seedlings.

In order to have the desired effects on insect pest densities, two requisites are to be considered: the height of the undersown vegetation relative to that of the main crop, and the percent ground coverage by the undersown vegetation (Finch and Kienegger, 1997). During the Spring and Summer experiments, the flea beetles were not affected by the different treatments until 41 days after transplanting (figures 1 and 3). We attribute this response to the young stage of clover (i.e. small height relative to the crops, and low percent ground coverage, data not presented), which was not sufficient to adequately camouflage the crops. Later in both seasons, the presence of clover significantly reduced the densities of the flea beetles, likely because the height of the clover relative to that of the crops created barriers to the colonising beetles. This effect could be evidenced from the height of the clover later in the experimental period. In spring at 73 days after transplanting, on the average, clover has reached 35% and 26% of the vertical height of cabbage and cauliflower, respectively, in plots of simultaneous seeding of clover compared to a percent of 25% and 16% in plots of cabbage and cauliflower, respectively, where the clover was seeded 9 days after transplanting. In summer at the same stage, on the average, the clover has reached a height of 149% and 93% relative to that of cabbage and cauliflower, respectively, when seeded simultaneously with transplanting compared to a height of 130% and 85% relative to that of cabbage and cauliflower, respectively, when the clover was seeded 9 days after transplanting. By the end of the experimental period, in plots of both crops, the ground coverage was estimated at 85-95% and 90-95% in spring and summer, respectively, for clover seeded simultaneously with transplanting. Based on the height of clover and the percentage of ground coverage, the clover could have created a physical barrier to the beetle movement.

In the Spring experiment, numbers of flea beetles 55 days after transplanting were consistent with the percentage of infested plants. A significant reduction of the beetle numbers was observed in plots with clover seeded before transplanting and those with simultaneous seeding of

the clover. By then, the clover was growing vigorously in these plots, and its dense and tall cover likely acted as an obstacle to beetle movement, therefore, reduced the rate of colonisation.

In the Summer experiment, only after 37 days after transplanting did the treatments produce a pronounced decline in the infestation in plots simultaneously undersown with clover. The beetle population started to decline towards the end of the experimental period, and the percentage of infested plants decreased. The reduction in flea beetle population in undersown plots in this study is consistent with the reduced numbers of flea beetles in undersown plots of mixed cropping systems (Lehmhus *et al.*, 1996), and on cabbage grown with living mulches (Andow *et al.*, 1986).

Our results revealed that the flea beetles have exhibited seasonal preferences to the seedlings of cabbage and cauliflower. In early spring the cabbage seedlings have suffered a higher attack compared to cauliflower; in early summer, however, the reverse is true. This behaviour could be possibly explained by, among other factors, seasonal changes in the chemical profiles of these crops. Renwick (2002) reported that changes in plant chemistry, due to seasonal and environmental factors or nutrition, may affect the acceptance or rejection of a plant.

The diamondback moth did not show any response to the treatments. In other experiments the results have been equivocal: intercropping was found to reduce the densities of the diamondback moth (Åsman *et al.*, 2001; Bukovinszky *et al.*, 2004), to show no effect (Lehmhus *et al.*, 1996; Wiech and Kalmuk, 2003), or to vary depending on the intercropped plant (Theunissen and Schelling, 1996). As the summer of 2003 was exceptionally dry and warm in the region, we suspect that frequent sprinkler irrigation, extended after sunset, may have reduced the moth density. Talekar and Shelton (1993) and Kabori and Amano (2003) reported that sprinkler irrigation and rainfall during late hours of the day can negatively affect the abundance of the diamondback moth by disrupting the activity of adult moths and washing the larvae (except the first instar) off of the leaves.

The results of our experiments suggest that competition negatively affected the final yield of cabbage in some treatments, consistently with the results of Theunissen *et al.* (1995) and Hooks and Johnson (2002). However, these authors introduced the clover well before the main cruciferous crop, about 3-4 and 9-10 weeks, respectively. Our results showed that introducing clover 9 days after transplanting did not cause a significant reduction of yield. Nonetheless, the negative competitive effects of clover could be partly outweighed by increased soil nitrogen as a result of clover's ability to fix nitrogen.

Conclusions

The implementation of crop diversification in vegetable production systems imposes many difficulties concerning the timing of sowing or planting the undersown crop as well as managing the weeds in the undersown plots. Optimisation of the timing is particularly important because growers face a trade-off between reduced infesta-

tion by phytophages and increased competition (and hence, potentially reduced yield) due to undersowing.

We conclude that introducing clover after transplanting of the main crop will result in reasonable yield of the crop while effectively reducing the attack by some insect pests, especially the flea beetles, later in the season.

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