AgriScience Program - Cluster Component

Final Performance Report

This template covers the annual performance reporting for the final year of the activity and two additional questions to satisfy the final performance reporting requirements.

Section A: Annual Performance Reporting

This section is the same as previous Annual Reports completed to date and is intended to capture only those results that were achieved during the final year of the activity.

Name of Recipient: Organic Federation of Canada	
Project Title: Organic Science Cluster III: Connecting Environmental Sustainability with the Science of Organic Production	
Project Number: ASC-13	**Final Period Covered by the Report**: 2022/04/01 to 2023/03/31
Activity #: 18 **Name of Activity:** Potential of predatory bugs (*Nabis* and *Orius*) as biological control agents of the tarnished plant bug (*Lygus lineolaris*) in organic strawberry field	**Principal Investigator:** Dr. Caroline Provost, Dr. François Dumont
Activity Start Date: 2018/04/01	**Activity End Date:** 2023/03/31

1. Activity Summary

Please provide a high-level summary of the work carried out during the reporting period in this activity that includes an introduction, objectives, methodology, deliverables, results and discussion. Give an update regarding the year's deliverables that are listed in the activity workplan. Technical language can be used in this section. The suggested length for the summary is 2 to 5 pages with a maximum of 4 tables and figures.

Biological control of tarnished plant bugs with *Nabis americoferus* in organic strawberry fields.

Abstract

The tarnished plant bug is known to be a pest of several host plants including strawberries. Producers of organic strawberry fields must incur high costs using bioinsecticides, hence the interest in finding biological control alternatives. Laboratory and experimental field studies have shown the potential of *Nabis americoferus* as a biological control agent against tarnished plant bugs. This project aimed to test the effectiveness of *Nabis* in the fields by simulating the biological control process already in use for other commercialized predators. Therefore, organic strawberry growers received shipments of *Nabis* nymphs in 2021 and 2022 for several consecutive weeks to regulate populations of the tarnished plant bug. Our results show reduced tarnished plant bug populations and lesser fruit damage. However, as shown in our data, the results can be affected by many parameters, including the mortality rate during transport and the synchronization of the nymphs' stages between *Nabis* and the tarnished plant bug. Additional experiments will be needed to improve these parameters and optimize *Nabis*' releases.

Introduction

The tarnished plant bugs (TPB) *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) are one of the main pests of strawberries in organic production (Lambert et al. 2007). Bioinsecticides registered in this crop against TPB are expensive, which limits their use. In addition, the frequency of their use should be limited to avoid the development of resistance (Zhu et al. 2011; Dorman et al. 2020; George et al. 2021; Tan et al. 2021). Ecological factors such as landscape characteristics and host availability are important for regulating TPB populations (Grab et al. 2018a, 2018b). Several predators regulate these populations (Leigh and Gonzalez 1976; Fleischer and Gaylor 1987; Hagler et al. 2018; Gómez-Domínguez et al. 2021). The potential of *Nabis americoferus* (Carayon) (Hemiptera: Nabidae) (hereafter referred to as *Nabis*) has been demonstrated in our laboratory and field experiments. However, no demonstration of biological control in real in-field situations has been made.

The improvement of *Nabis* rearing makes it possible to produce enough individuals to practice biological control in the field. We have defined that releasing 0.5 adult *Nabis* per plant effectively reduces TPB populations. However, adults are more expensive than nymphs to produce, and the risk of cannibalism during transport may be higher. The well-synchronized introduction of *Nabis* nymphs could achieve the objectives of regulating TPB. *Nabis* released into the field will feed on TPB of equivalent or lower developmental stages. To ensure optimal synchronization for biological control, *Nabis* nymphs can be introduced multiple times until TPB populations reach adulthood. The *Nabis* will therefore be able to impact the pest populations over a long period and have an effect until the harvest of the fruits.

This project aimed to simulate the application of biological control against the TPB in actual organic strawberry crops. By simulating the biological control process, i.e., preparing predators in the laboratory and shipping them in containers by transport, we hypothesized that *Nabis* could impact TPB populations to reduce strawberry damage.

Materials & methods

Study sites

The experiment was conducted in six commercial organic strawberry fields in the summer of 2021 and 2022. In 2021, two fields were at the Nordvie farm in St-Bruno-de-Guigues (Témiscamingue) (47.49078, -79.48334), two fields at the Jean-Pierre Plante farm in St-Laurent-de-l'Île d'Orléans (Capitale-Nationale) (46.86035, -71.05060), one field at the Matto-Val farm in Val-Alain (Chaudière-Appalaches) (46.45558, -71.75060) and one field at the Patrice Coursol farm in Mirabel (Laurentides) (45.622777, -73.976643).

Nabis rearing, preparation, and shipping

The *Nabis* released in this project were raised in a laboratory at the Centre de recherche agroalimentaire de Mirabel (CRAM) in Mirabel. The *Nabis* were raised in cages with six eggplant plants and romaine lettuce. They were fed *Ephestia* eggs. The temperature in the rearing room was maintained at 25°C, the relative humidity at 55%, and 16 hours of light per day were provided.

The *Nabis* were shipped to the various producers via the transport company UPS. Packages were delivered in less than two days to sites in Témiscamingue and Quebec City. The *Nabis* were introduced on the same day for the site in the Laurentians.

In 2021, 16 nymphs of stages L3 to L5 were put in containers of 250 ml. Buckwheat scales and *Ephestia* eggs were added to the containers. The containers were arranged in coolers and kept cool using IcePack. In 2022, 32 nymphs of stages L2 to L3 were put in the 250 ml containers with buckwheat's scales and *Ephestia* eggs. The shipments were prepared in the same way as 2021.

Experimental design

In each field, two plots of 12 rows by 20 m long were defined. These plots were at least 50 m apart from each other. The *Nabis* treatment was applied in one of the plots, while the control treatment (without the introduction of *Nabis*) was assigned to the other plot.

In 2021, three introductions of *Nabis* nymphs (L2-L4) were made in plots with *Nabis* treatment. Nine containers of 16 *Nabis* nymphs were systematically distributed in the plots. Therefore, a total of 144 *Nabis* was released with each introduction. These introductions were made every two weeks: May 4th, May 18th, and June 1st for the Mirabel (Laurentians region), Île d'Orleans (Quebec region), and Val-Alain (Chaudiere-Appalache region) sites, and on May 12th, May 26th, and June 9th for the Témiscamingue sites.

In 2022, improvements in our breeding techniques have allowed us to introduce more individuals into the fields. In each field, 12 containers of 32 *Nabis* were distributed in the plots. One container for each row. Introductions were done for four consecutive weeks: from May 10th to May 31st for the Mirabel, and from May 18th to June 8th for Île d'Orléans, Val-Alain and Témiscamingue sites. If we assumed that all shipped individuals were introduced in the field, each treated plots received 1536 *Nabis*.

Monitoring populations and damage

The tarnished plant bug populations were monitored weekly for five weeks (except for the Témiscamingue fields which were followed for four weeks) by threshing. In each plot, 25 beatings were made. They were evenly distributed all over the rows (to cover the entire plot). The number of nymphs and adults of TPB and the presence of *Nabis* were noted. Monitoring began on May 18th (Mirabel), May 19th (Val-Alain), May 25th (Île d'Orléans) and May 31st (St-Bruno-de-Guignes).

For damage tracking, in 8 of the 12 rows, 50 randomly selected strawberries were observed to detect noticeable traces of TPB damage. The number of damaged strawberries per row was noted. Two damages monitoring were carried out on June 7th and 16th in Mirabel, June 29th in Île d'Orléans (so only one follow-up to this site), July 2nd and 9th for Val-Alain, and June 14th and 22nd for St-Bruno-de-Guignes.

Statistical analyses

A mixed linear generalized model (GLMER) for Poisson distribution was used to test the effect of the *Nabis* treatment on the density of TPB nymphs observed per plant. The date (linear and three-degree polynomial relationship) and the interaction between the *Nabis* treatment and the date were included in the model. The farm and field were included in the model as random variables. Different models were run for 2021 and 2022 because we used a different release rate.

A GLMER model for Poisson distribution was used to test the effect of releasing *Nabis* on the number of damaged strawberries. The treatment was included as a fixed effect and the farm and fields were included as random effects. Different models were done for 2021 and 2022.

For all models, the statistical significance of fixed effects was estimated with the likelihood ratio rest (LRT) using the function *drop1* in R.

All analyses were performed in R (R Core Team 2022).

Results

Effectiveness of the Nabis

In 2021, temporal variations in TPB abundance followed a quadratic curve ($LRT_2 = 73.44$; $p < 0.0001$) (Figure 1). After peaking in early June, populations declined in abundance. The introduction of *Nabis* had a significant effect on TPB ($LRT_1 = 6.44$; $p = 0.01$) (Figure 2). The difference between *Nabis* and control treatments was most pronounced during the peak period (Figure 2). An average reduction of 31% in population's abundance was observed. Differences in treatment effectiveness between sites are revealed by the significance of random slopes ($Chi_2 = 28.52$; $p < 0.0001$) (Figure 3).

In 2022, TPB abundance increased over time following a three-stage polynomial relationship (LRT_3 = 241,93; $p < 0.0001$) (Figure 1). *Nabis* treatment had no statistical effect on TPB (LRT_1 = 3,03; $p = 0.08$) (Figure 2). The random slopes were not statistically significant indicating that the effects of treatments did not vary following the fields (Chi_2 = 1,95; p = 0,38) (Figure 3).

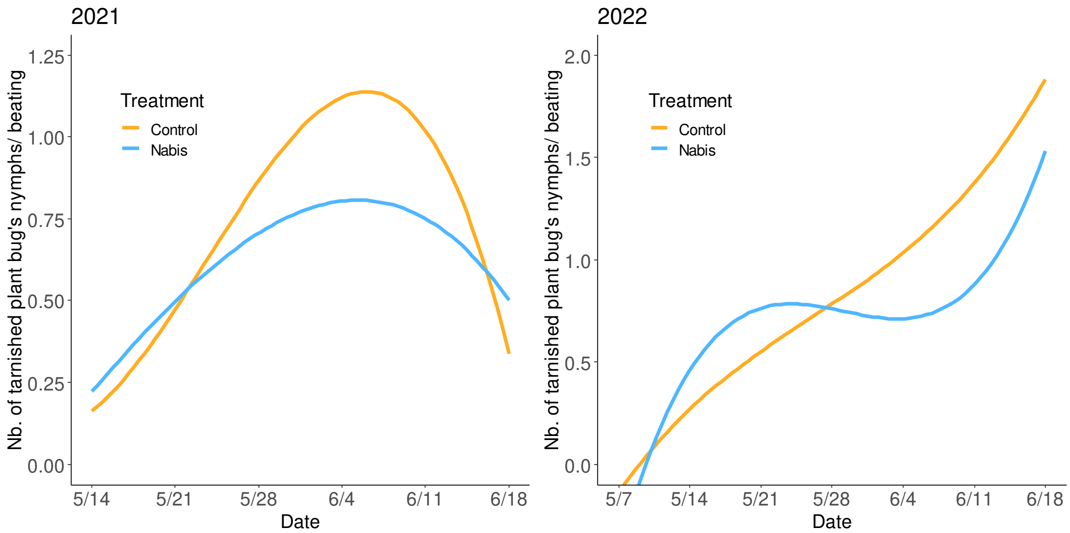

Figure 1. Modelling the temporal variation of tarnished plant bug nymphs' abundance by beating as a function of *Nabis* release treatment in 2021 and 2022.

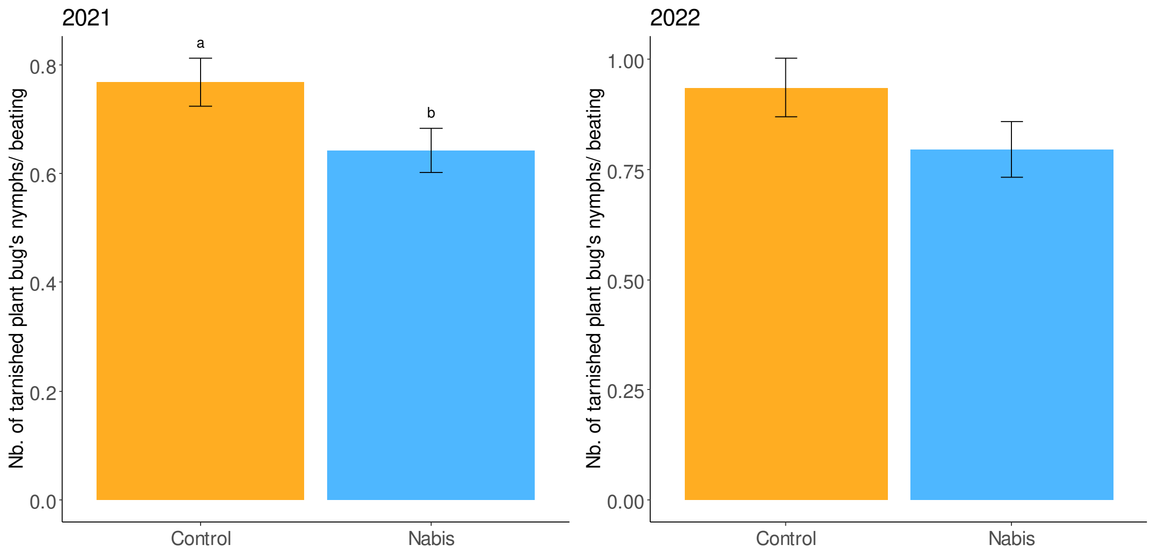

Figure 2. Average abundance of tarnished plant bug nymphs based on *Nabis* release treatment in 2021 and 2022. Different letters indicate a statistical difference ($\alpha = 0.05$).

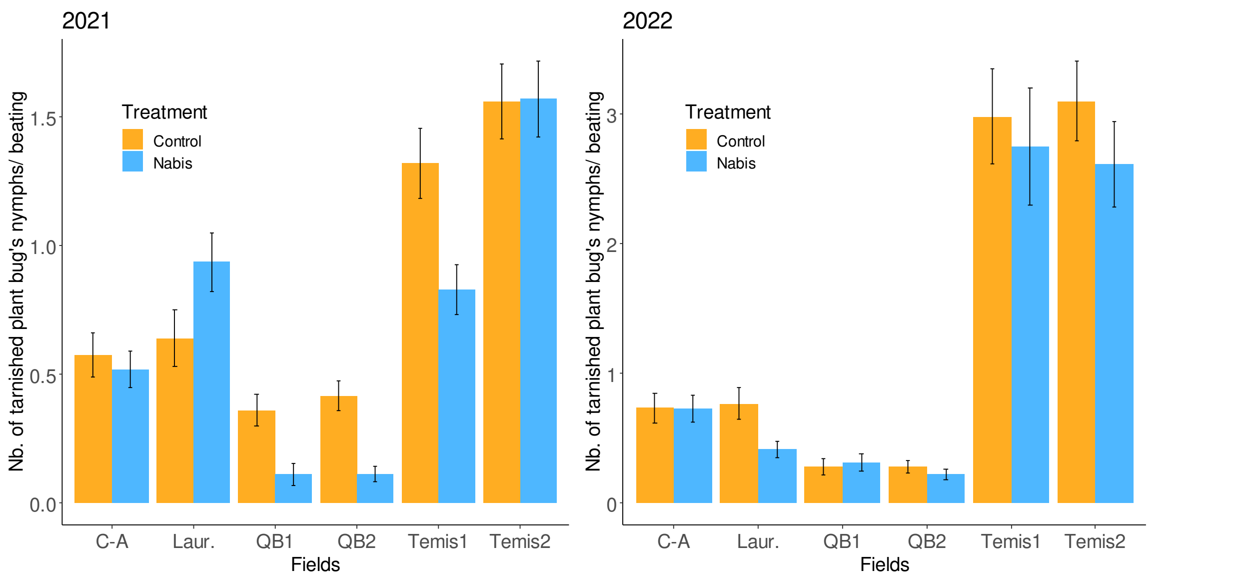

Figure 3. Average abundance of tarnished plant bug nymphs based on *Nabis* release treatment and fields in 2021 and 2022.

Effect on fruit damage

In 2021, introductions of *Nabis* resulted in reduced damage to treated plots ($LRT_1 = 9.53$; $p = 0.002$) (Figure 4). A significant portion of the variability was explained by differences between fields ($Chi^2 = 6.32$; df = 1; $p = 0.01$) (Figure 5).

In 2022, *Nabis* treatments had no effect on the amount of strawberry damage ($LRT_1 = 1.48$; $p = 0.22$) (Figure 4). Differences between sites explained a significant portion of the variability in the data ($Chi^2 = 57.52$; df = 1; $p < 0.0001$) (Figure 5).

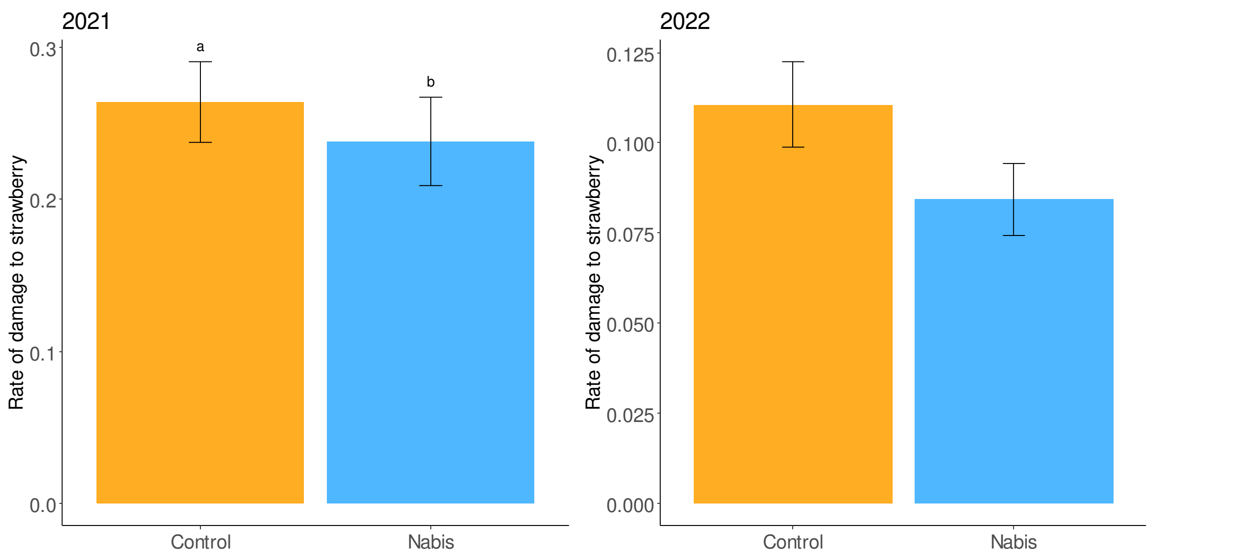

Figure 4. Strawberry damage rate caused by tarnished plant bugs based on *Nabis* release treatment in 2021 and 2022. Different letters indicate a statistical difference ($\alpha = 0.05$).

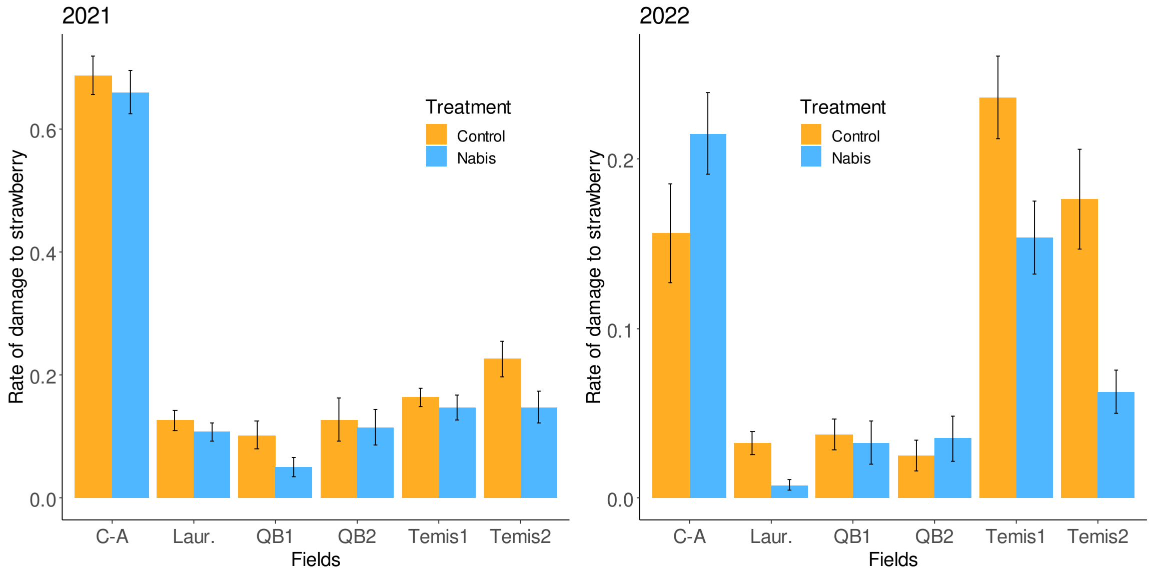

Figure 5. Strawberry damage rate caused by tarnished plant bugs based on *Nabis* and field release treatment in 2021 and 2022.

Discussion

The release of predators for biological control in fields can affect pest populations at a different time and space scales. Released predators can have a rapid (within days or weeks) and localized (where they were introduced) effect, but also a longer lasting and larger area effect. As part of our experiment, we measured the localized and short-term effect of successive releases of *Nabis*. Releases of *Nabis* in 2021 effectively reduced the density of TPB during the peak period, resulting in reduced fruit damage. However, in 2022, when more than 3.5 times more *Nabis* were released, a marginal non-statistically significant reduction in TPB densities was observed. This year-to-year difference in efficiency could be explained by reduced efficiency of young (L2) nymphs of *Nabis* released in 2022, while the individuals released in 2021 were nymphs of more advanced stages (L3-L4). Weather and mortality during transport may also explain this decrease in efficiency between 2021 and 2022. All these assumptions are mutually inclusive.

Young *Nabis* nymphs are less voracious than nymphs of more advanced stages of development or adults (Dumont et al. Submitted). It is therefore possible that releases of young nymphs have a less pronounced local and short-term effect than releases of more developed individuals. Good synchronization between the developmental stages of *Nabis* and TPB is also more difficult to achieve with young nymphs. *Nabis* nymphs consume TPB of equivalent or lower stages (Dumont et al. Submitted). Thus, to be effective immediately, young *Nabis* nymphs must be released at the time that TPB are in the L1 and L2 nymphal stages. In 2022, it is possible that the majority of released *Nabis* were from lower stages than TPB found in the field, which could explain why our releases were not effective.

The cold and rainy spring of 2022 may have reduced the efficiency of the *Nabis* in the field. *Nabis* are ambush predators. Therefore, they wait for their prey and catches them when they arrive to meet them. This type of predator is most effective when prey is active, which increases the chances of encounter (Greene 1986; Avgar et al. 2008; Wearmouth et al. 2014). In cold and rainy conditions, TPB were probably less active than in better conditions. The result could therefore be a reduced encounter rate that translates into a reduced predation rate. Favorable or unfavorable weather conditions for the biological control role of the *Nabis* against the TPB are not known. Finally, the poor conditions of 2022 have potentially generated mortality among the released *Nabis* who had already suffered from the transport.

The transport of biological control agents from the point of production to the field is a significant challenge that limits their effectiveness (Van Lenteren 2000). In this demonstration project, we intended to simulate conventional releases where growers obtain biological control agents from a supplier and receive them by mail (in less than 48 hours). Agronomists who released *Nabis* into the fields report observing some level of mortality in containers in 2022. Thus, a large proportion of the 1536 *Nabis* shipped by field probably died during transport or shortly after their release. The exact causes of this mortality have yet to be determined. The 2022 containers contained 32 nymphs at the start compared to 16 nymphs in 2021. It is possible that this density leads to a higher rate of cannibalism among nymphs. In addition, young nymphs are more fragile than large nymphs. Transport could therefore have a significant negative impact on these forms. Moreover, on the Mirabel site, where introductions are made directly from the laboratory to the field, without going through the mail transport stage, the *Nabis* released in 2022 have been very effective. A reduction of approximately 50% in the TPB population has been observed at this site. Thus, mortality during transport would probably be the most significant factor in explaining the lack of efficiency of *Nabis* in other regions of Quebec in 2022.

In general, releases of Nabis into organic strawberry fields have been effective in the biological control of TPB. However, parameters remain to be defined to improve their effectiveness in spring conditions, reduce mortality during transport and increase the cost effectiveness of the approach. The latter aspect could be based on precision biological control. This approach is based on precise knowledge about the distribution of pest bugs in strawberry fields and the localized introduction of predators. Our experiments have shown that under the right conditions, *Nabis* releases can have rapid and sustained localized impacts (Dumont et al. Submitted). In addition, the spatial distribution of TPB can be influenced by the development of trap crops (Swezey et al. 2013, 2014; Hagler et al. 2018; Dumont and Provost 2019, 2022). A high concentration of pest bugs in these areas also promotes predation by *Nabis* (Hagler et al. 2018).

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Section B: Final Performance Reporting

The following three questions are supplemental to the standard APR questions, to gather additional information as required for the final year of performance reporting.

5. Final Summary of the Research Activity and Results

Summarize the final results of your research activity in plain language. Include: Problem statement (why), Objectives (what), Methods (how), Results and conclusions (word them as recommendations for producers if applicable), Future research needed. Maximum 1000 words. This summary may be edited for brevity and clarity. It will be published on the OSC3 website in both official languages, so please respond in whichever language is more comfortable for you. Please also include: a) the list of all researchers, co-investigators, co-authors who should be acknowledged for the project, b) the list of collaborating partners to be acknowledged, c) 2-3 good quality pictures that we can post with the summary along with photo caption including a brief description of the photo, location, names of anyone in the photo, month/season and year, name of the person who took the photo.

La demande pour les fraises biologiques a augmenté rapidement au cours de la dernière décennie. La punaise terne *Lygus lineolaris* (Palisot de Beauvois) (Hémiptères: Miridae) est la principale barrière qui empêche les producteurs de fraises conventionnels d'adopter une gestion biologique. La punaise terne est attaquée par plusieurs espèces prédatrices et parasitoïdes, en particulier les punaises et les araignées prédatrices. Les punaises prédatrices, *Nabis americoferus* (Carayon) (Hemiptera: Nabidae) et *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), colonisent naturellement les parcelles exploitées par la punaise terne et sont soupçonnées d'être les principaux contributeurs à la mortalité de celle-ci. Le potentiel de ces prédateurs n'est pas encore exploité, mais pourrait constituer une partie importante de la solution dans la gestion de la punaise terne dans les champs de fraises biologiques. L'objectif principal de ce projet était de déterminer le potentiel de deux hémiptères prédateurs, *N. americoferus* et *O. insidiosus*, en tant qu'agents de lutte biologique contre la punaise ternie et d'optimiser leur rôle dans les champs de fraises biologiques. Deux grands volets ont été développés : 1) établir le potentiel des prédateurs en laboratoire et en champs; et 2) explorer le potentiel d'améliorer génétique des *N. americoferus* par la sélection artificielle basée sur le niveau d'agressivité.

Pour le premier volet du projet, nous avons mené des tests de laboratoire pour déterminer la voracité des adultes et larves de *N. americoferus* et *O. insidiosus* pour différents stades de punaises ternes. Ensuite, nous avons réalisé des tests en champ expérimental pour définir le taux et le moment d'introduction optimal. Enfin, nous avons testé notre approche en champs de fraises biologiques. Pour le second volet, nous avons développé un test éthologique mesurant l'agressivité des *N. americoferus* adultes. Par sélection artificielle positive et négative, nous avons développé des lignées agressives et dociles. La propension à la prédation intraguilde envers *O. insidiosus* par les lignées (tests de laboratoire) et leur efficacité en champ ont été testées (champs expérimentaux).

Nous avons démontré que tous les stades de *N. americoferus* consomment des punaises ternes de stades équivalents ou inférieurs. Les adultes *O. insidiosus* s'attaquent aux jeunes larves de punaises ternes (L1-L2). En champs, toutes les densités testées de *N. americoferus* ont efficacement réduit les populations de punaises ternes pendant plusieurs semaines comparativement au témoin, mais *O. insidiosus* avait un effet marginal sur le ravageur. De plus, pour toutes les périodes d'introduction testées, Nabis s'est avéré efficace pour réduire la population de punaises ternes. En champs de fraises biologiques, les introductions de *N. americoferus* ont généralement réduit les densités de punaises ternes et les dommages aux fruits qui en découlent. Cependant, la mortalité des *N. americoferus* dans le transport a représenté un défi pour l'application de la lutte biologique avec ce prédateur. En laboratoire, l'héritabilité réalisée était de 0,16 et 0,27 pour l'agressivité et la docilité chez les *N. americoferus*. Les mâles sont plus agressifs que les femelles. Les lignées agressives attaquent davantage les *O. insidiosus*, ce qui résulte en plus de prédation intraguilde. Toutefois, les lignées dociles ont une meilleure capacité d'ajuster leur réponse aux conditions. Quand les proies extraguildes étaient disponibles (punaise terne), ce qui avait pour effet d'augmenter la densité de proies (le total des punaises ternes et *O. insidiosus*) les Nabis dociles ajustaient leur taux d'attaque à la hausse. En champ, les *N. americoferus* agressives réduisaient davantage la densité de punaises ternes que les *N. americoferus* dociles. Les dommages aux fraises par la punaise terne étaient réduits dans les parcelles avec les *N. americoferus* agressives. Le caractère agressif et docile était conservé pendant plusieurs semaines dans les parcelles où les *N. americoferus* ont été introduites.

Les *N. americoferus* sont des prédateurs efficaces de punaises ternes en champ de fraises biologiques. L'introduction de larves de stades avancés (L4 – L5) ou d'adultes est préférable à l'introduction de jeunes larves (L2 – L3). Les effets locaux (là où les *N. americoferus* sont introduites) sont observés pendant plusieurs semaines à une densité de 0,25 Nabis par plants. Il est conseillé de traiter les foyers d'infestation de punaise terne dès que les jeunes larves du ravageur sont observées (L1 à L3). La sélection de *N. americoferus* agressives pourrait accroître les bénéfices procurés par ce prédateur. Toutefois, les lignées agressives sont plus difficiles à élever et elles font davantage de prédation intraguilde envers les *O. insidiosus* que les lignées dociles. Les *O. insidiosus* sont des prédateurs secondaires de la punaise terne et elles ont est effets plus limités en champ. Il est conseillé d'aménager le paysage de façon à maintenir et promouvoir ces deux prédateurs indigènes. L'aménagement de plants de molène serait bénéfique pour les *N. americoferus* à l'automne.

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Chercheurs et auteurs

- François Dumont, Ph. D.
- Caroline Provost, Ph. D.

Co-investigateurs

- Maud Lemay, M. Env.
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- Maggie Bolduc, agronome
- Madeleine Olivier & Sylvie Côté, ferme Nordvie

Collaborateurs

- Ferme Nordvie (Témiscamingue)
- Ferme Jean-Pierre Plante (Île D'Orléans)
- Ferme Matto-Val (Val-Alain)
- Ferme Patrice Coursol (Mirabel)

Nymphe de *Nabis americoferus* sur une feuille de fraises après un lâcher dans un champ de fraises biologiques. Ferme Patrice Coursol, Mirabel, mai 2021. Photo prise par Maud Lemay.

Dispositif de l’envoi des nymphes de *Nabis americoferus* : petit pot de plastique contenant des écailles de sarrasin et des œufs d’*Ephestia*. Des nymphes de Nabis sont encore présentes à la surface. Laboratoire du Centre de recherche agroalimentaire de Mirabel, Mirabel, juin 2022. Photo prise par Maud Lemay.

Champ de fraises biologiques à la ferme de Patrice Coursol à Mirabel après un lâcher de *Nabis americoferus*. Mai 2022. Photo prise par François Dumont.