

**LYGUS LINEOLARIS NUTRITIONAL NEEDS AND HOST CHOICES FOR IPM STRATEGIES WITH
PREDATORS AND TRAP CROPS**

M. Solà^{1,2}, F. Dumont¹, C. Provost¹, E. Lucas²

1- Centre de recherche agroalimentaire de Mirabel, 2- Université du Québec à Montréal

ABSTRACT

The need for efficient strategies to control *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) (TPB) is crucial. This study aimed to reveal TPB host choices to provide information for an optimized IPM program. First, to reveal if TPB preferences were driven to fulfill nutritional requirements, the effect of different diets on the TPB performance was assessed. Later, TPB host choices were observed in arenas simulating a strawberry field with predators, *Nabis americanoferus* (Carayon) (Hemiptera: Nabidae), and trap crops (buckwheat and canola). The results suggest that diet influences TPB performance. Specifically, canola and the omnivorous diet improves TPB fitness, while strawberries and spider mites are low-quality hosts. When offered a choice, TPB prefers trap crops, especially canola, and flower is the preferred plant structure. These preferences are shifted to less preferred hosts in the presence of predators.

Keywords: Tarnished plant bug, biological control, trap crops, predator, strawberry

RÉSUMÉ

Le besoin de stratégies efficaces pour lutter contre *L. lineolaris* (Palisot de Beauvois) (Hemiptera : Miridae) (TPB) est crucial. L'objectif du projet était de révéler les choix d'hôte de TPB permettant de fournir des informations nécessaires à l'élaboration d'un programme de lutte intégrée optimisé. Premièrement, pour révéler si les préférences de TPB étaient pour répondre aux besoins nutritionnels, l'effet des différentes diètes sur la performance de TPB ont été évalués. Ensuite, ses choix dans des arénas simulant un champ de fraises avec prédateurs (*N. americanoferus* (Carayon) (Hemiptera : Nabidae)), et des cultures pièges (sarrasin et canola) ont été observés. Les résultats suggèrent que la diète influence la performance de TPB. Plus précisément, le canola et les mélanges omnivores améliorent la performance tandis que les fraises et les tétranyques sont des mauvaises ressources alimentaires. Lorsqu'on lui offre du choix, TPB préfère les cultures pièges, particulièrement le canola. La fleur est la structure végétale préférée. Les préférences changent vers des hôtes moins préférés en présence du prédateur.

Mots-clés : Punaise terne, lutte biologique, plantes pièges, prédateur, fraise

INTRODUCTION

The Tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a cosmopolitan and highly polyphagous pest of field and greenhouse crops. TPB feeds on more than 350 hosts, of which at least 130 are economically significant (Young, 1986). Among them, strawberries, which represent an important source of income for the agriculture of Canada. TPB nymphs and adults' feeds especially on apical plant tissues and developing reproductive organs, resulting in malformation of fruit and abnormal growth habits (Young, 1986, Strong, 1968). As a result, this species entails one of the most critical phytosanitary problems in North America, and no efficient control strategy is

available. Moreover, the organic alternatives purposed fail to control the pest because of not considering the overall complexity of this species.

Indeed, host choices may be led by sensorial preferences, such as visual or olfactory (Blackmer et al., 2004; Frati et al., 2009) or to fulfill nutrient requirements (Burla et al., 2014). In addition, their choices may also depend on the target action, e.g. feeding or reproduction. Alternatively, their behavior may be driven as a response to external factors such as resource scarcity, due to the presence of multi hosts (Bernays et al., 1997) or competitors and predators (Brent et al., 2010). In fact, these external stressor factors could be modulated and used as strategies of biological control against this pest.

Given the limited information available on TPB behavior, learning the feeding requirements on its biology may facilitate the comprehension of their choices and contribute to the knowledge of trophic connections, a central element in the structure of ecosystems (Hagler et al., 2010). From one side, polyphagy at the individual level may be beneficial because it allows insects the opportunity to select different foods that are complementary or that best fit changing physiological needs (Bernays et al., 1997). On the other, eating a variety of different plants allows dilution of potentially poisonous chemicals. Altogether, host choices may provide the insect different degrees of performance by enhancing survival, shortening the development period, and increasing their reproduction (Burla et al., 2014).

Among the panoply of Integrated Pest Management strategies (IPM) available, increasing the plant biodiversity in the environment as means of trap crops may reduce TPB impact on the crop (Accinelli et al., 2015; Rasmert et al., 2001). Trap crops concept consists of insects' attraction to a preferred host plant habitat or oviposition site leaving the main crop relatively free of damage (Sevacherian et al., 1973). This cultural control strategy has successfully been used for managing *Lygus spp.* in organically grown strawberry fields in California with blooming alfalfa (*Medicago sativa*) (L.) (Swezey et al., 2007). Also, it has been reported that mustard (*Sinapis alba*) (L.) and buckwheat (*Fagopyrum esculentum*) (Moench) can be used as lures to alter the TPB distribution in the field (Dumont and Provost, 2017). On the other side, several studies demonstrate that broadening vegetation species also increases the number of natural enemies (Nicholls et al., 2001; Bowie et al., 1999). Particularly, when non-predaceous stages are present or when prey densities are low (Coll & Guershon, 2002; Armer et al., 1998; Naranjo & Gibson, 1996; Wiedenmann and O'Neil, 1990).

The use of predatory heteropterans, such as *Nabis americanoferus* (Carayon) (Hemiptera: Nabidae), as natural enemies can be of interest because these species are rarely host-specific and can display considerable plasticity in the types of prey and plant food that they utilize in nature (Lundgren, 2011). Dumont and Provost (2017) observed that the damsel bug, *Nabis americanoferus*, together with the minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), naturally colonize strawberry plots exploited by TPB and are suspected to be the main contributors to the TPB's mortality (about 50% from large nymphs to adults). The damsel bug feeds on every TPB developmental stage (Guppy, 1986) and follows seasonal trends with peak densities matching with the TPB seasonality (Gonzalez et al., 1997). Overall, the potential of this heteropteran native and non-invasive predator found across North America is yet unexploited. Aside from the reduction of pest populations by predation, the presence of *Nabis* may induce changes of host selection by TPB that could, for example, induce this species to select less preferred hosts, and this needs to be adequately assessed.

The objective of this project was to assess the impact of different hosts on the TPB fitness and then, to reveal the preferred TPB host under the presence of trap crops and the predator *Nabis americanoferus*.

Overall, TPB host decisions are susceptible to change pest population dynamics and their distribution in the space and, consequently affect the degree of damage on the economic crop. Therefore, to start, revealing TPB physiological needs according to diets may provide the first step to understand TPB host choices and then help to improve the management of this pest in an environmentally benign program.

MATERIAL AND METHODS

Rearing and maintenance

Insects. Tarnished plant bugs and *Nabis* breeding were initially started from individuals captured at the beginning of the summer season from fields margins in the Laurentides region (Quebec, Canada). TPB rearing consisted of immature stages stored in small containers (6"x6"x4") ventilated with muslin under controlled conditions. To obtain adult TPB of known age, L5 stage were transferred to larger ventilated plexiglass cages. Then, they were separated according to their age (<7 days, 7> 14 days and > 14 days). Romaine salad was used as substrate and renewed 2-3 times a week. *Nabis americanoferus* (Carayon) (Hemiptera: Nabidae) were provided weekly by the Agri-Food Research Center of Mirabel (CRAM) from their breeding with eggplant (*Solanum melongena*) infested with green peach aphids, *Myzus persicae* (Sulzer) (Homoptera: Aphididae) and *Ephesia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs. Spider mites, *Tetranychus urticae* (Koch) (Prostigmata: Tetranychidae), were purchased from Anatis Bioprotection (Quebec, Canada), while green peach aphids were raised in the laboratory on eggplant plants.

Plants were grown in greenhouses (UQAM, Montreal) from their seed in a mixture of compost and soil. Plants were watered daily and only used at flowering stage.

Rearing's and experiments were maintained under standard environmental conditions in a growth cabinet at 25°C, 70% RH and 16:8 h (L:D).

Biological trials

TPB performance according to diet

The following variables were used to measure host suitability: duration of the nymphal development, survival curves, sex ratio, adult weight, and adult body length. For this purpose, nymphal development was followed from the beginning of N2 stage to adulthood. For that purpose, TPB rearing cages were checked daily for nymph emergence. Newly emerged N1 nymphs were isolated in petri dishes with romaine salad for 5 days. Then, N2 nymphs were singly transferred into test arenas. These consisted in aerated petri dishes (9mm diameter) containing a base of agar gel, a strawberry leaf (*Fragaria vesca* L.) with the abaxial side exposed and a specific diet (treatment). Cotton moistened with water was introduced in each arena. Treatments consisted in : i) strawberry flower, ii) buckwheat flower, iii) canola flower, iv) ~20 *Myzus persicae*, v) ~20 *Tetranychus urticae*, vi) strawberry flower + ~20 *Myzus persicae*. TPB nymphs were allowed to feed freely until the adult stage. To ensure optimal conditions, every 2–3 days TPB were transferred into a new petri dish with fresh agar and diet. Mortality and changes in the developmental stage were daily notated. For each treatment, 20 individuals were tested.

On the same day of adult observation, measures of weight, sex and body length were assessed. To facilitate insect manipulation, adults were previously exposed to CO₂ for a short period. Size measures of body length were performed with Image J (Schneider et al., 2012), while weight was assessed with a precision balance. Three measures per insect were performed, and their mean was used.

Host preferences observations

The preferred host species and plant structure of TPB were assessed in arenas simulating a strawberry field with an integrated pest management program with trap crops, potential preys, and predators. In each arena, 30 fastened (24h) adults TPB (<7 days of emergence) at 1:1 sex ratio were allowed to choose between 3 plants: strawberry (*Fragaria × ananassa* Weston), canola (*Brassica napus* L.) & buckwheat (*Fagopyrum esculentum* Moench) and 4 plant structures (flower, stem, adaxial and abaxial side leaf) under two different treatments. Treatments differed in the presence of 3 adults *N. americanoferus*. Arenas consisted of a cage (60h 40*30L) with an aerated mesh at the top and 2 transparent acrylic sides to facilitate observation. In each cage, three equidistant plants at the flowering stage were offered. Each plant was previously infested (72h before) with 50 preys (*T. urticae*

urticae and *M. persicae*) using a fine paintbrush. TPB Adults were pre-sexed 24h before observation. For this purpose, TPB were exposed for 4-5 seconds to CO₂. Males were easily recognized by marking them with pink fluorescent powder. When the treatment included *Nabis americanoferus*, these were introduced simultaneously to TPB. Insect's position (TPB and *Nabis*) was observed 4 times a day (8:00, 11:00, 14:00 and 17:00h ($\pm 1h$)) for 72h. Cages were put on benches at chest height to facilitate the direct focal observations. Arena's position and the arrangement of plants within each cage were randomized. At each observation, cages were clockwise turned 90°. Ten cages per treatment were prepared.

Statistical analyses

All analyses were conducted using R (R Core Team, 2017). The relationship between nymph mortality and diet was assessed by a Chi squared test. Then, for each diet, nymph mortality was analyzed using a Generalized Linear Model, assuming a binomial distribution for the variable 'number of dead nymphs of the total number of nymphs studied'. Nymphal survival curves were estimated via the proportional hazards model (Cox, 1972) with "survminer" and "survival" R packages (Kassambara et al., 2017; Therneau and Lumley, 2015). Nymphs fed on strawberries were used as the reference treatment (diets). Individuals who did not die by the end of the nymph period were censored (0 = death event did not occur; 1 = death event occurred).

Effects of the developmental time to adult, sex, weight, and body size were only determined for those insects that completed development to adulthood with Kruskal-Wallis test (Kruskal and Wallis, 1952). Since there were no significant differences in developmental time nor adult weight among sexes, males and females were pooled ($X^2_1=1.922$, p-value=0.166, $X^2_1=1.132$, p-value=0.287, respectively). Means separation was analyzed using a Wilcoxon test with $\alpha = 0.05$ as the threshold for significance differences. These did not include TPB fed on spider mites since only 2 individuals survived to adulthood. A Spearman correlation analysis was conducted to determine relationships among the number of days to adulthood and adult measures (weight and body length).

Generalised linear mixed models with a Poisson distribution were implemented to test the effect of host treatment (trap plants or trap plants + *Nabis*), host species (canola, buckwheat, and strawberry), and plant structures (flower, adaxial leaf, abaxial leaf, stem) and their interaction to the number of tarnished plant bugs or *Nabis* in each position. Models were performed with lme4 R package (Bates, 2020). Models included cage as random effect. Significant differences in the mean number of insects (TPB or *Nabis*) of each fixed factor were identified by the Tukey multiple-comparison procedure (Tukey, 1953) with the R package "multcomp" (Hothorn et al., 2008). Percentages of insects observed in the plant structures per each plant were assessed for plotting purposes.

RESULTS

TPB performance according to diet

Results show that the feeding host influences the survivorship rate, the number of days to adulthood and adults' weight and body size (Table I).

Diet influenced the mortality of nymphs ($X^2 = 43.754$, df = 5, 181, $p < 0.0001$). More precisely, feeding on spider mites significantly increased the mortality rate of TPB nymphs ($p=0.012$) while the rest of diets significantly reduced it except for the treatments with aphids or strawberry flowers that this reduction was not significant ($p=0.200$, $p=1$, respectively). Nymphs fed with canola, or a mixture of strawberry flower and aphids presented the lowest mortality rate ($p < 0.0115$). The reduction of TPB mortality fed on buckwheat presented an estimate of 1.73 ($p=0.024$) (Table I).

The assessed diets affected the survival curves of TPB nymphs (LR= 30.14, df=5, $p < 0.0001$). Indeed, feeding on spider mites significantly reduced survivorship at a hazard ratio of 3.057 ($p<0.005$) while the rest of the diets increased it, although this was not statistically significant. Moreover, median survival times of TPB nymphs fed on spider mites was 11[10-17 CI95%] days while for the rest of the diets was superior to 16 days [14 Lower CI 95%].

Diet also significantly affected the duration of developmental time to adulthood ($X^2=26.322$, $df=5$, $p<0.0001$) which differed in 3.52 days among diets. Diets based on canola and the phytozoophagus mixture of strawberry flowers and aphids presented the lowest developmental time to adulthood (<14days). Aphids and buckwheat significantly increased in one day the TPB development time. TPB fed on strawberry flowers required the longest developmental time which was similar to the time needed for the 2 TPB nymphs fed on spider mites that arrived at adulthood (~16 days) (Table I).

Of the 78 *Lygus lineolaris* that completed development to the adult stage, 43 were males and 35 were females indicating an approximative sex ratio of 1:1.

Different diets significantly affected adult's weight and body length ($X^2_5=33.826$, $p\text{-value}<0.001$, $X^2_5=28.633$, $p\text{-value}<0.001$, respectively). A difference of 2.32g was observed in adults TPB among diets. The highest TPB weight was found with adults fed on canola and the mixture of strawberry flower and aphids, while the lightest adults corresponded to those fed on strawberry and spider mites. Adults fed with buckwheat or aphids presented an intermedial weight of 4.5 and 4.25, respectively (Table I). Similar trends were observed on adult's body length. Yet, a difference of 0.85mm in TPB body length was observed among diets. Canola and the zoophytophagous diets presented the largest adults. These adults were slightly larger than those fed on aphids or buckwheat. The strawberry diet provided the shortest adults, which were slightly shorter than the two individuals fed on spider mites that arrived at adulthood (Table I). Females were significantly larger than males ($X^2_1=4.51$, $p<0.5$).

The Spearman correlation revealed that the number of days to adulthood was inversely related to both adult measures ($r^2= -0.503$, $p<0,0001$ and $r^2=-0.360$, $p<0.005$, for adult weight and body length, respectively) and that both adults measures were positively correlated ($r^2=0.55$, $p<0.0001$).

Table I. Number of TPB that completed the development to adulthood (N), percentage of mortality, mean number of days ($\pm$ error) to adulthood, mean adult's weight ($\pm$ error) and mean adult's body length ($\pm$ error) per each treatment. Means followed by the same letter are not significantly different (Wilcoxon, $\alpha= 0.05$).

Tableau I. Nombre de nymphes TPB ayant atteint l'âge adulte (N), pourcentage de mortalité, nombre moyen de jours ($\pm$ erreur) jusqu'à l'âge adulte, poids moyen de l'adulte ($\pm$ erreur) et longueur moyenne du corps de l'adulte ($\pm$ erreur) pour chaque traitement. Les moyennes suivies de la même lettre ne sont pas significativement différentes (Wilcoxon, $\alpha=0,05$).

Treatment	N	% mortality	Number of days to adult	Adult 's weight (g)	Adult's body length (mm)
Canola	18	10	13.39 $\pm$ 0.27a	5.60 $\pm$ 0.30a	4.56 $\pm$ 0.09a
Strawberry+aphids	18	10	13.95 $\pm$ 0.26ab	5.16 $\pm$ 0.16ab	4.32 $\pm$ 0.07ab
Aphids	14	30	14.36 $\pm$ 0.23b	4.5 $\pm$ 0.24bc	4.02 $\pm$ 0.18b
Buckweat	17	15	14.7 $\pm$ 0.35b	4.25 $\pm$ 0.16c	4.06 $\pm$ 0.12b
Spider mites	2	90	16.5 $\pm$ 0.5	3.62 $\pm$ 0.28	4.26 $\pm$ 0.32
Strawberries	10	50	16.9 $\pm$ 0.72c	3.28 $\pm$ 0.19d	3.65 $\pm$ 0.11c

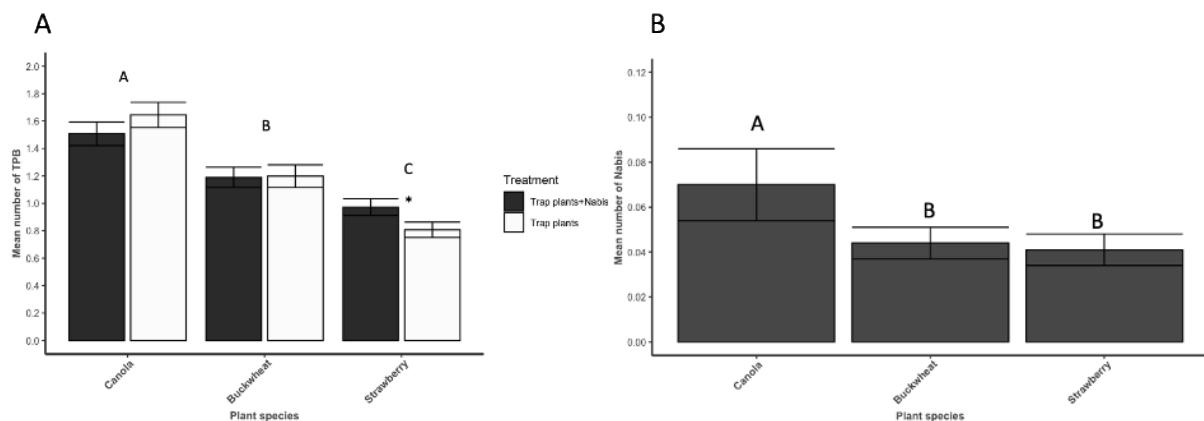
Host preferences observations

Results show that TPB distributes the exploitation of host across the different species and plant structures (Fig.1). Additionally, most part of the time, TPB was found on the hosts. The same behavior was observed for the predator. Yet, across observations, less than 30% of insects were found outside of the plants. Trap crops, especially canola, were the preferred host for TPB while the least preferred feeding host remained the strawberry ($\beta = -0,35 \pm 0,11$; $z = -2,96$; $p = 0,003$, $\beta = -0,43 \pm 0,18$; $z = -2,36$; $p = 0,002$, $\beta = -0,405 \pm 0,18$; $z = -2,26$; $p = 0,024$ for canola, buckwheat, and strawberry, respectively). When *Nabis* was introduced to the system, the number of TPB in strawberries significantly increased ($\beta = -0,86 \pm 0,32$; $z = -2,69$; $p = 0,007$) while the number of TPB in canola were slightly reduced. The presence of TPB in buckwheat did not differed among treatments (Fig.1). *Nabis* preference for plant

presented the same trends as TPB. Thus, canola was significantly the preferred plant ($\beta = -2,76 \pm 0,29$; $z = -9,71$; $p = 0,001$) while buckwheat and strawberries presented the lowest number of *Nabis*.

Fig. 1. Mean number ($\pm$ error) of TPB (A) and *Nabis* (B) on each plant species per cage ($n = 10$ cages) according to each treatment (trap plants or trap plants + *Nabis*). Different letters stand for statistical differences on hosts while asterisks denote differences among treatments per each plant species (Tukey $p < 0.05$).

Fig. 1. Nombre moyen ($\pm$ erreur) de TPB (A) et de *Nabis* (B) sur chaque espèce végétale par cage ($n = 10$ cages) selon chaque traitement (plantes pièges ou plantes pièges + *Nabis*). Différentes lettres représentent des différences statistiques entre hôtes tandis que les astérisques indiquent des différences entre les traitements pour chaque espèce végétale (Tukey $p < 0,05$).

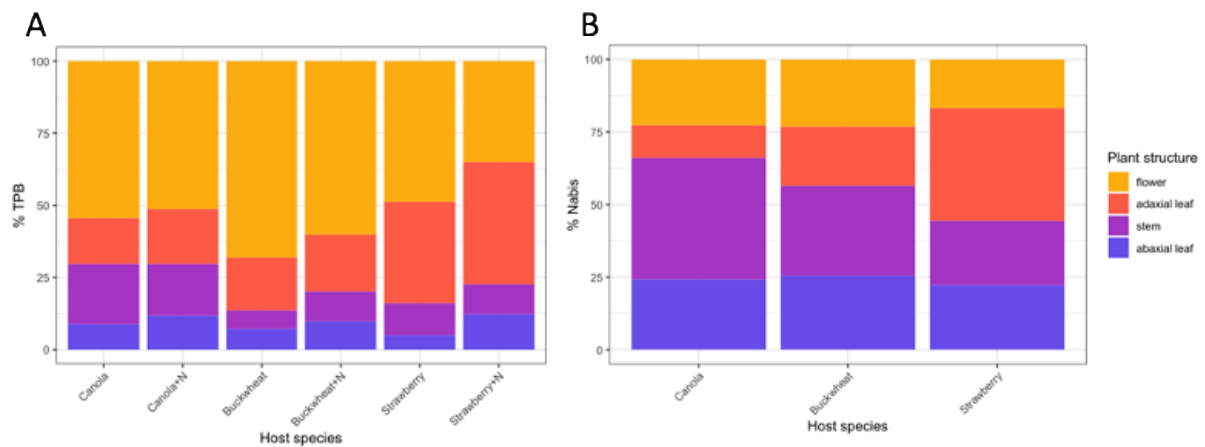


The preferred TPB plant structure was, with difference, the flower (53% of occurrence) ($\beta = 1,47 \pm 0,13$; $z = 11,73$; $p < 0,001$). The frequency of occurrence of TPB among the less preferred structures depended on the plant species. For canola, the second preferred plant position was the stem (21%) and the adaxial side of the leaf (16%) while the least preferred position was the abaxial side of the leaf (9%). For buckwheat and strawberry, the adaxial side of the leaf presented a high frequency of TPB, specially in strawberry (18 and 35%, respectively) while the stem (6 and 10%, respectively) and the abaxial side of the leaf (7 and 5%, respectively) were the least preferred plant positions. This differences among structures were statistically significant only for strawberries ($\beta = 0,77 \pm 0,21$; $z = 3,62$; $p < 0,001$, $\beta = -0,42 \pm 0,21$; $z = -2,06$; $p = 0,04$, $\beta = -0,56 \pm 0,25$; $z = -2,27$; $p < 0,024$ for adaxial side of the leaves, flower and stem, respectively). On the other side, for *Nabis*, the preferences among plant structures were more diluted and presented differences between trap plants or strawberries. Specifically, for canola and buckwheat the frequency of occurrence was : stem (41 and 31%, respectively) > abaxial side of the leaf (24 and 26%, respectively) > flower (22 and 23%, respectively) > adaxial side of the leaf (11 and 21%, respectively) while for strawberries: adaxial side of the leaf (39%) $\beta = 1,32 \pm 0,63$; $z = 2,09$; $p < 0,04$ > stem and abaxial side of the leaf (22%) > flower (17%).

When *Nabis* was added to the system, TPB shifted its plant structure preferences. Precisely, the frequency of TPB found in the abaxial side of the leaf was slightly increased in detriment of the stem structure ($\beta = 0,44 \pm 0,21$; $z = 2,092$; $p = 0,03$). Additionally, when the predator was present, the number of TPB found in the flower was reduced compared to the rest positions. This was only statistically supported for the strawberry plant ($\beta = 0,87 \pm 0,35$; $z = 2,49$; $p = 0,01$) (Fig.2).

Fig. 2. Frequency of occurrence of TPB (A) and *Nabis* (B) on the 4 different plant structures per each plant species according to the treatment. (A) Plant species followed by "+N" stands for plants in the trap plants + *Nabis* treatment while the rest correspond to the plants in the treatment without the predator.

Fig. 2. Fréquence de TPB (A) et *Nabis* (B) sur les 4 structures végétales pour chaque espèce végétale en fonction du traitement. (A) Les espèces végétales suivies de "+N" représentent les plantes dans le traitement des plantes pièges + *Nabis* tandis que le reste correspond aux plantes dans le traitement sans le prédateur.



DISCUSSION

The purpose of this study was to reveal the nature of the TPB host choices to provide information for an optimized IPM program to reduce the pest pressure on strawberry fields. First, to reveal if TPB preferences were driven to fulfill nutritional prerequisites, the effect of different diets on the TPB performance was assessed. The results suggest that diet influences TPB performance as a means of developmental time, rate of mortality and survival curves and physiological adult measures. Yet, in the present study, high-quality resources, such as canola or the phytozoophagous mixture of aphids and strawberries reduced the developmental time to adulthood and presented larger and heavier TPB adults (Table I). A similar relation has been reported in the literature for other species. Burla et al. (2014) observed shortest nymphal instar and longer adult stage of *Tupiocoris cucurbitaceus* (Spinola) (Hemiptera: Miridae) when insect preys were provided on tomato or tobacco leaves than when the leaves were the only available food source. Their results also support the idea that omnivorous mixtures (polyphagy) may increase insect performance. This has also been observed in the present study: TPB individuals fed on a phytozoophagus mixture presented better performance than individuals feed on the resources provided alone (aphids or strawberry flowers) (Table I). Polyphagy may benefit individuals by complementing nutrients that best fit changing physiological needs (Bernays et al., 1997) among other factors.

It is important to distinguish between polyphagy at the individual level (i.e., diet mixing) and generalist species (Mitchel et al., 2006). *Lygus lineolaris* and the members of the heteropteran guild have been described as generalist omnivores, opportunists balancing their diet between prey and non-prey food sources (Robinson et al., 2017; Lundgren, 2011; Hagler et al. 2010; Coll and Guershon, 2002; Agustí and Cohen 2000; Fauvel, 1999; Alomar and Albajes, 1996; Naranjo and Gibson, 1996; Wiedenmann and Wilson, 1996). Polyphagy in heteropteran species is of great interest from both an economic and evolutionary standpoint. This is because several guild members are used as natural enemies while others, such as TPB, are important pests. When important agricultural pests are omnivores instead of strict herbivores, their impact on the host plant may change as the omnivore shifts between consumption of plants and prey consumption (Rosenheim et al., 2014). Due to the importance of TPB as a pest, most of the literature describing the biology and ecology of this species has focused on their role as phytophagous. In contrast, very few studies have quantified the contribution of predation to their total diet (Wheeler, 2001). Nevertheless, the carnivorous tendencies of *Lygus* sp. have been already documented (Rosenheim et al., 2014) and confirmed in this study (Table I). Unfortunately, little is known about the factors that govern the switch between plant feeding and predation among heteropteran species. The results presented in this work may help build a theoretical framework to

predict the behavior of mirids according to their degree of phyto-zoophagy and, therefore, help reduce their impact as pests.

Interestingly, keeping in mind that TPB threatens the strawberry market and that this nutritional resource is low quality (high mortality rate, longer developmental time, and smaller and lighter adults) (Table I), other factors, and the combination of them, may be implicated in the behavior of TPB. Then, to deepen the comprehension of this pest choice, a semi-field experiment simulating a strawberry field with trap crops, potential preys and predators was performed. The results have revealed that the introduction of trap crops in strawberry fields may reduce the pressure on the target culture. When given a choice, a higher number of TPB were observed on trap crops (Figure 1.A). This result is in accordance with the findings of Dumont and Provost (2017). The authors found that *L. lineolaris* was more abundant on buckwheat than on strawberry plants. On the other side, the experiment on nutrient requirements has shown that trap crops, especially canola, represent better quality resources than strawberries (Table I). If *L. lineolaris* choices were based on physiological needs or host preferences, it would be expected that the use of these plant species may reduce the pest pressure on strawberries. However, it is important to keep in mind that trap crops, by improving insect fitness may increase this pest population and intensify crop damage. Simultaneously, several studies on trap crops have revealed that these species also host a panoply of natural enemies. Indeed, the present work has shown that the highest number of the predator *N. americanoferus* were found on canola plants (Figure 1.B). Then, it is expected that the synergy of IPM strategies with trap crops and predators may help to reduce the pest population on strawberries.

Our results have revealed that the preferred plant structure, potentially for feeding purposes, is the flower (more than 50% of TPB occurrence) (Fig. 2.A). The same preference has been observed for *L. hesperus* (Hagler et al., 2016). This preference might explain that even if strawberry represents a low-quality nutrient (Table I) and that this species is the less TPB preferred host (Fig.2.A), the strawberry market, resulting from fecundated flowers, is heavily threatened by this pest.

Under semi-field conditions, it has been revealed that TPB distributes the exploitation of hosts across the different species and plant structures (Fig.1.A and Fig.2.A). This behavior may be explained by the benefits provided with polyphagy, as discussed above, and avoid competition. Brent et al. (2010) observed that *Lygus* nymphs reared under increasing densities exhibited incremental delays in maturation, heightened mortality rates, and reductions in body mass and various size parameters. Thus, collectively indicating that population density can dramatically influence an individual's development and then influence host choices.

On the other side, as already announced, *L. lineolaris* host choices may also be drifted to avoid the predation risk. Here, it has been shown that when *Nabis* was added to the system, TPB shifted its host preferences to less preferred plant hosts and structures. Specifically, in the presence of *Nabis*, the number of TPB in strawberries significantly increased while the number of TPB in canola was slightly reduced (Fig.1.A). Additionally, when the predator was present, the frequency of TPB found in the abaxial side of the leaf was slightly increased in detriment of the stem structure (Fig 2.A) which appeared to be the preferred plant structure for *Nabis* (Fig.1.B).

All in all, this work has revealed the behavioral complexity of TPB. Further studies on nutrients requirements with polyphagous mixtures, host preferences for reproduction, olfactory choices, ecological studies performed outside the laboratory and simulation models are needed to reveal *L. lineolaris* choices in agrosystems under multidimensional situations. In summary, this study has revealed the nutritional prerequisites of TPB. Also, it has suggested that TPB feeding choices may not only be driven for fitness improvement since strawberry has revealed to be a low-quality diet. Additionally, the evaluation of the nutritional adequacy of the diet of TPB has provided important information for mass rearing, especially for other heteropteran species susceptible to be used as natural enemies. Furthermore, this information may help build a theoretical framework to predict the behavior of mirids according to their degree of phyto-zoophagy and, therefore, help reduce their impact as pests. On the other side, it has been shown that the use of trap crops, especially canola, may reduce the pression on strawberries and that the presence of the predator *Nabis americanoferus* may induce changes in TPB behavior, shifting its preferences to less preferred hosts plant and structures.

REFERENCES

- Accinelli, G., Lanzoni, A., Ramilli, F., Dradi, D., & Burgio, G. 2005. Trap crop: an agroecological approach to the management of *Lygus rugulipennis* on lettuce. *Bull. Insectol*, 58(1), 9-14.
- Agustí N, Cohen AC. 2000. *Lygus hesperus* and *L. lineolaris* (Hemiptera: Miridae), phytophages, zoophages, or omnivores: evidence of feeding adaptations suggested by the salivary and midgut digestive enzymes. *Journal of Entomological Science* 35: 176-186.
- Alomar, O., & Albajes, R. 1996. Greenhouse whitefly (Homoptera: Aleyrodidae) predation and tomato fruit injury by the zoophytophagous predator *Dicyphus tamaninii* (Heteroptera: Miridae). Zoophytophagous Heteroptera: implications for life history and integrated pest management, 155-177.
- Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H. & Grothendieck, G. (2012). Package 'lme4'. CRAN. R Foundation for Statistical Computing, Vienna, Austria.
- Blackmer, J. L., Rodriguez-Saona, C., Byers, J. A., Shope, K. L., & Smith, J. P. (2004). Behavioral response of *Lygus hesperus* to conspecifics and headspace volatiles of alfalfa in a Y-tube olfactometer. *Journal of Chemical Ecology*, 30(8), 1547-1564.
- Bernays, E. A., & Minkenberg, O. P. J. M. 1997. Insect herbivores: different reasons for being a generalist. *Ecology*, 78(4), 1157-1169.
- Bowie, M. H., Gurr, G. M., Hossain, Z., Baggen, L. R., & Frampton, C. M. 1999. Effects of distance from field edge on aphidophagous insects in a wheat crop and observations on trap 1186 B.A. Choate and J.G. Lundgren *International Journal of Pest Management*, 45, 69–73.
- Brent CS. 2010. Stage-specific effects of population density on the development and fertility of the western tarnished plant bug, *Lygus hesperus*. *Journal of Insect Science* 10:49 available online: insectscience.org/10.49
- Burla, J. P., Grille, G., Lorenzo, M. E., Franco, J., Bonato, O., & Basso, C. 2014. Effect of different diets on the development, mortality, survival, food uptake and fecundity of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae). *Florida Entomologist*, 1816-1824.
- Coll, M., & Guershon, M. 2002. Omnivory in terrestrial arthropods: mixing plant and prey diets. *Annual review of entomology*, 47(1), 267-297.
- Cox, D. R. 1972. Regression models and life-tables. *Journal of the Royal Statistical Society: Series B (Methodological)*, 34(2), 187-202.
- Dumont, F., Provost, C. 2017. Combinaison de méthodes de lutte pour contrer la punaise terne en fraisière. Rapport Final. Prime-Vert, projet no. CRAM-1-14-1699.
- Fauvel, G. 1999. Diversity of Heteroptera in agroecosystems: Role of sustainability and bioindication. *Agric. Ecosys. Environ.* 74, 275– 303.
- Fрати, F., Chamberlain, K., Birkett, M., Dufour, S., Mayon, P., Woodcock, C., & Bin, F. 2009. *Vicia faba*–*Lygus rugulipennis* interactions: induced plant volatiles and sex pheromone enhancement. *Journal of chemical ecology*, 35(2), 201-208.
- Gonzalez, D., Ramsey, D. A., Leigh, T. F., Ekbohm, B. S., & Van Den Bosch, R. 1977. A comparison of vacuum and whole-plant methods for sampling predaceous arthropods on cotton. *Environmental Entomology*, 6(5), 750-760.
- Guppy, J.C. 1986. Bionomics of the damsel bug, *Nabis americanoferus* Carayon (Hemiptera: Nabidae), a predator of the alfalfa blotch leafminer (Diptera: Agromyzidae). *The Canadian Entomologist*, 118(8): 745-751.
- Kassambara, A., Kosinski, M., Biecek, P., & Fabian, S. 2017. Package 'survminer'. Drawing Survival Curves using 'ggplot2'. (R package version 0.3. 1.).
- Kruskal, W. H., & Wallis, W. A. 1952. Use of ranks in one-criterion variance analysis. *Journal of the American statistical Association*, 47(260), 583-621.
- Hagler JR, Jackson CG, Blackmer JL. 2010. Diet selection exhibited by juvenile and adult lifestages of the omnivores western tarnished plant bug, *Lygus hesperus* and tarnished plant bug, *Lygus lineolaris*. *Journal of Insect Science* 10:127.
- Hagler, J. R., Tassone, E. E., Coffelt, T. A., & Lipka, A. E. 2016. Host plant preference of *Lygus hesperus* exposed to three desert-adapted industrial crops. *Industrial Crops and Products*, 89, 363-367.

Hothorn T, Bretz F, Westfal P. 2012. Package 'multcomp': simultaneous inference in general parametric models.

Lundgren, J. G. 2011. Reproductive ecology of predaceous Heteroptera. *Biological Control*, 59(1), 37-52.

Naranjo SE, Gibson R. 1996. Phytophagy in predaceous Heteroptera: effects on life history and population dynamics. Alomar O, Wiedenmann R., editors. Zoophytophagous Heteroptera: Implications for Life History and Integrated Pest Management, 57-93. Proceedings Thomas Say Publication in Entomology, Entomological Society of America.

Nicholls, C. I., Parrella, M., & Altieri, M. A. 2001. The effects of a vegetational corridor on the abundance and dispersal of insect biodiversity within a northern California organic vineyard. *Landscape Ecology*, 16, 133-146.

Pimm, S. L., and John H. Lawton. 1978. On feeding on more than one trophic level. *Nature* 275.5680: 542.

Ra mert, B., Hellqvist, S., Ekbom, B., & Banks, J. E. 2001. Assessment of trap crops for *Lygus* spp. in lettuce. *International Journal of Pest Management*, 47(4), 273-276.

Robinson, A., Inouye, D. W., Ogilvie, J. E., & Mooney, E. H. 2017. Multitrophic interactions mediate the effects of climate change on herbivore abundance. *Oecologia*, 185(2), 181-190.

Rosenheim, J. A., Goeriz, R. E., & Thacher, E. F. 2004. Omnivore or herbivore? Field observations of foraging by *Lygus hesperus* (Hemiptera: Miridae). *Environmental Entomology*, 33(5), 1362-1370.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671-675.

Sevacherian, V., & Stern, V. M. 1975. Movements of *Lygus* bugs between alfalfa and cotton. *Environmental Entomology*, 4(1), 163-165.

Strong, R. E. 1968. The selective advantage occurring to *Lygus* bugs that cause blasting of floral parts. *J. Econ. Entomol.* 61: 315-6.

Swezey, S.L., Nieto, D.J., Bryer, J.A. 2007. Control of western tarnished plant bug *Lygus hesperus* Knight (Hemiptera: Miridae) in California organic strawberries using alfalfa trap crops and tractor-mounted vacuums. *Environmental Entomology*, 36: 1457-1465.

Team, R. C. (2017). R: A language and environment for statistical computing.

Therneau, T. M., & Lumley, T. 2015. Package 'survival'. R Top Doc, 128(10), 28-33.

Tukey, J. W. 1953. Section of mathematics and engineering: Some selected quick and easy methods of statistical analysis. Transactions of the New York Academy of Sciences, 16(2 Series II), 88-97.

Wiedenmann RN, O'Neil RJ. 1990. Effects of low rates of predation on selected life-history characteristics of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). *Canadian Entomologist* 122: 271-283.

Wiedenmann RN, Wilson LT. 1996. Zoophytophagous Heteroptera: summary and future research needs. In: Alomar O, Wiedenmann R, editors. Zoophytophagous Heteroptera: Implications for Life History and Integrated Pest Management, 190- 202. Proceedings Thomas Say Publication in Entomology, Entomological Society of America.

Young, O. P. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae). *Annals of the Entomological Society of America*, 79(4), 747-762.

Zhu, Y. C., West, S., Snodgrass, G., & Luttrell, R. 2011. Variability in resistance-related enzyme activities in field populations of the tarnished plant bug, *Lygus lineolaris*. *Pesticide Biochemistry and Physiology*, 99(3), 265-273.